The inclusion of seligi leaf flour fermentation (*Phyllanthus buxifolius*) in feed on fat and energy retention in catfish (*Pangasius pangasius*)

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Abstract. Catfish (*Pangasius pangasius*) is one of the popular freshwater fisheries commodities and its growth relatively fast. Fish growth and survival were affected by feed. The high-quality feed is expensive so it can be added with alternative feed ingredients such as Seligi leaf flour. Seligi leaf flour has 11.53% protein and 15.48% crude fibre. However, high crude fiber can be overcome by fermentation using probiotics. This study aims to determine the use of Seligi leaf flour fermentation (*Phyllanthus buxifolius*) in feed on fat retention and energy retention of catfish (*Pangasius pangasius*). This study used an experimental method with a completely randomized design (CRD) consisting of four treatments (0%, 2%, 4%, and 6%) with five replications. Data analysis used Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test. The results of the research that had been carried out were significant differences (P <0.05) on fat retention and energy retention of catfish. The highest fat retention and energy retention was in P2 treatment, while the lowest fat retention and energy retention was in P0 treatment.

1. Introduction
Catfish (*Pangasius pangasius*) is one of the popular freshwater fisheries commodities. Catfish meat contains 12.03% protein, 5.22% fat, and 75.63% water [1]. Catfish are easy to be cultivated, resistant to environmental change and can grow fast [7]. Fish’s growth and survival are affected by feed. Reducing costs in making feed can be done by using alternative feed ingredients [8]. One of the alternative feed ingredients that can be used is Seligi leaf flour. Seligi leaf flour contains 11.57% of crude proteins and its probably able to reduce fat and cholesterol levels [14]. Meanwhile, the weakness of seligi is it contains high crude fiber, approximately around 15.48%. This weakness can be overcome by fermentation using probiotics. Fat retention in fish illustrates the fish ability to store and utilize fat on feed [10]. Energy retention is the ratio between the amount of energy stored in fish tissue and the amount of energy in the feed consumed [3,4]. Thus, this study aimed at investigating the effect of fermented Seligi Leaf inclusion on fat and energy retention of feed in catfish.
2. Material and methods

2.1. Material
The materials used in this study were catfish with a size of 12-15 cm obtained from fish seed centre (BBI), Puri Mojokerto East Java, Seligi leaf flour fermentation, soybean meal flour, fish meal, cornflour, rice bran, fish oil, mineral mix, multivitamins, and CMC.

2.2. Method

2.2.1. Preparation
The first preparation for the study was to adapt the fish and prepare 20 aquariums with 50x30x30 cm³ size. Clean the equipment and aquariums with a disinfectant (chlorine) and let it sit for 24 hours then rinsed it using clean water and dry it. The dried aquarium was filled with water from the reservoir to a height of 20 cm and aerated for 24 hours. Fish maintenance was carried out by providing additional feed with seligi leaf flour fermentation.

2.1.2. Seligi leaf flour fermentation
The production of Seligi leaf flour fermentation was conducted by drying the fresh Seligi leaf under sunlight until dry. Then the Seligi leaves were finely ground and weighed according to the desired feed requirements. Seligi leaf flour was fermented using probiotic bioMC4 at a dose of 2% plus a dose of 2% molasses and 30% aquades, then fermented for seven days. The fermented Seligi leaf flour was dried in the sun. After drying, proximate analysis was carried out and it was ready to be mixed in the feed preparation.

2.1.3. Producing feed
The feed preparation is done by preparing ingredients such as soybean meal flour, fish meal, cornflour, fermented Seligi leaf flour, bran, fish oil, mineral mix, multivitamins and CMC. First, calculate the feed ration for each treatment and weigh the feed ingredients needed. Mixing feed ingredients starting from the ingredients that have the lowest to highest ration values. Then formed into a lump like a ball shape. Then cut the material using a pellet mill. The pellets are then cut according to the fish's mouth, then aired or dried in the sun until dry. The dried pellets then stored in a plastic bag and labelled.

2.1.4. Data analysis
Data was analyzed using Analysis of Variance (ANOVA) to determine the effect of experimental treatment, followed by Duncan's multiple range test if there was any significant difference among treatments [1].

3. Result and discussion

3.1. Fat retention
The results of statistical analysis of data on fat retention of catfish (Pangasius pangasius) in this study that had been carried out for 40 days obtained values ranging from 0.203-0.302%. The average data of catfish meat fat retention can be seen in Table 1 and Figure 1.

![Figure 1. Fat retention value of catfish meat (Pangasius pangasius)](image-url)
The highest average fat retention was obtained in P2 treatment of 0.302% which was significantly different from P0, P1 and P3. While the lowest average fat retention value was obtained in treatment P0, namely 0.203% which was not significantly different from P1 and P3. The results of the Analysis of Variance (ANOVA) calculation showed that there was a significant difference (p < 0.05) between each treatment.

