Evaluation of kombucha tea as a feed additive for improving the protein and lipid retention of African catfish (*Clarias gariepinus*)

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Abstract. Kombucha tea is the product of sugared tea fermentation by a symbiotic culture of bacteria and yeast. This beverage contains a high source of the microbial community, such as acetic acid bacteria, lactic acid bacteria, and yeast, which may serve as an alternate source of probiotics. The present study aimed to investigate the effect of dietary enriched kombucha tea at different levels on the protein and lipid retention of African catfish (*Clarias gariepinus*). A total of 400 fish, with an average initial weight of 5.26±0.11 g, were randomly divided into five treatments. The diet treatment containing different volumes of added kombucha tea (4 mL, 6 mL, 8 mL, and 10 mL per kg of feed) was given and compared to the control, without kombucha addition. The fed was given at satiation three times a day for 30 days. The result showed a significant increase (P<0.05) in protein and lipid retention in the group that received kombucha tea addition, compared to the control group. Kombucha tea addition at 8 mL/kg of feed resulted in the highest protein and lipid retention, but the addition above this value showed a slight decrease in protein and lipid retention. Overall, the present study proved that the addition of kombucha at the optimum levels in diet could improve the protein and lipid retention of fish, which are related to the fish’s somatic growth. These results provide evidence of the potential application of kombucha tea in the aquaculture industry as a feed additive, especially for probiotic sources.

1. Introduction

Fish feeding is a crucial component of aquaculture because it provides all the required nutrients, including protein, lipid, carbohydrate, vitamin, and minerals, for fish growth and maintenance. Protein is the main ingredient in fish feed due to the high protein requirement in fish compared to terrestrial animals [1]. Dietary protein requirements for most fish species have been reported to be between 30-55% of the diet. However, this varies depending on the fish species, fish stages, dietary protein source, and environmental conditions [2]. Protein supplies essential and non-essential amino acids for protein synthesis, growth, and energy for maintenance. Besides the necessity of dietary protein, lipid on feed
also contributes to fish growth unless the lipid requirement is not as high as protein. In addition to being an energy source, lipid provides essential fatty acid to maintain the biological structure and normal function of cell membranes. Dietary lipid has a protein-sparing effect that can minimize the use of protein to provide energy so that the protein can be optimally used for growth [3]. In aquaculture, it is preferable that the majority of the food, especially protein, can be used for growing and the maintenance energy is obtained from non-protein sources like lipid and carbohydrates. Therefore, the presence of balanced and optimum nutrients in the diet, including protein and lipid, is a crucial factor in the success of fish cultivation.

Generally, the completeness of nutrient content in diet determines the quality level of feed, which affected the feed price. In formulating a diet, the protein source is known to be an expensive component in fish feed. Meanwhile, the feeding cost constitutes about 40-60% of the total production cost [4]. These can be a problem in cultivation because of the expensive cost of feeding. The addition of feed additive such as probiotics, a beneficial living microorganism on the intestinal system, might be the best approach for minimizing the feeding cost. Probiotics would be enriched the quality of feed, which promotes nutrient utilization and results in better fish growth [5–7]. Many studies claimed that probiotics enhance the growth and health status of the fish through different mode of action, for instance, enhancing the feed utilization and fish weight gain by increasing the digestibility, preventing the growth of pathogens, improving the water quality, and improving the immune system [8–10].

According to Wang et al. [5], using mixed-species probiotics is more effective than a single probiotic strain because many bacteria have a symbiotic relationship with each other and function synergistically. Kombucha tea is a slight sweetness and sourness beverage made from the fermentation of sugared tea by a symbiotic culture of bacteria and yeast [11]. It has been documented that kombucha contains a wide range of microbes, including acetic acid, lactic acid bacteria, and yeast, which have been used as probiotics in aquaculture for a long time [12].

