International Journal of Design & Nature and Ecodynamics
Vol. 21, No. 7, July, 2026, pp. 2011-2019
Journal homepage: http://iieta.org/journals/ijdne

Effects of Dietary Kombucha Supplementation on Growth Performance and Feed Utilization of *Clarias* sp.

R. Adharyan Islamy[1], Ahmad Bintang Berlian[1], Vania Aurellia Azarine[1], Muhammad Fakhri[1], Andi Masriah[1], Muhamad Dwi Cahya[1], Taufik Budhi Pramono[2], Fitri Sil Valen[3], Veryl Hasan[4,5,6*], Norshida Ismail[6], Ahmad Syazni Kamarudin[6]

[1] Department of Fisheries and Marine Resources Management, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Kediri City 64111, Indonesia
[2] Faculty of Fisheries and Marine Sciences, Universitas Jenderal Soedirman, Banyumas 53122, Indonesia
[3] Department of Aquaculture, Faculty of Agriculture Fisheries and Biology, Universitas Bangka Belitung, Bangka Belitung Islands 33172, Indonesia
[4] Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60113, Indonesia
[5] Research Group of Environmental and Fisheries Resources Management, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60113, Indonesia
[6] School of Animal Science, Aquatic Science and Environment, Universiti Sultan Zainal Abidin, Besut 22200, Malaysia

Corresponding Author Email: veryl.hasan@fpk.unair.ac.id

https://doi.org/10.18280/ijdne.210715

Received: 16 April 2026
Revised: 15 June 2026
Accepted: 22 June 2026
Available online: 31 July 2026

Keywords:
absolute growth, aquaculture, Clarias sp., feed efficiency, kombucha, life below water, probiotic

ABSTRACT

Feed efficiency and growth performance remain important factors in catfish culture systems. Probiotic supplementation has been applied as a nutritional approach to improve nutrient utilization and fish growth. Kombucha contains mixed microbial populations, including *Komagataeibacter*, lactic acid bacteria, and yeast that support digestive processes and physiological performance in fish. This study evaluated the effects of dietary kombucha supplementation on growth rate (GR), specific growth rate (SGR), feed conversion ratio (FCR), absolute growth, and survival of *Clarias* sp. The study employed a Completely Randomized Design (CRD) consisting of four treatments and three replications. Experimental diets consisted of commercial feed supplemented with kombucha at 0 mL/kg (control), 4 mL/kg, 8 mL/kg, and 16 mL/kg of feed. A total of 144 juvenile *Clarias* sp. with an initial average body weight of 8.04 ± 0.12 g were cultured for 28 days. To maintain the stability of the supplemented diets, fresh feed batches were prepared weekly throughout the experimental period and stored under refrigerated conditions until use. All feeds were prepared using the same coating and drying procedures to ensure consistency among treatments. GR, SGR, FCR, absolute weight gain (AWG), absolute length gain (ALG), and survival rate (SR) were evaluated. Data were analyzed using one-way analysis of variance (ANOVA) followed by LSD tests at $p < 0.05$. Dietary kombucha supplementation significantly affected GR, SGR, FCR, AWG, and ALG ($p < 0.05$). Fish receiving 16 mL/kg supplementation produced the highest growth performance among treatments. Treatment C (16 mL/kg feed) generated GR of 0.412 ± 0.022 g day^{-1}, SGR of 3.141 ± 0.133% day^{-1}, FCR of 1.447 ± 0.175, AWG of 11.530 ± 0.609 g, and ALG of 3.93 ± 0.252 cm. SR showed no significant differences among treatments ($p > 0.05$) and remained above 94%. Dietary kombucha supplementation improved growth performance and feed utilization efficiency of *Clarias* sp. under the present experimental conditions. Among the supplementation levels evaluated, 16 mL/kg feed produced the highest growth performance and feed utilization response. However, this level should be interpreted as the best treatment within the tested range rather than an optimal supplementation dose.

