The effect of bioprocess product of coconut husk on the stability of ration water, durability and floatability of fish feed

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Abstract. Physical characteristics pelleted non-extrusion containing bioprocess product of coconut husk, has been investigated. The study was designed experimentally using a Completely Randomized Design (CRD), five treatments were repeated three times. Five treatments included the use of bioprocess product of coconut husk fermentation (CHF) as much as 0%, 25%, 50% and 75% as substitution of conventional raw material energy source on pelleted formulation, and control (floating pellet). The parameters measured were sink speed test, durability test, stability test and moisture content test. The effect of each treatment on parameters was analyzed using Analysis of Variance (ANOVA) and continued with Duncan's Multiple Distance Test. The results showed that bioprocess did not show a significant difference on durability, but product of coconut husk additive feed increased stability of ration water, breaking speed and sinking speed. Stability of ration water with the addition of coconut husk fermentation 75% CHF was significantly higher than other CHF treatments, but not significant different with control pelleted. Stability of ration water in 25-75% CHF treatments. after 120 minutes ranging from 75.93\% - 81.77\%, with pellet durability of 93.33-98.84\%.

1. Introduction

Feed is a combination of several ingredients that contain nutrients both quantitatively and qualitatively is needed to achieve optimum growth. Growth potential and metabolic rate need to be supported by feed energy content, temperature and water quality. However, the production of independent feed by fisheries sector business actors is constrained by a number of factors, including the physical quality of feed which is easily broken and dissolved in water so that it can quickly reduce water quality; and feed that is easily drowned before consumption by fish. The problem faced along with the intensification of cultivation is the increase in waste material, which comes from the rest of the feed, feces and metabolic waste in the form of phosphate, H\textsubscript{2}S, nitrite and NH\textsubscript{3} and can be toxic to accelerate the occurrence of eutrophication. In these conditions, not all nutrients in the feed are used efficiently for growth. Starting from overfeeding with penetration of water into it causes leftover food and nutrient leaching. So from the appropriate floating feed technology needed that does not require investment and operational costs as expensive as the manufacture of commercial large-scale commercial feed which involves the extrusion process using extruder machines. Bioprocess through fermentation does not require sterilization of tools or materials,
without considering the type and composition of the binder, carried out non-extruding or without using a floating feed making machine (extruder), and without deep frying stages so that it is more energy efficient and much more practical [1].

Feed for cultured fish requires certain process steps to ensure that the pellet produced has good stability in water. This stability aims to give the fish time to feed. From the results of this study the granules of fish feed ingredients need to be glued together in order to maintain or stabilize the components of the feed ingredients so they are not easily broken down destroyed in water and ensure that as little as possible of the nutrients in the lost feed dissolved into pool water. Physical properties tested include absorption of water (water absorption), stability in water (water stability), speed of sinking (floatability) and durability. The low stability of pellets in water causes imbalance problems in the water medium, however high pellet dispersion values are also unfavorable in terms of production costs and availability of bound nutrients [2]. The level of resistance in water and optimal feed hardness can maximize the use of feed by fish when the feed given has a balance of nutrients needed by fish and can be digested properly in the digestive system of fish. Precondition is very important to do to make it easier to digest food in the digestive tract of fish, one of which is through bioprocess technology.

Bioprocess technology is an appropriate way of utilizing waste feed ingredients to be valuable for fish feed. One of the waste-material that is a potential source of energy is coconut husk or coconut pairing waste (skin aging and coconut fruit) because the carbohydrate and fat content determine whether the waste is useful or becomes a pollutant. Molds that have been known to ferment fatty and fibrous materials (such as peanut meal) are Neurospora sitophyla or known as red ‘oncom’ molds. This type of mold is able to produce amylolytic, proteolytic, and lipolytic enzymes and is adaptive to the aerobic environment so that it can decompose food components in the substrate to be simpler [3]. Fats and fatty acids as part of lipids can function as sparing proteins, sources of essential fatty acids, and coating pellets in fish feed. The benefits of fermentation in fatty sources such as coconut, will also be of use value in the extraction of organic acids. Fermentation is very well developed for the manufacture of bioproducts, such as bioethanol, lactic acid, citric acid, fatty acids, etc.) both from simple carbohydrates (glucose) and from waste materials as carbon sources [3]. Coconut husk is one of the right ingredients, is the outer shell of a coconut seed attached to the inside of the coconut shell which is often called ‘testa’. This skin is a waste resulting from the coconut shell solubility which so far has not been widely used.

