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Research Article

Dietary effect of kombucha tea supplementation on growth performance and intestinal histomorphology in giant gourami (*Osphronemus gourami*)

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Abstract

Improving feed quality and efficiency is crucial for sustainable aquaculture practices, particularly for giant gourami (*Osphronemus gourami*) cultivation. One of the useful strategies is the inclusion of kombucha tea in the diet. This study aimed to evaluate the effect of different levels of kombucha tea supplementation on the intestinal histomorphology and growth performance of giant gourami. A completely randomized design (CRD) with five treatments and four replications was used. The treatments consist of a control diet (T0) and diets supplemented with 10 ml (T10), 15 ml (T15), 20 ml (T20), and 25 ml (T25) of kombucha tea per kg of feed. The fish were fed three times daily to satiation for 40 days. The result showed that kombucha tea considerably increased the villi length (p<0.05), enhancing nutrient absorption and fish growth performance (p<0.05). The highest specific growth rate (2.21±0.12 %.day^{-1}) and feed conversion ratio (1.42±0.09) were observed in T25 group, while statistically similar to T20 (p>0.05). These findings suggest that supplementing kombucha tea on a diet improves intestinal morphology, promoting better feed utilization and growth performance. This strategy offers a sustainable approach to enhancing feed efficiency and productivity in giant gourami aquaculture.

Introduction

Giant gourami (*Osphronemus gourami*) is one of the distinguished freshwater fish commodities in Indonesia. It has a high nutrient content, a good taste, and is usually served as grilled fish in restaurants, so the market demand for this fish tends to increase every year. On the other hand, the slow growth of giant gourami is the main obstacle to its production. It takes about a year to rear the gourami until it reaches a market size of about 500 g (Alimuddin *et al*., 2011). The growth performance of fish was affected by various factors, such as genetics, hormones, environmental conditions, and feed intake. Adequate quality and quantity of feed are the most crucial factors in achieving maximum growth (Aryani *et al*., 2017).

In this recent study, kombucha tea was used as the probiotic source. Greenwalt *et al*. (2000) discovered that kombucha tea is rich in microbial composition, including bacteria and yeast. Furthermore, kombucha also contains various bioactive compounds, such as organic acid, polyphenols, sugars, vitamins, minerals, and hydrolytic enzymes, which have a beneficial impact on health (Leal *et al*., 2018). Some studies have claimed that one of the microbial contents in kombucha is lactic acid bacteria (LAB) (Puspawati and Arihantana, 2016; Cvetković *et al*., 2019; Bishop *et al*., 2022a). LAB is non-spore-forming bacteria that belong to Gram-positive which has homo- and hetero-fermentative activity. It has been known and used in aquaculture as a probiotic because it is safe, environmentally friendly, and suitable for aquaculture systems, including improving nutrition, water treatment, and disease prevention (Chizhayeva *et al*., 2022). A previous study has reported that kombucha tea diet supplementation could increase the nutrient utilization of catfish, and as a result, it has better growth performance and feed efficiency than fish without kombucha tea supplementation (Aprianus *et al*., 2021; Ramadhan *et al*., 2021). To our knowledge, improving nutrient utilization in fish can be affected by the performance of the digestive tract, mainly intestinal morphology. Therefore, this recent study aimed to investigate the effect of kombucha tea supplementation on the mucosal fold of fish, which is related to fish growth performance and feed utilization. The study on kombucha tea supplementation in giant gourami contributes to the ongoing efforts to optimize aquaculture practices, offering a natural and potentially sustainable solution to enhance fish growth and feed utilization. The findings may have broader applications and could impact both the aquaculture industry and the scientific understanding of probiotic effects on fish health and performance.

Material and methods

The study was conducted in May-July 2022 at the Laboratory of the Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga. This research received ethical approval and oversight from Airlangga University under authorization number 8/UN3.1.16/2023.

Fish preparation

A total of 200 gourami fish, with an average initial weight of 2.28±0.12 g, were obtained from Banyuwangi (East Java, Indonesia) farmers. Fish were randomly divided into

20 tanks (40×25×30 cm^3) with a stocking density 1 fish.L^{-1} and acclimatized for a week before the experiment. During the acclimatization period, fish fed with commercial pellet (Hi-Pro-Vite, Indonesia) with 32% crude protein three times daily to satiation.