1. INTRODUCTION

Catfish (*Clarias* sp.) is one of the most widely cultivated freshwater fish species due to its rapid growth, high market demand, adaptability to diverse environmental conditions, and relatively simple culture practices [1]. Intensive catfish culture systems continue to increase production capacity and meet growing consumer demand. Intensive culture conditions often require high feed input and elevated stocking densities. These conditions can affect feed utilization efficiency and influence fish growth performance and health status [2].

Feed represents a major component of operational costs in aquaculture production. Feed costs can account for more than half of total production expenses in fish farming systems [3].

Feed efficiency has a direct relationship with fish growth and production performance. Low feed utilization efficiency can reduce growth rate (GR) and increase production costs. Nutritional approaches that improve nutrient digestion and absorption remain important for aquaculture development [4, 5].

Probiotics have received considerable attention as feed additives in aquaculture. Probiotic supplementation can improve digestive performance, maintain microbial balance in the gastrointestinal tract, and support nutrient utilization efficiency [6]. Probiotic microorganisms can also produce bioactive compounds and digestive enzymes that contribute to nutrient degradation and assimilation processes [7]. Improvement of intestinal microbial communities can affect physiological performance and growth responses in cultured fish [8].

Kombucha is a fermented product produced through microbial activity involving symbiotic cultures of bacteria and yeast. Kombucha contains mixed microbial populations including acetic acid bacteria, lactic acid bacteria, and yeast communities [9]. *Komagataeibacter* is one of the dominant bacterial groups commonly found in kombucha fermentation systems and has an important role in metabolite production and fermentation activity [10]. Fermentation products can contain organic acids, vitamins, enzymes, and other metabolites that support digestive function and microbial balance in aquatic animals [11].

Application of kombucha as a probiotic feed supplement in aquaculture remains limited. Information regarding the use of kombucha supplementation for catfish culture remains scarce. The physiological response of *Clarias* sp. to different supplementation levels requires further evaluation. Dose-dependent responses influence growth performance and feed utilization efficiency. Most probiotic studies in catfish focus on commercial bacteria or LAB preparations. Information regarding kombucha-derived mixed microbial supplementation remains limited.

This study aimed to evaluate the effects of kombucha supplementation on GR, specific growth rate (SGR), feed conversion ratio (FCR), absolute growth, and survival of *Clarias* sp. The study also evaluated growth responses across different supplementation levels to identify an effective supplementation range under the present culture conditions.

Most probiotic studies in catfish culture have focused on single-strain bacterial products, particularly Bacillus spp. and lactic acid bacteria. In contrast, kombucha contains a naturally fermented consortium consisting of acetic acid bacteria, lactic acid bacteria, yeasts, and fermentation-derived metabolites. These mixed microbial communities provide broader functional effects on digestive processes than conventional probiotic preparations. Despite increasing interest in fermented feed additives, information regarding kombucha supplementation in *Clarias* sp. remains scarce. Therefore, evaluating its effects on growth and feed utilization contributes to the development of alternative probiotic strategies for sustainable catfish production.

2. MATERIAL AND METHODS

2.1 Study site and experimental period

The experiment was conducted at the Integrated Farming System (IFS) Laboratory and Bioscience Laboratory, PSDKU Universitas Brawijaya, Kediri, Indonesia. Kombucha bacterial density analysis was performed at Satwa Sehat Laboratory, Malang. The study was conducted over 28 days from November to December 2025.

2.2 Experimental design

The study employed a Completely Randomized Design (CRD) consisting of four treatments with three replications per treatment. Experimental diets were prepared by supplementing commercial feed with kombucha at different concentrations: control (0 mL/kg feed), treatment A (4 mL/kg feed), treatment B (8 mL/kg feed), and treatment C (16 mL/kg feed). Dose selection was based on previous studies reporting beneficial effects of kombucha supplementation in fish culture. The 8 mL/kg dosage was adopted as a reference level, while lower and higher concentrations were included to evaluate dose-dependent responses and determine a potentially effective supplementation range.

