Replacement of fish meal with the waste of smoked fish processing and snail (*Pila ampulacea*) flesh meal in the fermented practical diet of walking Catfish (*Clarias* sp.)

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Abstract. Waste of smoked fish processing still contains nutritious ingredients for fish feed. Aims of this research were to determine the effect of giving pellets from fish fumigation waste to the growth and proximate levels of fish. Dumbo catfishes that feed by recycled pellets and characterizing fungal content that might contaminate recycled pellets. Catfishes were grouped into five groups, and feed with a variation of smoked fish waste contents in pellets K.0 (Control), K.1 (0% of recycled waste), K.2 (30% of recycled waste), K.3 (60% of recycled waste), and K.4 (90% of recycled waste). Feeding and nourishment were performed for 30 days. Results indicated that K4 yields the highest yield of weight and length for catfishes with a length of 22.28±0.69 cm and weight 31.74±0.93 gr, in addition to the lowest FCR value 0.77.

**Keywords:** *Clarias gariepinus*, Dumbo catfishes, organic waste of smoked fish, recycled, fish pellets, proximate analyses, fungi contents.

1. Introduction

Fishmeal is the major protein source in fish feed [1]. An increase in the demand for fish meal as consequence of aquaculture and poultry industries development makes the price of the fish meal increase consistently [1]. The utilization of waste and by-product of fish processing industries is a way to utilize optimally the sources of fish meal [1]. The smoked fish in the Kenjeran beach area, Surabaya is one of the traditional products of the fish processing industry in Indonesian countries [2]. This waste contains high organic content from the internal organs, heads, tails and fish fins. These wastes contain nutritious component such as 35.68% of protein; 7.63% of fat; 10.3% of water; 11.7% of ash; 6.73% of fiber and 19.15% of carbohydrate, in which can be used as a source of ingredient especially protein for fish feed [3]. Therefore, the wastes might be utilized for the ingredient in fish pellets to provide cheaper fish feed and to reduce the environmental impact of the wastes.
Feed nutrition is one of the factors that play an important role in aquaculture [4]. High fish growth will be obtained through appropriate feeding in the terms of quantity and quality of feed, and nutrient contents such as protein, fat, calories, vitamins and minerals. [1, 4, 5]. In modern aquaculture, artificial feed in the form of pellets is used widely in the world [1].

Walking catfish *C. gariepinus* is the common commodity in freshwater aquaculture in several countries such as Indonesia, Thailand, and Vietnam [6]. This fish grows fast, resistance to diseases, a high market demand, and ecologically wide tolerance to environmental conditions [2, 6]. However, the high price and high feed conversion ratio of artificial feed are the main constrain in catfish aquaculture. Therefore, smoked fished waste is one of the alternative ingredient sources for cheaper feed to increase the profitability of walking catfish aquaculture. This research aimed to evaluate the nutrition content of smoked fish waste, utilize the smoked fish processing waste for the main source of protein in catfish feed, evaluate the growth performance of catfish fed the feed, and evaluate the nutrition of catfish carcass.

Pellets with a composition of 60% fish waste (fins and tails), 10% rice snails, 27% bran, 2% vitamins and 1% tapioca flour can contribute to the development of fish growth with the highest relative weight, namely 72.64%, and 488.97%. Besides, this composition also provides the highest protein value in catfish meat which is equal to 20.79% [2]. Protein analyzed nutrient content in catfish meat [2]. Therefore, proximate analysis of pellets and catfish meat [7, 8] should be examined. In this study, there were variations concentrations on fish waste contents. This research is important because it can find the combination of pellet material from fish fumigation waste, which can produce maximum catfish growth and to know the proximate content of fish pellets and fish meat. The purpose of this study was to determine the nutrient content of pellets made with the main components of fish fuming waste, and evaluate the growth performance of catfish fed, the quality of catfish produced in African catfish (*Clarias* sp.).

2. Materials and methods
The research was conducted from January-December 2018 at the Zoology and Animal Engineering Laboratory, Department of Biology, Faculty of Science, Institut Teknologi Sepuluh November (FS-ITS) and in the Veterinary Analysis Unit, Faculty of Veterinary Medicine, Airlangga University Surabaya.