Based on this background, fermented coconut husk with mold (Neurospora sitophyla) can be applied either as an alternative feed material or as a fish feed substitute for extrusion pellets, because it does not require high heat, without considering the type and composition of the binder, without the need for tools chemical extraction or solvent in producing a source of fatty acids as a pellet coating, and is carried out in a non-extrusion manner without using an extruder, and without deep frying stages so that it is more energy efficient and far more practical. The purpose of this study are analyzing the nutrient and saturated-unsaturated fatty acid content of coconut husk product as a raw material for fish feed, and also analyzing the effect of the ratio of coconut husk fermentation (CHF) as a raw material in the formulation to the physical quality (durability and stability) of fish feed.

2. Research Methods

The preparation of research tools begins with the procurement of bioprocess equipment and materials and pellet making equipment. Bioprocess of coconut husk begins from making inoculum and propagation of bioprocess. Materials to be used in the manufacture of bioproducts include: fermentation (coconut husk), rice flour, rice bran, mold N.sitophyla, agar nutrients and standard mineral solutions. Other materials that will be used include aquadest, glucose, agar extract, and buffer pH 7. The tools to be used are, autoclaves, gobbets, bunsen burners, petri dishes, porcelain cup, centrifuge Nimac CR 21G, funnel, Knick pH-meter, Novaspec II spectrophotometer, test tube, furnace, fermentor cabinet, and milling machine. Bioprocess results were chemical analyzed using Proximate Test and GCMS.

The fermented feed process was starting by bioprocessing the N. sitophyla inoculum with coconut husk then fermented on dose and time of fermentation 1%, five days, scale up Bioprocess coconut husks with N. sitophyla for 5 days, and mixing the results of Bioprocess stages 1 (25%, 50% and 75% in
formulations with filler material (rice bran), and protein supplements (soybean meal) and advanced bioprocess for a minimum of 2 days to obtain fish feed. The durability test (the number of pellets that are returned intact after being stirred mechanically (pneumatic)) [4] was calculated by equation 1:

\[
\text{% Durability} = \frac{\text{Weight of pellets after rotating}}{\text{Weight of pellets before rotating}} \times 100\% \tag{1}
\]

Before dipping to be tested based on immersion time, the sample for each treatment was divided into three equal parts. Soaking time is 30, 60, 120 minutes after immersion. After soaking the pellets are removed and then dried and dried so that the water content of the pellets before soaking is the same as after soaking, then the dry weight and stability of the pellets are calculated [5]. The water stability was calculated by equation 2.

\[
\text{Water Stability} = \frac{\text{Final dry weight after soaking}}{\text{Initial dry weight before soaking}} \times 100\% \tag{2}
\]

3. Results and discussion

1.1. Chemical Analyzed Description of Coconut Husk Fermentation

Mixing coconut husk flour, bran, corn flour and rice flour starter with and mineral solution produces a mixed substrate in the form of wet granules that are separated from each other and are not sticking with the dominant brown colour. After fermentation using \( N. \) sitophyla the initially separated substrate granules at the end of the fermentation turn into a solid entity (Figure 1.).

![Figure 1. N. sitophyla inoculum](image)

Fermentation of fiber material is usually carried out by microorganisms in the form of mold, because it includes fermentation of solid media. One mold that has been known to have high cellulolytic activity and is often used in fermentation of fiber material was \( N. \) sitophyla or known as red “oncom” mold. This type of mold is able to produce amylolytic, proteolytic, and lipolytic enzymes and is adaptive to the aerobic environment so that it can decompose food components in the substrate to be simpler [3].