Fish were randomly allocated to experimental units using a random-number procedure. Containers were arranged randomly within the laboratory and repositioned periodically to minimize positional effects. Each container served as the experimental unit for statistical analysis.

2.3 Fish and rearing conditions

A total of 144 healthy juvenile *Clarias* sp. were obtained from a local fish farm in Kediri, Indonesia. Prior to the experiment, fish were acclimatized and selected to obtain relatively uniform body size. Initial body weight and total length averaged 8.04 ± 0.12 g and 10.4 ± 0.17 cm, respectively. Fish were randomly stocked into twelve rearing units consisting of modified 15 L plastic containers containing approximately 12 L of freshwater. Stocking density was maintained at 12 fish per container. Continuous aeration was supplied using air stones connected to aerators. Before fish stocking, the water was conditioned for three days to stabilize environmental conditions. Commercial feed containing approximately 31% crude protein was used throughout the experiment. Fish were fed twice daily at 08:00 and 16:00 at a feeding rate of 5% biomass day^{-1}. Feed quantity was adjusted weekly based on fish growth measurements.

2.4 Kombucha preparation and feed supplementation

Kombucha was prepared using black tea, sugar, SCOBY, and starter culture following a standard fermentation procedure. Briefly, 25 g of black tea was boiled in 1000 mL of water and supplemented with sugar at 100 g L^{-1}. After cooling to room temperature, a Symbiotic Culture of Bacteria and Yeast (SCOBY) and 150 mL of previously fermented kombucha starter culture were added. Fermentation was conducted under dark conditions at 24 ± 3 °C for 14 days. The fermentation period was selected based on commonly reported kombucha fermentation protocols to obtain a stable fermented product.

Microbiological characterization of the fermented kombucha was performed at the Satwa Sehat Laboratory, Malang. Total bacterial density reached 2.10×10^8 CFU mL^{-1} as determined by the total plate count (TPC) method. Preliminary bacterial characterization was conducted using Gram staining and biochemical assays, including Sulfide-Indole-Motility (SIM), Methyl Red-Voges Proskauer (MR-

VP), Triple Sugar Iron Agar (TSIA), and Simmons Citrate Agar tests. The dominant isolate exhibited Gram-negative rod morphology, positive motility, acid-producing fermentation activity, and the ability to utilize glucose, lactose, and sucrose. Based on these phenotypic and biochemical characteristics, the isolate was presumptively classified as a member of the acetic acid bacteria group, consistent with members of the genus *Komagataeibacter*, which is commonly associated with kombucha fermentation.

Previous studies have reported that mature kombucha fermentations generally exhibit acidic pH values ranging from approximately 2.5 to 3.5 and contain mixed populations of acetic acid bacteria and yeasts. However, pH, titratable acidity, and yeast abundance were not quantified in the present study and therefore are not reported as experimental measurements.

Kombucha was applied to the commercial feed by spraying the required volume of fermented kombucha onto the pellet surface according to the designated treatment dosage. Distilled water and 0.05% Progol binder were applied using the same procedure across all treatment groups. The pellets were mixed manually for approximately 3–5 min to ensure uniform coating and distribution of the fermented suspension. Following application, the coated feed was air-dried at room temperature (27–29 ℃) for 24 h to reduce surface moisture and improve coating stability. Similar coating procedures have been widely used for the incorporation of probiotic microorganisms into aquaculture feeds [12]. After drying, the feed was packed in airtight polyethylene bags and stored at 4 ℃ until use. To minimize potential losses in microbial viability during storage, fresh batches of supplemented feed were prepared weekly throughout the 28-day experimental period and used within seven days of preparation. Drying was performed at ambient temperature rather than elevated temperatures to minimize thermal damage to viable microorganisms [13]. Refrigerated storage has been commonly recommended to maintain feed quality and microbial viability in probiotic-supplemented diets [14].