African catfish (Clarias gariepinus) is a common freshwater fish widely cultivated in Indonesia due to their high tolerance in various density culture settings, disease resistance, rapid growth rate, and ability to accept a diverse range of diet [13]. The previous study reported that dietary kombucha improves weight gain, specific growth rate, and feed efficiency in African catfish cultivation [14,15]. However, the effect of dietary kombucha on nutrient utilization remains unexplored. Optimizing nutrient retention help to avoid inputting an excessive nutrient in culture effluents. As a result, the present study aimed to investigate the impact of dietary enriched kombucha tea on the protein and lipid retention of African catfish (Clarias gariepinus).

2. Material and methods

2.1. Kombucha tea preparation
The kombucha tea was prepared using the methods described by Ramadhan et al. [15]. About 50 g of dry tea leaves and 150 g of sugar were added to 1 L of boiled water and heated for 10 min. Then, the tea extract was filtered and allowed to cool at room temperature. The SCOBY, a starter culture of kombucha, was added to the tea extract in the glass jar and covered with clean cotton. The mixture was incubated for 12 days at room temperature. At the end of the incubation period, the mixture formed as two portions, a floating cellulosic pellicle layer, and the liquid broth. After separation, the liquid broth was used for this study.

2.2. Diet preparation
The kombucha tea was included to the commercial diet (MS Prima Feed, Matahari Sakti, Indonesia), containing 36% of protein and 5% of lipid, by spraying method [16]. The mixture of kombucha tea and prokol, as the binder, was sprayed in different volumes of 4 mL, 6 mL, 8 mL, and 10 mL to the one kg of commercial diet. Then, pellets were dried at room temperature.
### 2.3. Fish acclimatization and feeding experiment

African catfish seeds were purchased from a fish farm in Kabat, Banyuwangi. Fish were acclimatized for seven days in 4 aerated tanks with 100 L capacity before experiments. During the acclimatization period, fish were fed with a commercial diet three times a day as much as 5% of body weight. After acclimatization, fish (n=400; 5.26±0.11 g of weight) were randomly distributed into five feeding treatments. Each of these groups was then divided into four replication tanks, containing 20 L of water. The first group (K0) served as the control group, without the kombucha addition, while the other groups (K4, K6, K8, and K10) were fed on the experimental diet containing 4 ml, 6 ml, 8 ml, and 10 ml of added kombucha tea, respectively. Fish were fed the experimental diet for 30 days, at satiation, and three times daily. Water quality parameters, such as water temperature, pH, and dissolved oxygen, were monitored daily and maintained at 27-28 °C, pH 6-7, and dissolved oxygen 4-6 ppm during the experiments. Removal residual and fecal matter were performed every day to maintain the water quality.

### 2.4. Protein and lipid retention measurement

Sample of fish muscle was collected twice, at the beginning and the end of the experiment. Fish muscle samples were taken from both the control and experimental groups. Proximate analysis including crude protein, lipid, and moisture were performed according to AOAC 1990 [17] with a slight modification. Crude protein was determined by Kjeldahl method, crude lipid was analysed by ether extraction using Soxhlet extractor, and moisture was determined by oven drying at 105 °C for 24 hours. The protein and lipid retention were determined following equations according to Abidi and Khan [1].

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Protein\ Retention = \frac{Protein\ gain}{Protein\ fed} \times 100
\]

\[
Lipid\ Retention = \frac{Lipid\ gain}{Lipid\ fed} \times 100
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### 2.5. Statistical analysis

All the data were subjected to analysis of variance (ANOVA) followed by Duncan’s multiple range test to evaluate significant differences among the treatments. All significance tests were at P>0.05. The statistical analysis was performed using SPSS version 13.

### 3. Result and discussion

In this study, the addition of kombucha tea to the diet gave a significant effect on the increased protein and lipid retention level of catfish. Protein and lipid retention of African catfish fed with different volume of dietary kombucha tea for 30 days of culture are displayed in Table 1.

| Treatment | Sample size (N) | Protein Retention (%) | Lipid Retention (%) |
|-----------|-----------------|-----------------------|---------------------|
| K0 (control) | 4 | 10.28±0.66<sup>d</sup> | 5.29±1.09<sup>c</sup> |
| K4 | 4 | 13.02±0.43<sup>c</sup> | 8.78±2.31<sup>b</sup> |
| K6 | 4 | 14.97±0.92<sup>b</sup> | 10.74±0.85<sup>ab</sup> |
| K8 | 4 | 17.75±0.41<sup>a</sup> | 13.52±1.75<sup>a</sup> |
| K10 | 4 | 15.76±0.49<sup>b</sup> | 12.28±3.25<sup>a</sup> |
| P-value | | <0.01 | <0.01 |

<sup>a</sup> Protein and lipid retention value are presented as mean ± SD
<sup>b</sup> The different superscript notation at the same column describes a significant difference between the treatment.