Experimental design

This study used a completely randomized design with 5 treatments and four replicates. Treatments including T0 as the control group and (T10, T15, T20, and T25) containing 10 ml, 15 ml, 20 ml, and 25 ml of kombucha tea per kg of feed, respectively. The experimental diets were prepared by spraying the kombucha tea according to the prescribed dose to the one kg commercial diet (Dotta *et al*., 2018) and were air dried for 30 minutes. Fish were fed the experimental diet three times a day using at satiation method for 40 days. Removal of residual and fecal matter was performed every day to maintain the water quality. During the maintenance period, fish body weight was measured at intervals of ten days, while total feed consumed and survived fish were noted daily.

Growth performance and feed utilization

The parameters observed for growth performance and feed utilization consist of weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), protein efficiency ratio (PER), protein retention (PR), feed conversion ratio (FCR), and survival rate (SR). All parameters were calculated as the following formulas:

$$WG = Wt - Wo$$

$$AGR = \frac{Wt-Wo}{t}$$

$$SGR = \frac{\ln Wt - \ln Wo}{t} \times 100$$

$$PER = \frac{Weight\ gain}{Protein\ intake}$$

$$PR = \frac{Total\ protein\ in\ body\ gain}{Total\ protein\ consumed} \times 100$$

$$FCR = \frac{Feed\ intake}{Body\ weight\ gain}$$

$$SR = \frac{Final\ count}{Initial\ count} \times 100$$

Intestinal histomorphology

Intestinal samples for histological examination (n= 4/treatment) were collected at the end of the experiment. The fish was stunned by immersion in ice water before being dissected. A sampling tissue for histological examination was carried out from the middle part of the intestine and fixed in 10% Neutral Buffered Formaldehyde (NBF) solution. The histological section of the intestine was prepared through a series of procedures such as dehydration, paraffin embedding, sectioning with microtome and staining with Mayers Hematoxylin Eosin. The examination of intestinal tissue sections was performed using a microscope (Eclipse E200-LED, Nikon, Japan) connected to a video monitor (400× magnification). The mucosal fold (villi) length was measured from the folding tip to the bottom as described by Pirarat *et al*. (2011).

Statistical analysis

Data was analyzed using SPSS software (SPSS version 20 for Windows) by one-way ANOVA. The significant differences between treatments were further analyzed using the Duncan multiple range test. Significance differences were considered when p<0.05. The results are shown as mean±SD.

Results

Growth performance and feed utilization

Dietary kombucha tea on giant gourami for 40 days resulted in a significant increase in growth performance and feed utilization (Table 1). The growth performance parameters involved weight gain (WG), absolute growth rate (AGR), and specific growth rate (SGR) which showed a significant increase compared to the control group without kombucha supplementation (p<0.05). The growth performance of giant gourami increased along with the increasing doses of kombucha given on the diet. Fish fed on the diet containing 25 ml of kombucha (T25) showed the best WG, AGR, and SGR with the value 3.05±0.27 g, 0.076±0.007 g.day^{-1}, and 2.21±0.12 %.day^{-1}, respectively. However, this treatment was not significantly different from fish fed with 20 mL of kombucha (T20).

Table 1: Growth performance and feed utilization of giant gourami fed with kombucha tea supplementation for 40 days.

Parameters	Treatment					*P*-Value
	T0	T10	T15	T20	T25	
Initial weight (g)	2.32±0.05	2.39±0.14	2.29±0.11	2.22±0.08	2.15±0.10	
Final weight (g)	4.78±0.29	4.80±0.26	4.88±0.20	5.05±0.27	5.20±0.32	
WG (g)	2.46^{b}±0.24	2.41^{b}±0.25	2.59^{b}±0.29	2.82^{ab}±0.26	3.05^{a}±0.27	0.018
AGR (g.day^{-1})	0.061^{b}±0.006	0.060^{b}±0.006	0.065^{b}±0.007	0.071^{ab}±0.007	0.076^{a}±0.007	0.018
SGR (%.day^{-1})	1.80^{c}±0.10	1.74^{c}±0.15	1.90^{bc}±0.20	2.05^{ab}±0.14	2.21^{a}±0.12	0.003
PER	1.67^{b}±0.19	1.71^{b}±0.16	1.94^{ab}±0.18	2.23^{a}±0.28	2.25^{a}±0.27	0.004
PR (%)	29.87^{c}±3.16	31.91^{c}±4.13	36.28^{c}±1.74	43.00^{b}±7.15	51.01^{a}±2.29	0.001
FCR	1.84^{ab}±0.10	1.90^{a}±0.15	1.69^{b}±0.14	1.44^{c}±0.08	1.42^{c}±0.09	0.001
SR (%)	87.5±5	90±0	90±0	87.5±12.58	87.5±5	0.945

Data are presented as mean ± SD. The superscripts in the data indicate significant differences among the treatments in each row (p<0.05). Initial and final weight were not statistically analyzed.