2.1. Smoked fish waste, diet preparation, and fermentation
Waste of smoked fish processing (WSFP) was collected from the smoked fish industry in Kenjeran, Surabaya, Indonesia. The waste was packed in a plastic container. The fish species processed in this industry were *Scomberomorus commerson*, *Arius thalassinus*, *Himantura gerrardi*, *Lutjanus* sp, *Siganus* sp, and *Euthynnus affinis*. Snails, *Pila ampulacea* were collected from rice fields, the flesh was separated from the shell and washed with fresh water. The smoked fish waste and the snail flesh were dried by oven at 40°C, milled and sieved with manual meat rollers. Rice bran was obtained from local rice farmers. The composition of basic material for production fish pellets from the flour of *Pila ampulacea*, rice bran, tapioca, vitamin was 40; 54; 2 and 4%, respectively. The diets were formulated with the various substitution level of fish meal by WSFP meal and snail flesh meal (SFM) (table 1). The vitamin (traditional market) added in this diet contained. The ingredients were mixed homogenously, and the moist mashes were fermented with *Saccharomyces cerevisiae* (traditional market) and *Rhizopus* sp (traditional market) at 1.25 g/kg of feed. The fermentation was carried out for 12 hours in aerobic conditions. Then the fermented moist mashes were passed through a manual pelletizer with a 3.0 mm sieve diameter. Wet crumbled pellets were collected and dried in a hot air-drying oven at 45°C till the moisture content was approximately 10%. Pellets were stored at room temperature, then wrapped in plastic packaging until used [9].
2.2. Feeding trial
Walking Catfish in the average body weight of ± 8gr and an average total length of ± 9 cm was used in this feeding trial. The catfish were grouped into 5 treatments (K.0, K.1, K.2, K.3, and K.4) in triplicates. Catfish was reared in 50 cm x 50 cm containers at a stocking density of 7 catfish/container. At the beginning of the experiment, catfish were fasting for 24 hours to remove remaining food in the digestive tract. The feeding trial was carried out for 30 days. The catfish were fed with the respective diet twice a day [10] at 20 % of the feeding rate. At the beginning and the end of the experiment, weight and length of the fishes were measured [11].

| Pellets   | Composition (%) |
|-----------|-----------------|
|           | K.0 | K.1 | K.2 | K.3 | K.4 |
| WSFP meal | 0.  | 0.  | 30  | 60  | 90  |
| SFM       | 0   | 100 | 70  | 40  | 10  |

K.0 = Control/Commercial Pellets, K.1 = Combination 1 (organic waste 0%); K.2 = Combination 2 (organic waste 30%); K.3 = Combination 3 (organic waste 60%); K.4 = Combination 4 (organic waste 90%).

3. Data Analysis

3.1. Feed conversion ratio (FCR)
Feed conversion measurements (FCR) were carried out on the 30th day using the following formula [12].

$$\text{FCR} = \frac{F}{\text{Total weight of fishes growth}}$$

3.2. Survival rate
The survival rate was measured with the formula [12].

$$\text{SR} = \frac{\text{Nt} \times 100\%}{\text{No}}$$

SR = Survival rate (%). No = Number of fish at the beginning. Nt = Number of fish at the end of the experiment.

4. Results and discussions

4.1. The nutritional content of the diet
The protein levels of fermented diets ranged from 23.66 to 34.73 %. The protein content of the control diet was 30.53%, indicating that the treatment diets contained protein in an appropriate range for the evaluation of low and high content protein for walking catfish. Walking catfish requires a protein level of >25 % in the diet [13] (table 2). The fat and carbohydrate of the diets ranged from 20.3-33.6 % and 8.35-13.4%, respectively.

Fermentation can increase the protein, fat, and ash content in fish pellets, but reduce fiber and carbohydrate levels because of Rhizopus sp. Produces cellulases, hemicellulases, pectinase, tannase, phytase, amylase, lipase, protease and other enzymes of immense industrial importance [14]. Hong et al. [15] reported that the fermentation of soybean is able to increase protein content up to 10% [16]. Rhizopus sp. also increases short-chain fatty acids content such as lactic, acetlic, butyrate, formate and propionate acid [15]. Likewise, increased levels of ash (minerals) were also caused by soybean yeast (Rhizopus sp.) that produces an enzyme called phytase. Phytase is an enzyme that able to catalyze the
hydrolysis of phytic acid in pellet materials into mio-inositol mono, di, tri, tetra and pentaphosphate, as well as organic phosphate [17]. The addition of phytase enzymes to pellet ingredients can increase the content of Ca, P, and Mg [18]. The fermentation process reduces carbohydrate content because the fermentation process breaks down carbohydrates into glucose which is used as an energy source [19]. The decreasing value of fiber in pellets is caused by Rhizopus sp producing cellulase enzymes that can hydrolyze cellulose in pellets” fibers by breaking the glycosidic bonds [20, 21].