The calculation of the number of colonies aims to determine the best dose of use of \( N. \) sitophyla on coconut husk as fermented microbes as a preliminary test at the Laboratory of Poultry Nutrition and Feed Technology, Faculty of Animal Husbandry, Padjadjaran University. During the beginning of the fermentation process, a decrease in pH caused by microbes that metabolize sucrose into organic acids. High levels of sugar in the solution cause an increase in microorganism activity and organic acid production thereby reducing the pH of the solution [6]. The results of coconut husk fermentation (CHF) by \( N. \) sitophyla obtained an increase in protein content of 3.63% and a decrease in fat content, crude fiber and ash content. When preparing raw materials for feed components, there is no consideration of adding special binder components, or deliberate mechanical and thermal treatment to glue and unite the granules with separate granular textures. But after fermentation, the substrate grains are united to form a solid, compact and sturdy texture.

This shows that \( N. \) sitophyla mycelia is able to replace the role of adhesives and binders which are usually added in the manufacture of pellet fish feed whose raw material initially is fine flour. The mechanical and thermal procedures commonly used to bring about compactness, robustness, and buoyancy in pellets are also not required in this fermentation. Fermentation of feed using room
temperature, which is around 20-25 °C. The formation of the orange red *N. sitophyla* mycelia actually makes the fermentation feed change colour to be attractive (red-orange).

**Table 1.** Nutrient composition of Coconut husk

| No. | Nutrient content | Coconut husk | Coconut husk fermentation (CHF) |
|-----|------------------|--------------|---------------------------------|
| 1   | Crude Protein    | 7.7          | 11.32                           |
| 2   | Crude Fat        | 26.48        | 18.47                           |
| 3   | Crude Fiber      | 15.19        | 11.79                           |
| 4   | Ash              | 2.79         | 2.16                            |

Source: Laboratory of Animal Nutrition Ruminant and Chemical Food Faculty of Animal Husbandry Unpad (2019).

**Table 2.** Fatty acid composition of coconut husk fermentation (CHF)

| No. | Organic Acid         | %          |
|-----|----------------------|------------|
| 1.  | Saturated Fatty acids|            |
|     | Hexanoic acid        | Caproic    |
|     | Octanoic acid        | Caprilic   |
|     | Decanoic acid        | Capric     |
|     | Dodecanoic acid      | Lauric     |
|     | Tetradecanoic acid   | Myristic   |
|     | Hexadecanoic acid    | Palmitic   |
| 2.  | Unsaturated Fatty acids| 9,12 octadecadienoic| o-6| 1.86 |
|     | 9 octadecenoic       | o-9        | 6.69                            |
|     | Octadecatrienoic     | o-3        | -                               |

The balance of energy and protein in the formulation is of central importance in feeding. Not only is the adequacy of the quantity and quality of protein that must be guaranteed, often the energy value is not as predicted, so the addition of high-calorie ingredients is important. In addition to increasing caloric value, dietary fat reduces the effect of dusting / improves texture and increases palatability, thereby reducing feed lost both in the pelleting process and its administration [7,8].

3.2. Physical Quality of Pellets

(1) Durability

The physical quality of feed is one of the factors that can affect fish performance. Degradation is a measure of the physical quality of pellets in fish feed which is known from the durability and stability of feed in water. The effect of the coconut husk formulation and the fermented product bran on the physical quality of the pellet is presented in Figure 2 and Table 3. The results of statistical analysis (Figure 2; Table 3) show that there was a significant influence on durability. Fermented buoyancy feed with bran substitution by 25%, 50%, and 75% CHF, has a durability that was no different (p > 0.05) with basal feed 0%CHF (A). This shows that the fermented coconut husk can match the sinking pellet, even though the floating pellet (control feed) was significantly different (p < 0.05) in its durability value.

The fat content in fermented coconut husk can affect the cohesiveness of feed material, oil or fat reduces the dusty effect of ration and can improve the texture of pellets [1]. Figure 2 and Table 3, show that the feed product mix of fillers and 25%, 50%, and 75% CHF of coconut, fermented by *N. sitophyla* had a durability value equal to the mixture of ingredients without coconut extract printed into sinking pellet (A). Extrusion pellets or floating pellets (C) durability and hardness was higher than others. The durability of the printed pellets related with the elasticity of the protein in various reactions. The
elasticity (plasticizes) of proteins and the process of disruption of starch structure are the causes of starch gelatinization in the process of making pellets that involve thermal, chemical and mechanical energy conditions [9].