Protein and lipid retention was significantly improved at fish fed containing kombucha tea compared to the control group (P<0.05). The protein and lipid retention level was increased along with the increase
of kombucha tea level on a diet. The highest protein (17.75±0.41%) was observed in a group that received kombucha addition at 8 ml/kg of feed. However, the addition of more than 8 ml/kg resulted in a significant decrease of protein retention to 2% (15.76±0.49%). A similar trend was also observed in the lipid retention level. Kombucha addition at 8 ml/kg of feed resulted in the highest lipid retention (13.52±1.75%) compared to the other treatment, but at the addition of 10 ml/kg, there was a slight decrease of 1%. Whereas, there was no significant difference in comparison with K8 and K10.

The increased protein and lipid retention of catfish can be expected due to the various microbial and chemical content in kombucha. According to Villarreal-Soto et al. [18], a large range of yeast (Zygosaccharomyces, Saccharomyces, Candida) and bacteria including acetic acid bacteria (Gluconacetobacter, Acetobacter) and lactic acid bacteria (Bifidobacterium, Lactobacillus) are presented in kombucha. Some of the microbes, for example, Lactobacillus, Bifidobacterium, and Saccharomyces have been applied as probiotics in aquaculture. As previously reported, lactic acid bacteria could excrete proteolytic enzymes that hydrolyze extracellular protein into free amino acids [19, 20]. Moreover, Meyers et al. [21] stated that lactic acid bacteria also produce enzymes with lipolytic activity but the activity was not as strong as the extracellular lipase produced by Candida, that also present in kombucha. Therefore, the presence of various microbes in kombucha can create a synergistic activity to increase the feed digestibility by producing various hydrolytic enzymes that breaking down the un-digestible component in feed. In consequence, there is an increase in the absorption of protein and lipid from the diet which is then converted into protein and lipid in fish muscle.

In the kombucha system, the interaction of the microbes with the sugared tea, as a substrate, are led to produce the various chemical component in kombucha, such as organic acids, amino acids, vitamins, minerals, and polyphenols, which give the beneficial effect when it consumed [22]. Acetic acid is the predominant organic acid in kombucha. The presence of this compound can become the precursor of cell production in yeasts, which are known to have the ability to produce lysine as an essential amino acid for fish [23]. Besides that, some of the organic acids in kombucha are known to have an antimicrobial activity that can protect the fish from pathogens [24]. Furthermore, kombucha also contains polyphenols which are related to oxidative stress. Fish are more susceptible to oxidative stress stimulated by poor water quality, pathogens, and poor handling. The presence of polyphenol could protect the fish body component, especially DNA, protein, and lipid from the free radical compounds caused by oxidative stress [14]. Hence, the presence of those compounds also contributed to the improvement of protein and lipid retention.

According to this study, the administration of kombucha at more than 8 ml/kg of feed decreased protein and lipid retention. This result proved that the excessive consumption of kombucha harmed the fish. Despite the various advantages of kombucha, the acidity of kombucha might lead to lesions on digestive organs so that the absorption of nutrients can be disturbed [25]. Therefore, the quantity of kombucha tea became a concern on the application of kombucha as a feed additive to produce the optimum fish’s somatic growth.

4. Conclusion

Kombucha tea has good potential as a feed additive for improving the protein and lipid retention of African catfish with the optimum dose at 8 ml/kg of feed. The presence of various bacteria and yeast in kombucha become an alternative source of probiotics contributed to feeding utilization and nutrient absorption in fish.

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