### Table 2. Proximate value of pellets

| Composition | K.0 (%) | K.1 (%) | K.2 (%) | K.3 (%) | K.4 (%) |
|-------------|---------|---------|---------|---------|---------|
| Protein     | 30.53   | 23.66   | 28.51   | 30.63   | 34.73   |
| Fat         | 13.47   | 9.38    | 8.92    | 8.56    | 8.35    |
| Carbohydrate| 31.71   | 33.64   | 27.77   | 25.79   | 20.38   |
| Ash         | 8.89    | 6.63    | 7.66    | 9.94    | 11.87   |
| Fiber       | 7.23    | 8.27    | 7.96    | 7.78    | 6.44    |
| Ca          | 0.14    | 7.76    | 8.78    | 7.84    | 9.71    |
| Water       | 8.03    | 10.66   | 10.40   | 9.46    | 8.52    |

K0=Control/Commercial Pellets, K.1 = Combination 1 (organic waste 0%); K.2 = Combination 2 (organic waste 30%); K.3 = Combination 3 (organic waste 60%); K.4 = Combination 4 (organic waste 90%)

4.2. The growth rate of *C. gariepinus*

The nutritional contents of fish smoke waste affected the growth of fish weight and length. The growth of weight in each group of treatment was highly affected by the proportion of the smoked fish waste processing (P <0.05). Treatment K.1, K.2, and K.3 resulted in lower weight growth than the control treatment. The only treatment K.4 provide a higher weight growth rate than the control treatment. This result indicated that the substitution of fishmeal with 90% fish smokes processing waste and fermentation resulted in the growth rate of catfish higher than the catfish fed with a commercial feed (table 3). The similar results were found for the length growth.

### Table 3. Length and weight of *C. gariepinus* development after feeding by fish pellets.

| Treatment | Weight (gr) | Length (cm) |
|-----------|-------------|-------------|
| K.0       | 31.05±0.88a | 20.06±1.11d |
| K.1       | 14.20±1.38a | 14.36±0.28a |
| K.2       | 17.27±0.91b | 15.70±0.40b |
| K.3       | 21.04±1.54c | 17.20±0.60c |
| K.4       | 31.74±0.93d | 22.28±0.69e |

(Description: different superscripts in the same column show significantly different (P <0.05). K0=Control/Commercial Pellets, K.1 = Combination 1 (organic waste 0%); K.2 = Combination 2 (organic waste 30%); K.3 = Combination 3 (organic waste 60%); K.4 = Combination 4 (organic waste 90%))

The high growth of weight and length of the fish in K.0 and K.4 treatment was caused by high protein content. The high protein content of K.4 diet of 34.73% resulted in the highest growth of African catfish. This protein synthesis also plays a role in tissue formation so that tissue mass will increase. Foods with high protein content have a more effective influence on growth rate [21]. Fish waste can also be used for production of various value-added products such as proteins, oil, amino acids, minerals, enzymes, bioactive peptides, collagen, and gelatine. There are three types of proteins in fish: structural proteins, sarcoplasmic proteins, and connective tissue proteins. There are 16-18 amino acids present in fish proteins. The amino acids present in the fish can be utilized in animal feed in the form of fishmeal and sauce or can be used in the production of various pharmaceuticals. The
fish oil contains two important polyunsaturated fatty acids called EPA and DHA or otherwise called as omega-3 fatty acids are found in all parts of the fish. There are three types of proteins in fish: structural proteins, sarcoplasmic proteins and connective tissue proteins [22].

4.3. Survival rate
Fish survival rates were not affected by the diet treatment as all of the treatment group reached 100% of survival rate. The lower protein contents did not lower the survival rate. The survival rate of fishes is influenced by the nutritional quality of fish feed. If fish pellets contain high nutrients, it accelerates growth and survival rate of fish [23]. This study shows that the uses of The nutritional contents of fish smoke waste are raised are not only economical means of producing fish but also ensures higher survival rates. By this time some cost would have been saved and the fish farmer can be sure of a higher survival rate of fishes

4.4. Food conversion rate
The lowest FCR of 0.77 was found in the K.4 diet treatment. In contrast, the highest FCR value of 3.12 was found in K.1 diet treatment (table 4).