![Figure 2. Feed Durability of Bioprocess of Coconut Husk](image)

**Table 3. Coconut husk fermentation products on durability and stability of ration water.**

| Treatment | Durability (%) | Stability of ration water (%) |
|-----------|----------------|-------------------------------|
|           | 30 minutes     | 60 minutes | 120 minutes |
| A         | 92.78<sup>a</sup> | 86.80<sup>a</sup> | 82.87<sup>a</sup> | 71.70<sup>a</sup> |
| B         | 93.30<sup>a</sup> | 87.30<sup>a</sup> | 84.73<sup>a</sup> | 75.93<sup><sup>ab</sup></sup> |
| C         | 94.84<sup>a</sup> | 88.83<sup>a</sup> | 85.90<sup>a</sup> | 79.33<sup><sup>ab</sup></sup> |
| D         | 94.48<sup>b</sup> | 92.20<sup>b</sup> | 86.67<sup>a</sup> | 81.77<sup>b</sup> |
| E         | 99.57<sup>b</sup> | 96.53<sup>c</sup> | 91.33<sup>b</sup> | 86.93<sup>b</sup> |

A: pellet non-extrusion 0% CHF; B: 25% CHF; C: 50% CHF; D: 75% CHF; E: pellet control (floating pellet)

Substitution of bran filler with coconut husk fermentation by *N. sitophyla* as much as 25% -75% produces durability as well as conventional pellets (dry pellets). The fermented coconut husk feed material was more resistant to rotation on the tumbling tool, because its structure is more saturated and does not evaporate, it will immediately close the pores of the cell wall matrix and act as a lubricant between feed particles (mash), and heat conductor [8]. This has a positive effect on pellet hardness. However, fat-containing materials can improve the surface-active properties of pelletizing materials through the formation of emulsions. Although it can improve texture, fat as a hydrophobic compound can interfere with the binding of the nature of the water-soluble component in the feed (starch, protein and crude fiber) so that it can adversely affect the hardness and durability of the feed.

**(2) Stability of Ration Water**

The effect of the treatment on stability of ration water is presented in Table 3. Since the soaking 30 minutes there was a difference in the fermentation feed of 75% and the floating control pellet. Table 3 shows that the dispersion or stability value of pellets in water after 30 minutes and 60 minutes immersion showed significant differences between treatments, as well as the results of analysis of variance on pellet dispersions after immersion for 60 minutes. After 120 minutes the basal pellet (A) has begun to disintegrate so that it has low stability. Stability in fermented water pellets has the same pattern as conventional pellets (feed sink) (Figure 4) for 2 hours, which has decreased dramatically since one-hour immersion from 100% down to 82.87% for basal feed (A) and 84.73 - 86.67% for fermented floating feed. Between the first hour to the second hour, commercial floating feed (floating pellet) shows the stability value in water that was not different from fermented feed, even the use of fermented buoyancy feed containing 75% CHF has the same significance value as extrusion floating pellets where the difference was around 5%. In general, the existence of a difference that is not too large for 2 hours
immersion in water shows that the stability in water in fermented buoyancy feed approaches the value of stability in ration water in commercial buoyancy feed.

![Figure 3. Stability of Ration Water Coconut Husk Fermentation](image)

Feed formulation stability is influenced by feed composition, processing type, and water content. In the manufacture of pellet filler sources of starch (bran) by heating, it can act as a binder with the gelatinization process due to compression and thermal presence of friction between particles in pelleting, as happened in basal feed (A) without CHF. Control feed (E), as floating pellet have a high polarity occurs or energy interactions between the polysaccharide chains in CMC, thermal, and ionic liquid phases increase the stability of pellets in water. Whereas biological processing by *N. sitophyla*, results in compactness of pellet from hygiene that binds between particles of material. Comparison of the stability of pellets in water or Dispersion of Pellets after soaking 30 minutes, 60 minutes, and 120 minutes can be seen in Table 3. Control pellets (E) produce stability that tends to be high, but does not differ from buoyancy fermentation, 75% CHF to soaking 120 minutes. The nature of proteins in processing other than sensory and kinesthetics aspects (such as flavour, odour, colour, texture); also includes aspects of hydration, dispersibility, solubility and swelling. Substitution of protein-energy source fillers (bran and soy flour), by 75% CHF containing higher fat can increase stability.

