Potential utilization of kombucha as a feed supplement in diets on growth performance and feed efficiency of catfish (Clarias sp.)

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Abstract. Kombucha is a tea beverage obtained from the fermentation of sugared tea by a symbiotic culture of bacteria and yeast. Kombucha is known to have many beneficial effects in health due to its chemical content and a high source of acetic acid and lactic acid bacteria and yeast. This study aimed to investigate the effect of kombucha supplementation in feed on growth performance and feed efficiency of catfish (Clarias sp.). Four hundred fish were distributed randomly into five feeding treatment groups includes a basal diet (control) and kombucha supplementation in the fish diet at 4 mL, 6 mL, 8 mL, and 10 mL/kg of feed. The feed was administered for 30 days. Results showed that the administration of kombucha in feed significantly improved the growth performance of catfish (P<0.05). Fish fed with kombucha supplementation at 8 mL produced the highest weight gain, absolute growth rate, and specific growth rate. Feed conversion ratio and feed efficiency ratio were significantly enhanced in the administration of 8 mL kombucha in a fish diet compared to the control diet. This study indicated that administration of kombucha in fish diet had a potential for enhancing the growth performance and feed efficiency of catfish (Clarias sp.).

1. Introduction
Catfish (Clarias sp.) is the most popular freshwater fish commodity that is consumed and cultured in Indonesia. Catfish contain a high nutritional value such as protein, lipids, minerals, and vitamins which are good nutrients for human health [1]. To fulfill the fish consumption by humans, fish farming becomes an alternative solution. Catfish are known to be easily cultivated because it has high tolerance in various density culture conditions, high growth rate, resistance to disease, and ability to accept a wide variety of feed [2]. Feed plays a vital role in the success of fish farming. The optimum nutrient in feed could enhance the growth performance of fish and shorten the cultivation period. However, the high quality of fish feed has an expensive price which can lead to an increased production cost. Therefore, farmers usually add a quantity of eatable substance, for example probiotic, to enrich the quality of fish feed which can further improve the fish growth and feed efficiency [3,4].

Probiotics are defined as live microbial feed supplements that useful for the host, especially in balancing the intestinal microbes. Some previous studies reported that the administration of probiotics
in fish diet improved the growth performance and health status of fish [5–7]. The improvement of fish growth rate after probiotic inclusion was investigated due to the ability of probiotic to produce various digestive enzymes, which helped to improve the digestion system and nutrient absorption. One of the food product which can act as a probiotic is kombucha tea [8].

Kombucha tea is an oriental drink beverage made from the fermentation of sugared tea by a symbiotic culture of bacteria and yeast, called SCOBY. It becomes famous as a healthy drink because of its beneficial effects on human health which is contributed from polyphenols, sugars, organic acids, amino acids, vitamins, and minerals [9]. Some of the benefits have already been investigated such as anti-oxidant, anti-microbial, anti-carcinogenic, and hepato-protective agents [10]. Kombucha is also considered as a probiotic due to the presence of various microorganisms, including acetic acid bacteria, lactic acid bacteria, and yeast. Marsh et al. [11] reported that Lactobacillus had been found in kombucha with an abundance of about 30%, and Gluconacetobacter is the most abundant bacterial genus, present at up to 85%. Lactobacillus is a type of lactic acid bacteria that has been well-known as probiotics in aquaculture [12]. However, as far as we know, there is a limited study that documented the use of kombucha as a probiotic in aquaculture. For that reason, this study was conducted to investigate the effect of kombucha supplementation in feed on growth performance and feed efficiency of catfish (Clarias sp.).

2. Material and methods

2.1. Preparation of kombucha

All of the equipment used for kombucha production was sterilized by autoclave to avoid contamination. The symbiotic culture of bacteria and yeast (SCOBY), as a starter culture of kombucha, was purchased from Rumah Fermentasi, Tangerang, Indonesia. Dry tea leaves (50 g) infused into 1 L boiled water that has been added 150 g of white sugar and boiled for 10 min. After separating the tea leaves, the tea extract is allowed to cool at room temperature and moved into a glass jar. About 15 g of SCOBY was added to the glass jar and then covered with clean cotton. The mixture incubated at room temperature for 12 days. The kombucha tea obtained was filtered.