A commercial catfish feed (Hi-Pro-Vite 781-1, PT Central Proteina Prima, Indonesia) containing 31% crude protein according to the manufacturer's specification was used as the basal diet. Detailed proximate composition data (crude lipid, crude fiber, ash, moisture, and gross energy) were not provided by the manufacturer and were not independently analyzed in the present study. Therefore, only the crude protein content could be reported. All treatments received the same commercial basal diet, and the only difference among treatments was the level of kombucha supplementation (0, 4, 8, and 16 mL/kg feed). Because the quantity of kombucha and binder represented a small proportion of total feed mass, the nutritional composition of the basal diet was assumed to remain substantially comparable among treatments.

2.5 Enumeration of kombucha bacteria

The bacterial density of kombucha preparations was determined using the TPC method. Samples were serially diluted and inoculated on Tryptic Soy Agar (TSA) media using a spread plate technique. Plates were incubated at 28–30 ℃ for 24–48 h, and colonies ranging from 25–250 were counted. Total bacterial density was expressed as colony-forming units per milliliter (CFU/mL).

2.6 Growth performance and survival measurements

Fish sampling was conducted on days 0, 7, 14, 21, and 28. Body weight and total length measurements were performed to evaluate growth performance. Parameters observed included GR, SGR, FCR, absolute weight gain (AWG), absolute length gain (ALG), and survival rate (SR).

GR was calculated as:

$$GR = \frac{W_t - W_0}{t} \quad (1)$$

where, W_t is the final weight (g), W_0 is the initial weight (g), and t is the experimental duration (days).

AWG was calculated as:

$$AWG = W_t - W_0 \quad (2)$$

where, W_t is the final body weight (g), and W_0 is the initial body weight (g).

ALG was calculated as:

$$ALG = L_t - L_0 \quad (3)$$

where, L_t is final total length (cm), and L_0 is initial total length (cm).

FCR was calculated as:

$$FCR = \frac{F}{W_t - W_0} \quad (4)$$

where, F is the feed intake during the culture period. FCR was calculated using the total amount of feed offered during the experimental period. Feed was consumed rapidly after feeding, and no substantial feed residues were observed. Uneaten feed was therefore not quantified.

SR was determined as:

$$SR = \frac{N_t}{N_0} \times 100 \quad (5)$$

where, N_t and N_0 represent final and initial fish numbers, respectively.

AWG and ALG were calculated as the differences between final and initial values.

2.7 Water quality monitoring

Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were measured daily, while ammonia concentration was monitored every four days before siphoning activities. Partial water replacement was conducted every four days by removing approximately one-fourth of the water volume and replacing it with conditioned freshwater.

2.8 Statistical analysis

Data are presented as mean ± standard deviation (SD). Before analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Data that satisfied the assumptions of normality and homogeneity ($p > 0.05$) were subsequently analyzed using one-way analysis of variance (ANOVA). When significant treatment effects were detected ($p < 0.05$), means were compared using the Least Significant Difference (LSD) multiple comparison test. All statistical analyses were

performed using IBM SPSS Statistics version 25.0.

2.9 Animal welfare statement

Fish handling and husbandry procedures were conducted in accordance with standard aquaculture practices to minimize stress and injury throughout the experimental period. During sampling, fish were handled carefully, and measurements were completed as quickly as possible before the fish were returned to their respective culture containers. No invasive procedures were performed during the study, and no anesthetic agents were used. Mortality was monitored daily, and all efforts were made to maintain suitable environmental conditions and fish welfare throughout the experiment.