Protein efficiency ratio (PER) of giant gourami after dietary with kombucha showed significant differences between the treatments (p<0.05). The best PER 2.25±0.27 was recorded from fish fed with 25 ml of kombucha and this was no difference in the PER of T20 and T15 (p>0.05). The lowest PER was obtained from T0 and this was no significant difference from T10. Kombucha addition to gourami feed also increased protein retention (PR). Similar to the PER, the highest PR resulted in the group achieving the highest dose of kombucha (T25). There was a significant difference from the rest of the treatment (p<0.05). Supplementation of kombucha tea on gourami feed also affected the feed conversion ratio (FCR). Based on the result, the FCR declined along with an increase in the dose of kombucha given. The highest FCR was obtained in T10 which was not significantly different from the control group (T0). The survival rate of all treatments showed no significant difference (p>0.05).

Intestinal histomorphology

The effect of dietary kombucha on the intestinal morphology of giant gourami was observed by a light microscope (Fig. 1).

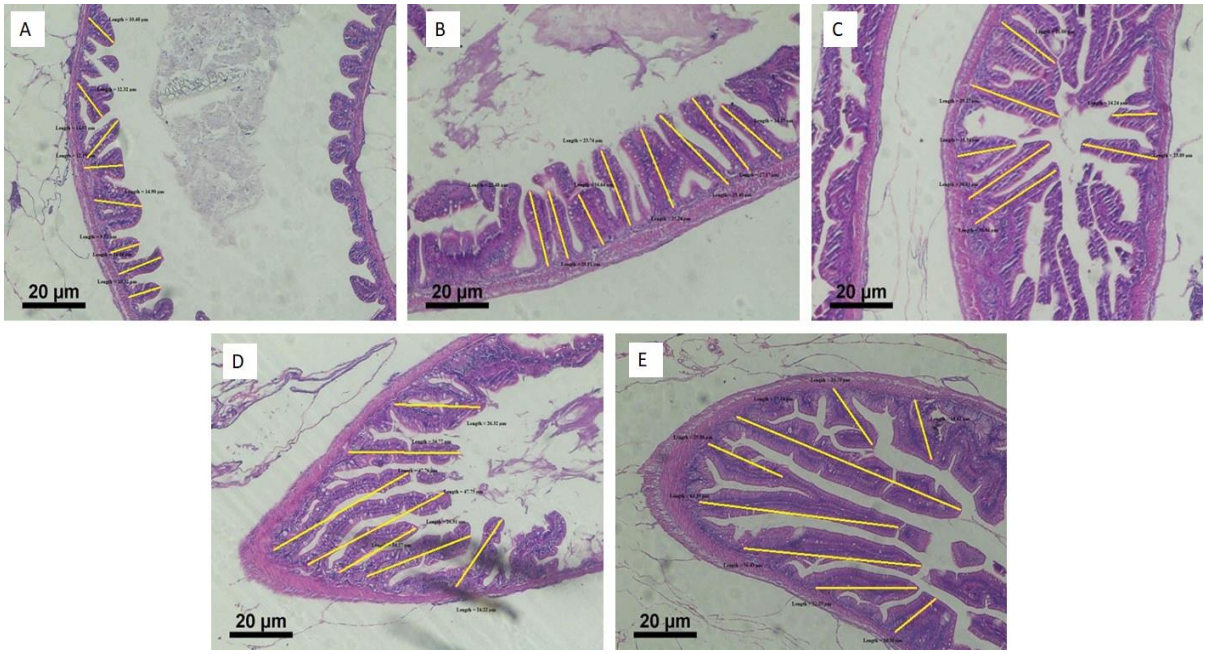

Figure 1: Increased giant gourami intestinal villi height after 40 days of kombucha diet feeding, A. T0: without kombucha; B. T10: supplemented with 10% of kombucha; C. T15: supplemented with 15% of kombucha; D. T20: supplemented with 20% of kombucha, and E. T25 supplemented with 25% of kombucha. The yellow line indicates villi length.

Based on this study, kombucha supplementation in the diet increased the height of the villus significantly compared to the control group (T0) (p<0.05). The data is presented in Figure 2. The highest villus resulted from the fish that received 25 mL of kombucha, but it was not statistically different from T20 group that received 20 mL of kombucha.