2.2. Microbial analysis of kombucha

The number of microbial cells contained in kombucha tea counted using the total plate count (TPC) method. The serial dilutions of kombucha tea were prepared by diluted 1 mL of kombucha tea in 9 mL of sterile NaCl solution (0.9%) serially up to 10^6. Then, 1 mL of each dilution was transferred aseptically onto duplicate sterile petri dish contained 20 mL of Trypticase soy agar (TSA) medium. The sample was trimmed thoroughly by a spatula cell spreader. After that, the plates were incubated at 37°C for 24 hours. The colony was counted and calculated using the formula as described in Chouhan [13]. Based on the condition that the number of counted colonies on a plate of 30-300 colonies.

2.3. Experimental diet preparation

This study used a commercial diet containing 30% of crude protein as the basal diet. Four experimental diets were prepared using a spraying method as described by Dotta et al. [14] with a slight modification. Kombucha was mixed with 0.05% of progol as the binder. Then, it sprayed to the feed pellets at different levels of 4 mL, 6 mL, 8 mL, and 10 mL (per kg of feed). Pellets were air-dried for 5-10 minutes at room temperatures.

2.4. Experimental design

A total of 400 hundred catfish (Clarias sp.) with an average initial weight of 5.26±0.11 g, were purchased from a fish farm at Kabat, Banyuwangi, Indonesia. The fish divided into 20 aerated tanks consist of five treatments and four replications. Before the feeding trials, all fish acclimatized for a week. During the acclimation period, fish were fed three times a day as many as 5% of body weight with a commercial diet. Removal of residual feed and fecal matter carried out once a day to maintain the water quality.
After acclimatization periods, all fish fasted for 24 hours before the feeding treatment. Five feeding treatments were carried out as followed: Group A was fed with a control diet (without kombucha) and other groups were fed 4 mL kombucha/kg feed (Group B), 6 mL kombucha/kg feed (Group C), 8 mL kombucha/kg feed (Group D), and 10 mL kombucha/kg feed (Group E). Feeding treatments were undertaken three times a day for 30 days using a satiation method. During the experimental period, water quality parameters were monitored every day and maintained at acceptable levels: water temperature 27-28°, pH 6-7, dissolved oxygen 4-6 ppm, and ammonia-nitrogen less than 0.04 ppm. In addition, 50% of the water was exchanged with de-chlorinated tap water to control the water quality.

2.5. The growth performance, feed utilization, and survival rate of fish

Fish growth performance assessed consists of weight gain (WG), absolute growth rate (AGR), and specific growth rate (SGR). During the maintenance period, fish body weight was measured at intervals seven days. Meanwhile, the feed utilization parameters measured include food conversion ratio (FCR) and feed efficiency ratio (FER). The number of survived fish and feed consumed noted daily. The growth performance, feed utilization, and survival rate of fish calculated as the following formula [15]

\[
WG = Wt - Wo \tag{1}
\]

\[
AGR = \frac{Wt - Wo}{t} \tag{2}
\]

\[
SGR = \frac{\ln Wt - \ln Wo}{t} \times 100 \tag{3}
\]

\[
FCR = \frac{\text{Feed intake}}{WG} \tag{4}
\]

\[
FER = \frac{WG}{\text{Feed intake}} \tag{5}
\]

\[
SR = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100 \tag{6}
\]

Where, Wt is final weight (g), Wo is initial weight (g), t is feeding treatment period (days).

2.6. Statistical Analysis

All the statistical analyses conducted using SPSS version 13. All the data were analyzed using a one-way ANOVA to evaluate the effect of kombucha supplementation on diet. Differences between means were deemed significant at P<0.05 through Duncan’s multiple range tests.