3. RESULTS AND DISCUSSION

3.1 Effects of kombucha supplementation on growth performance, feed utilization, and survival of fish

The effects of dietary kombucha supplementation on growth performance, feed utilization, and survival of fish are presented in Table 1. Statistical analysis demonstrated that supplementation significantly affected GR, SGR, FCR, AWG, and ALG ($p < 0.05$). However, no significant effect was observed on SR ($p > 0.05$). A dose-dependent response pattern was observed, where increasing kombucha supplementation levels progressively improved growth performance and feed utilization.

Table 1. Effects of kombucha supplementation on growth performance, feed utilization, and survival of fish

Parameter	Control	A (4 mL/kg)	B (8 mL/kg)	C (16 mL/kg)	*p*-Value
Growth Rate (GR, g day^{-1})	0.223 ± 0.007^{a}	0.231 ± 0.003^{a}	0.296 ± 0.013^{b}	0.412 ± 0.022^{c}	0.003
Specific Growth Rate (SGR, % day^{-1})	2.052 ± 0.068^{a}	2.116 ± 0.012^{a}	2.551 ± 0.091^{b}	3.141 ± 0.133^{c}	0.002
Feed Conversion Ratio (FCR)	2.466 ± 0.347^{a}	2.401 ± 0.182^{a}	1.954 ± 0.224^{b}	1.447 ± 0.175^{c}	0.001
Absolute Weight Gain (AWG, g)	6.234 ± 0.197^{a}	6.467 ± 0.093^{a}	8.300 ± 0.367^{b}	11.530 ± 0.609^{c}	0.004
Absolute Length Gain (ALG, cm)	2.70 ± 0.10^{a}	2.80 ± 0.10^{a}	3.28 ± 0.153^{b}	3.93 ± 0.252^{c}	0.006
Survival Rate (SR, %)	94.44 ± 4.88	94.44 ± 4.88	94.44 ± 4.88	97.22 ± 4.88	

Note: Values are presented as mean ± standard deviation (SD) (n = 3). Different superscript letters within the same row indicate significant differences among treatments according to the Least Significant Difference (LSD) test ($p < 0.05$). The *p*-values represent results from one-way analysis of variance (ANOVA).

Prior to statistical analysis, normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. The results of these assumption tests are presented in Appendix A (Table A1). All growth and feed utilization variables satisfied the assumptions for parametric analysis or exhibited only minor deviations considered acceptable for a balanced experimental design. Accordingly, treatment effects on growth performance and feed utilization were analyzed using one-way ANOVA followed by Fisher's LSD test at a significance level of $p < 0.05$. Survival rate was interpreted descriptively because values showed minimal variation among treatments.

GR increased significantly with increasing kombucha dosage. Fish receiving treatment C (16 mL/kg feed) exhibited the highest GR value (0.412 ± 0.022 g day^{-1}), significantly different from treatment B (0.296 ± 0.013 g day^{-1}), treatment A (0.231 ± 0.003 g day^{-1}), and the control treatment (0.223 ± 0.007 g day^{-1}). Compared with the control treatment, GR in treatment C increased by approximately 84.8%. Treatments A and the control did not differ significantly, while treatment B produced intermediate values.

A similar trend was observed for SGR values. Fish fed treatment C exhibited the highest SGR (3.141 ± 0.133% day^{-1}), followed by treatment B (2.551 ± 0.091% day^{-1}). The control and treatment A showed significantly lower values and were statistically similar. Compared with the control group, treatment C increased SGR by approximately 53.1%, indicating enhanced growth efficiency under higher supplementation levels.

Feed utilization efficiency improved as the kombucha dosage increased. Treatment C produced the lowest FCR value (1.447 ± 0.175), significantly lower than treatment B (1.954 ± 0.224), treatment A (2.401 ± 0.182), and the control (2.466 ± 0.347). Compared with the control treatment, FCR in treatment C decreased by approximately 41.3%, indicating a substantial improvement in feed efficiency. Statistical grouping showed that treatment A and the control remained similar, while treatments B and C displayed progressively improved responses.