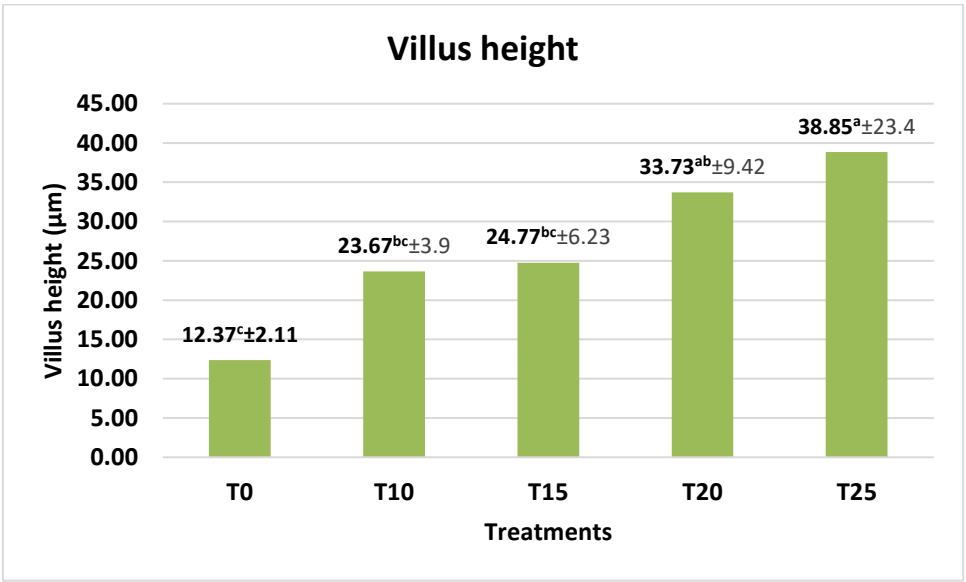

Figure 2: Average villus height of the intestine of giant gourami after feeding kombucha-supplemented diet for 40 days. Different notation in superscript indicates a significant difference (p<0.05).

Discussion

Growth was determined as the alteration in size of fish that resulted from a complex suite of numerous processes, including behavioral, morphological, and physiological processes, which are related to energy budgeting. This concept explains that growth was obtained from the excessive energy from the allocation of energy use in the fish (Enberg *et al*., 2012). The source energy of fish comes from nutritional components in feed, including proteins, lipids, and carbohydrates. These components are metabolically changed into energy through a series of mechanical, biological, and chemical processes in the ingestion, digestion, and absorption process (Rønnestad *et al*., 2013). Therefore, optimizing the digestive system of fish is important to get better fish growth performance.

In this present study, supplementation of kombucha tea on the diet exhibited substantial improvement in growth performance and nutrient utilization of giant gourami. This result was the consequence of probiotic action from kombucha tea. Kombucha tea contains a multi-strain of microbes including lactic acid bacteria (LAB), which is commonly used as aquaculture probiotics (Chizhayeva *et al*., 2022). Similar to this study, the administration of probiotics in the diet for different species of fish has been extensively studied by previous researchers who also reported the positive impact of probiotics inclusion on fish growth performance and nutrient utilization (Ramos *et al*., 2017; Zhao *et al*., 2021, Ghori *et al*., 2022; Gaffar *et al*., 2023; García-Meilán *et al*., 2023). In this study, the improvement of growth rate and nutrient utilization was in line with the increasing dose of kombucha given. The best growth rate and nutrient utilization were generated from 25 mL of kombucha diet treatment (Table 1), although there is an absence of a significant difference between T25 and T20. It could indicate a saturation point in the growth response, where additional kombucha supplementation provides no further benefit. This result proved that the inclusion of kombucha tea in the diet in higher doses increases the chance of microbes entering the fish digestive system and modulating the intestinal condition. Kombucha tea, as a probiotic, will compete with entero-pathogens for nutrients and space without affecting the normal microbiota in the gut. Then, probiotics secrete some digestive enzymes, which help to break down carbohydrates, protein, and lipids into smaller fragments that are absorbable in the fish gut (Allameh *et al*., 2017).