3. Result and discussion

The kombucha tea obtained from this study has some different character when compared to the unfermented tea, including its color, flavor, and taste. This result depicts that fermentation had an impact on the changing properties of the tea. Kombucha tea has a paler brown color compared to the unfermented tea. It also has a slightly sour flavor and taste. These results are similar to the study reported by Nurikasari et al. [16] which recorded the kombucha color changes from reddish-brown to light brown with the progression of fermentation. Chakravorty et al. [17] confirmed that this color alteration might be attributed to the conversion of thearubigin to theaflavin during the fermentation. Meanwhile, the sour taste of kombucha is related to the production of acidic substances like acetic acid, glucuronic acid, gluconic acid, and lactic acid by the microorganism involved in fermentation [18,19].

The growth performance and feed utilization of catfish fed with kombucha supplementation on diet were displayed in Table 1.
According to Dawood et al. [23], lactic acid bacteria and *Saccharomyces* has been used in aquaculture as probiotics for a long time. These probiotics can increase growth performance and feed utilization in catfish because they produce a wide variety of digestive enzymes such as amylase, lipase, and protease. These enzymes would help to denature the indigestible component in diet so it can optimally absorb in the digestive tract. Furthermore, they also have been found to increase the gastrointestinal barrier function from the harmful bacteria [24,25]. Besides the microbial content of kombucha, the chemical components produced by sugared tea fermentation also have a contribution to the growth enhancement of catfish. The presence of a variety of compounds, including organic acid, sugars, amino acids, polyphenols, vitamins, and minerals may become additional digestible nutrients that are easily absorbed by fish and affected the fish’s health. Muthusamy et al. [26] argued that polyphenols in kombucha might perform as antioxidant agents that prevent fish from oxidative stress caused by various stress factors, such as low water quality. The present study found that the administration of kombucha at a higher concentration than 8 mL/kg of feed could reduce the growth rate of fish. This result can be expected due to the high acidity of kombucha that might induce the internal organ lesions, particularly in digestive organs [21]. This fact showed that kombucha administration in high dosage leads to adverse effects on fish growth. Consequently, the appropriate dose in the application of kombucha administration as a fish feed supplement is crucial to ensure the optimal growth performance and feed efficiency of fish.

Table 1. Effect of kombucha supplementation in diets on growth performance and feed utilization of catfish

| Parameters   | Control (Group A) | 4 mL/kg (Group B) | 6 mL/kg (Group C) | 8 mL/kg (Group D) | 10 mL/kg (Group E) |
|--------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Initial weight (g) | 106.28±3.39      | 103.38±1.97      | 104.87±0.93      | 104.76±0.44      | 106.47±0.31      |
| Final weight (g)   | 207.25±1.33      | 218.85±6.57      | 236.65±2.60      | 253.69±3.75      | 253.23±4.33      |
| Weight gain (g)    | 100.96±2.24      | 115.47±4.73      | 131.77±2.01      | 148.93±3.77      | 146.75±4.05      |
| AGR (g/day/fish)   | 0.17±0.00        | 0.19±0.01        | 0.22±0.00        | 0.25±0.01        | 0.24±0.01        |
| SGR (%/day/fish)   | 2.23±0.09        | 2.49±0.04        | 2.71±0.02        | 2.95±0.05        | 2.89±0.04        |
| FCR               | 0.73±0.01        | 0.67±0.01        | 0.61±0.00        | 0.56±0.00        | 0.56±0.01        |
| FER               | 1.36±0.02        | 1.48±0.04        | 1.62±0.01        | 1.77±0.02        | 1.78±0.03        |
| SR (%)            | 96.25±4.78       | 92.50±6.45       | 93.75±4.78       | 93.75±4.75       | 90.00±4.08       |

Values are shown as mean ± SD. The different superscript notation at the same row expresses a significant difference between the treatment.
4. Conclusion
We can conclude that kombucha administration on a diet improved the growth performance and feed efficiency of catfish with the optimal dose at 8 ml/Kg of feed. The presence of diverse chemical content, bacteria and yeast in kombucha is believed to have a contribution to enhance the growth performance and feed efficiency.

5. Reference
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