This test provides the conclusion that buoyancy feed from bioprocess with coconut husk substrate and additives (bran and soybeans) fermented by *N. sitophyla* without the addition of a special binder and without using an extruder machine is able to match the level of stability in water in commercial buoyancy feed that is commonly used by fish farmers. The unification of feed grains in this experiment was a biological agent in the form of "oncom" mycelium of a mold *N. sitophyla*. *Molds* has been known to be able to unite soybean granules in the manufacture of soybean tempeh through fermentation at room temperature [6]. Rheological properties or processes that occur such as gelation or attachment and other properties (adhesion and cohesion) affect the formation of texture [10-12]. The advantage of fungi bioprocess was to produce mycelium as a binding biological agent and avoid damage to nutrients that are prone to heat as occurs in the extrusion process that can damage certain vitamins [6].

Besides being influenced by mycelium of fungi when, the attachment in this floating product of fermented was due to the presence of fat and fatty acid deposits. The dominant fatty acid content (Table 2) in the bioprocess of the coconut husk was lauric acid, which is a medium change saturated fatty acid and not volatile. Feed formulations supported by higher fat content (> 7%), have better stability than pellets with 45% protein.

(3) Description of Water absorption and Buoyancy

From the results of the durability and stability test, obtained the best comparison between 25% bran and soybean filler and the best 75% CHF (coconut husk) fermented feed. However, buoyancy feed containing AKF 25-75% absorbs water very quickly in the first minute, where fermented buoyancy feed is about three times stronger in absorbing water than commercial buoyancy of control feed (E). The process of absorption of water by both types of feed is increased but with a decreased absorption speed up to the 10th minute. In the 10th minute, fermented floating feed is able to absorb water up to 4 times
heavier than the dry weight of the feed. However, this absorption of fermented feed water decreases in the 20th minute.

Figure 3 shows that for two hours, neither fermented floating feed nor commercial floating pellet were completely submerged in calm water. This shows that in the floating test time span of 1 hour, fermentation is able to generate buoyancy which is equivalent to the floating ability that was raised by the extruder machine in the manufacturer's floating fish feed. The ability to float 100% of fermented feed in the test for 1 hour is longer than the 20 minutes buoyancy test time [13]. Non-extrusion float method using *N. sitophyla* fungi in this study also has better buoyancy than other non-extrusion techniques.

Floating without using an extruder machine has been done by utilizing the role of yeast and baking powder [12]. The best results obtained are 70% still floating in the 60th minute. The use of yeast *Saccharomyces cerevisiae* to float fish feed was also carried out [5]. The feed formulation used in the two studies included 35% flour as an adhesive and used the deep frying or frying stage in fish oil produced capable of floating for 6-8 hours. Although the buoyancy is longer, the appearance of buoyancy was less efficient compared to the buoyancy produced from coconut husk fermentation (CHF), which does not need to consider additional oil that requires energy, materials and other production facilities.

4. Conclusion

The results showed that bioprocess did not show a significant difference on durability, but the product of coconut husk additive feed increased stability of water ration, breaking speed and sinking speed. Stability of ration water with addition to coconut husk fermentation and stability in water, of 75% CHF treatments. after 120 minutes ranging from 75.93% -81.77%, with pellet durability of 93.33-98.84%. Coconut husk fermentation using *N. sitophyla* mixed with bran and soybean meal raises buoyancy in the fermented product. When compared to commercial buoyancy, 75% of coconut fermented buoyancy feed, has a graph of water ration stability similar to commercial buoyancy for 2 hours in water. Fermentation of fish feed ingredients using *N. sitophyla* deserves further development as an alternative flotation of fish feed so that its physical characteristics get better and approach the extrusion floating feed.

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