| Treatment | Food conversion rate (FCR) Value |
|-----------|----------------------------------|
| K.0       | 0.90                             |
| K.1       | 3.12                             |
| K.2       | 2.04                             |
| K.3       | 1.42                             |
| K.4       | 0.77                             |

K0=Control/Commercial Pellets, K.1 = Combination 1 (organic waste 0%); K.2 = Combination 2 (organic waste 30%); K.3 = Combination 3 (organic waste 60%); K.4 = Combination 4 (organic waste 90%)

Lower K.4 pellet FCR values indicated that feed efficiency can be converted into meat. FCR values can be influenced by species and feed quality given [24]. K.4 pellets contain the highest protein compared to pellets in other treatments. The FCR value of the treatment given pellet K.4, K.0, K.3, and K.2 is 0.77; 0.90; 1.42 and 2.04 belong to the good category. The good FCR value of fish is generally between 1-2 [24]. Weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) of the C. gariepinus catfish fed the test diet with different protein levels are given in table 4. These results implied that a high proportion of the dietary protein in fish smoke waste-containing artificial feed was metabolized to maintenance energy. The condition factor, which is described as an indicator of fatness, gross nutritional state, and the level of reserve nutrients, increased for the of C. gariepinus catfish in the present study as the dietary protein level increased, possibly indicating that higher dietary protein level increases the nutrient content in the fish body. Especially, the proximate chemical composition analysis indicated an increase of protein in WSFP meal 90% protein.

4.5. Proximate analyses on C. gariepinus flesh
The proximate analysis of C. gariepinus flesh showed that the highest protein and ash contents were 16.06 and 1.46% found in group K.4, respectively. However, the lower protein contents of flesh than that of the control group were found in the group of K1, K2, and K3. On the other hand, the highest fat content of 6.07% was found in K.0 group. The highest fiber and carbohydrate contents of 0.65 and 2.37% were yielded by K.1 group (table 5).

Quantifying proximate composition is important in ensuring the requirements of food regulations and commercial specification [25]. The moisture content of flesh is a good indicator of its relative content of energy, protein, and lipid [26]. Fish meat contains significantly low lipids and higher water
than beef or chicken and is favored over other white or red meals [27]. The total lipid of fish vary with the increasing weight or length of the fish, it may also vary with the season and varied habitats and feed [28]. Among the proximate composition, protein in fish is an excellent source, because of the amino acid composition and degree of digestibility [29]. Several studies on the proximate composition of fish have been from different parts of the world so far. The nutritional analysis of some freshwater fish was determined by previous study [30]. Proximate composition of different freshwater fish’s especially African catfish (Clarias gariepinus) was described earlier [31].

Table 5. Nutritional contents of C. gariepinus flesh after feeding with fish smoke waste-containing artificial feed

| Composition      | K.0 (%) | K.1 (%) | K.2 (%) | K.3 (%) | K.4 (%) | SNI(%) |
|------------------|---------|---------|---------|---------|---------|--------|
| Protein          | 14.01   | 13.84   | 13.91   | 13.93   | 16.06   | 17     |
| Fat              | 6.07    | 5.52    | 4.62    | 4.03    | 3.46    | 4.5    |
| Water            | 75.94   | 76.45   | 77.17   | 78.71   | 76.92   | 76     |
| Ash              | 1.22    | 1.13    | 1.17    | 1.23    | 1.46    | 1      |
| Fiber            | 0.44    | 0.65    | 0.46    | 0.41    | 0.22    | -      |
| Carbohydrate     | 2.29    | 2.37    | 2.23    | 1.77    | 1.5     | -      |
| Ca               | 0.03    | 0.04    | 0.44    | 0       | 0.38    | -      |

K0 = Control/Commercial Pellets, K.1 = Combination 1 (organic waste 0%); K.2 = Combination 2 (organic waste 30%); K.3 = Combination 3 (organic waste 60%); K.4 = Combination 4 (organic waste 90%).

5. Conclusion

Based on the research, it can be concluded that pellets from with additional organic waste produce the highest growth in weight and length are pellets with K.4 group. However, the lowest FCR value was found in the treatment of giving K.4 pellets with a value of 0.77. The proximate content of fish meat in all treatments still does not meet the standard proximate content of African catfish based on the Ministry of Health of the Republic of Indonesia in 1996 because it has a protein content that is still below the standard, which is less than 17%.

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