In our study, improvement of growth performance and nutrient utilization was also related to the fish intestinal structure alteration due to the addition of the kombucha diet. Fish with the kombucha addition had a greater villus height compared to the fish without kombucha (Fig. 2). The increasing villi length is an indicator of improvement of the intestinal surface area, which in turn promotes digestion and nutrient absorption in the intestine (Gaffar *et al*., 2023). According to Pirarat *et al*. (2011), probiotics may utilize sugar in the fish intestine and produce short-chain fatty acids as the main energy for intestinal epithelium cells. Additionally,

these fatty acids can trigger the secretion of gastrointestinal peptides or growth factors which impact cell proliferation. As a consequence, villi length was significantly increased and affected nutrient absorption and gastrointestinal health.

Besides the probiotic action, the improvement of villi length was due to the effect of bioactive compounds contained in kombucha. Based on Antolak *et al*. (2021), kombucha tea contains various bioactive compounds, such as polyphenols, organic acids, vitamins, enzymes, and bacteriocins. These compounds have been reported to have antioxidant properties that affect intestinal health. An antioxidant is any substance that can inhibit oxidation by scavenging free radicals and reactive oxygen species (Bishop *et al*., 2022b). The fish intestine is known to be susceptible to external adverse factors, for instance, the oxidation of feed, water environment, and intestinal flora, which may lead to damaging the intestinal villi structure (Long *et al*., 2022). Polyphenols in kombucha tea might play the role of antioxidants to protect fish intestine from oxidative stress. Our result proved that intestinal villi grew longer in fish fed with kombucha than without kombucha tea addition. Similar to the study by Jahazi *et al*. (2020), the inclusion of polyphenols in the diet improved the growth performance and antioxidant defense of common carp.

Improvement of the intestinal surface after a probiotic diet and the activity of digestive enzymes secreted from probiotics are believed to boost nutrient absorption synergistically. This may lead to an increase in protein retention (PR) and protein efficiency ratio (PER) observed in our study. The administration of 25 mL of kombucha on the diet generated the highest PR and PER value, 51.01±2.29 % and 2.25±0.27, respectively. Protein is the main component in fish feed which is required for fish growth. High protein retention indicates that the fish's body contains a large amount of protein obtained from fish feed (Pattipeilohy *et al*., 2020). Protein from feed will be broken down by protease enzymes, which are produced from fish's normal microbiota, into small peptides and amino acids which are easily absorbed by the intestine and then can be used or stored in the body (Wang *et al*., 2020). By adding the kombucha, digestive enzyme activity, especially protease, will increase due to the addition of exogenous enzymes secreted by microbes in kombucha. This agrees with the previous research obtained by Taoka *et al*. (2007) and Mariyam *et al*. (2021), which reported that dietary supplementation of probiotic stimulated the synthesis of digestive enzymes in the fish gastrointestinal tract higher than treatment without probiotic addition. Their findings also suggested that the dosage or concentration of probiotics administration is a key factor in enhancing digestive enzyme activity.

Increased digestive enzyme activity induced higher nutrient absorption, then impacted increasing fish growth performance and feed efficiency. In the current study, the decline in feed conversion ratio (FCR) occurred with increasing doses of kombucha. T25 generated the lowest FCR, although it was not significantly different from T20. FCR is an effective way to evaluate the efficiency of feed utilization. Lower FCR represents

better feed efficiency and conversion of nutrients into biomass. This finding confirmed that probiotic action from kombucha is beneficial for enhancing intestinal structure, fish growth performance, and feed utilization.

The positive outcomes of this study hold promising implications for the aquaculture industry, especially in the enlargement of giant gourami. The inclusion of 25 mL kombucha in the diet has been proven to increase fish growth performance and feed utilization by improving the structure of fish's intestines. However, it is still unclear about the kombucha's effects on fish digestive enzyme synthesis and fish gut microbial diversity for the detailed mechanism of kombucha in enhancing fish growth. In addition, further investigation could be carried out to determine the specific bacterial strains that play the most crucial role in better fish growth, remembering that kombucha consists of multi-strain bacteria.

Conclusions

In conclusion, the current finding discovered that the inclusion of kombucha tea in the giant gourami diet had a beneficial effect on intestinal morphology, as evidenced by a substantial increase in villus height. This morphological improvement correlates with the observed improvement in growth performance and nutrient efficiency. The recommended dosage of kombucha to increase growth and villus height is 25 $mL.kg^{-1}$ of feed. These findings contribute to a better understanding of the potential mechanism through which kombucha positively influences the physiological health of giant gourami by promoting gastrointestinal health and nutrient absorption.

Acknowledgment

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Conflicts of interest

No potential conflict of interest relevant to this article was reported

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