Table 1. Statistical analysis of fat retention data of catfish meat (Pangasius pangasius)

| Treatments | Energy Retention% ±SD | Arcsin Transformation ±SD |
|------------|------------------------|---------------------------|
| P0 (0%)    | 0.203 ± 0.036<sup>a</sup> | 2.580 ± 0.194<sup>a</sup> |
| P1 (2%)    | 0.217 ± 0.049<sup>a</sup> | 2.660 ± 0.296<sup>a</sup> |
| P2 (4%)    | 0.302 ± 0.027<sup>b</sup> | 3.145 ± 0.144<sup>b</sup> |
| P3 (6%)    | 0.221 ± 0.032<sup>a</sup> | 2.688 ± 0.200<sup>a</sup> |

The high value of fat retention in P2 treatment is due to the limited amount of the lipase enzyme which is responsible for hydrolyzing fat. So that the absorption of fat cannot be done optimally. This causes the remaining fat to be retained is greater. According to Komariyah and Al Setiawan [5] the usage of fat as an energy source is only as a "protein sparing", it means that fat has a function to replace protein as an energy source so that the usage of protein can be saved to maximize growth. The low content of fat retention in P0 is because the amount of fat used to produce energy is much greater than stored in the catfish's body. Meanwhile, according to Richter et al [11] which states that crude fibre can cause a significant reduction in fat retention and energy retention which results in decreased use of protein as an energy source.

3.2. Energy retention

The results of statistical analysis of energy retention data of catfish (Pangasius pangasius) in this study that has been carried out for 40 days obtained values ranging from 0.390-0.680%. Data on the average energy retention of catfish meat can be seen in Table 2 and Figure 2.

![Figure 2. Energy Retention Value of catfish meat (Pangasius pangasius)](image)

The highest average energy retention was obtained in P2 treatment of 0.680% which was significantly different from P0, P1, and P3. While the lowest average energy retention value is P0 of 0.390% which is not significantly different from P1 and P3. The results of the Analysis of Variance (ANOVA) calculation showed that there was a significant difference (p < 0.05) between each treatment.

The high energy retention value in P2 treatment is due to a balance between energy and protein levels in the catfish body. This balance is very important in the rate of growth. Because if the energy needs are less, the protein will be broken down and used as an energy source. This is supported by the opinion of Aslamyah [2] that the increase of fat use and carbohydrates as a source of energy, the feed protein can be more efficiently used and will be retained in the fish body for metabolic processes,
replacement of damaged cells or tissues, reproductive activities, and growth. The low energy retention content in the P0 treatment is because the energy produced is mostly expended by the body for metabolism, reproductive activities, biosynthesis and is lost in the form of heat. According to Subekti et al [13] that the energy stored is utilized in the synthesis of cell components and used as fuel in cell energy reproduction.

| Treatments | Energy Retention% ±SD | Arcsin Transformation ±SD |
|------------|-----------------------|---------------------------|
| P0 (0%)    | 0.390±0.073a          | 3.569 ± 0.332a            |
| P1 (2%)    | 0.482 ± 0.115a        | 3.962 ± 0.419a            |
| P2 (4%)    | 0.680 ± 0.052b        | 4.725 ± 0.182b            |
| P3 (6%)    | 0.467 ± 0.083a        | 3.905± 0.342a             |

3.3. Water quality
Water quality parameters measured during the research were temperature, degree of acidity (pH), dissolved oxygen (DO), and ammonia. Data on the range of water quality measured during the study can be seen in Table 3.

| Parameter      | Value         |
|----------------|---------------|
| Suhu (°C)      | 27.8 – 29.3   |
| pH             | 7             |
| DO (mg/L)      | 4.23 – 6.28   |
| Amoniak (mg/L) | 0 – 0.25      |

The results of the water quality during the study, the temperature obtained ranged from 27.8-29.3°C which was classified as a sufficiently optimal temperature for raising catfish. According to BNSI [4], the optimal temperature range for catfish maintenance is 25-33°C. Besides temperature, the pH and DO obtained were quite optimal, the pH is around 7 and DO ranging from 4.23 to 6.28 mg/L. This pH measurement is in accordance with the statement of BSNI [5] which states that the pH value for water for catfish growing media ranges from 6.5-8.5 while DO according to BNSI [5] requires a minimum of 3 mg/L of fish. Measurement of ammonia levels ranging from 0-0.25 mg / L is thought not to be dangerous for catfish growth media. Ammonia compounds that are considered dangerous for aquatic organisms are 0.6 mg/L [8].

4. Conclusion
This study can be concluded that the use of Seligi leaf flour fermentation (Phylanthus buxifolius) on feed with 4% dose can increase fat retention and energy retention of catfish (Pangasius pangasius).

5. References
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