Fish receiving treatment C achieved the highest AWG (11.530 ± 0.609 g), significantly greater than treatment B (8.300 ± 0.367 g), treatment A (6.467 ± 0.093 g), and the control treatment (6.234 ± 0.197 g). Relative to the control, AWG increased by approximately 84.9%. ALG also increased significantly and reached the highest value in treatment C (3.93 ± 0.252 cm), representing an increase of approximately 45.6% compared with the control treatment. Treatment B produced intermediate values, whereas treatment A and the control treatment remained statistically similar.

SR remained unaffected by dietary supplementation and ranged between 94.44 ± 4.88% and 97.22 ± 4.88%. Statistical analysis revealed no significant differences among treatments ($p > 0.05$). Survival values remained consistently high (>94%) throughout the experimental period, indicating favorable rearing conditions and suggesting that kombucha supplementation did not adversely affect fish viability.

3.2 Water quality parameters

Water quality parameters recorded during the experimental period are presented in Table 2. Mean values of temperature, pH, DO, and ammonia were generally comparable among treatments, indicating that dietary kombucha supplementation did not substantially alter water quality conditions.

Water quality parameters recorded during the 28-day feeding trial are presented in Table 2. One-way ANOVA revealed no significant differences among treatments in temperature ($p = 0.670$), pH ($p = 0.180$), DO ($p = 0.630$), or ammonia concentration ($p = 0.570$). These findings indicate that environmental conditions remained comparable among treatments throughout the experimental period. Temperature ranged from 28.1 to 30.2 °C, DO remained above 6 mg L^{-1}, and ammonia concentrations remained below 1 mg L^{-1}, which are within acceptable ranges for *Clarias* sp. culture.

Table 2. Water quality parameters during the experimental period

Parameter	Control	A (4 mL/kg)	B (8 mL/kg)	C (16 mL/kg)	*p*-Value
Temperature, morning (°C)	28.4 ± 0.97	28.3 ± 1.06	28.1 ± 0.99	28.1 ± 2.99	0.670
Temperature, afternoon (°C)	30.2 ± 1.44	30.0 ± 1.43	29.8 ± 1.47	30.1 ± 1.40	0.670
pH, morning	8.8 ± 0.40	8.6 ± 0.49	8.5 ± 0.48	8.2 ± 0.44	0.180
pH, afternoon	8.5 ± 0.49	8.4 ± 0.53	8.4 ± 0.48	8.1 ± 0.42	0.180
Dissolved oxygen (DO), morning (mg L^{-1})	7.2 ± 0.59	7.4 ± 0.56	7.3 ± 0.45	7.3 ± 0.58	0.630
Dissolved oxygen (DO), afternoon (mg L^{-1})	6.2 ± 0.57	6.2 ± 0.52	6.2 ± 0.52	6.1 ± 0.63	0.630
Ammonia (mg L^{-1})	0.42 ± 0.33	0.44 ± 0.30	0.44 ± 0.29	0.42 ± 0.27	0.570

Note: Values are expressed as mean ± standard deviation (SD). *p*-values were obtained using one-way analysis of variance (ANOVA) based on aquarium means (n = 3 per treatment). No significant differences among treatments were detected ($p > 0.05$).

Dietary kombucha supplementation increased GR and SGR in *Clarias* sp. The highest values occurred in fish receiving 16 mL/kg supplementation. The response suggests improved nutrient utilization during the culture period. Probiotic microorganisms in kombucha can modify intestinal microbial composition and support a more stable microbial environment in the digestive tract [15]. A balanced intestinal microbiota can support digestive processes and reduce microbial competition for nutrients in the gut lumen [16].

The fermented kombucha used in the present study was characterized by the presence of acetic acid bacteria presumptively identified as members of the genus *Komagataeibacter*, which are known to contribute to organic acid production during kombucha fermentation. However, microbial community composition, yeast abundance, and fermentation metabolites were not quantified; therefore, the underlying mechanisms responsible for the observed growth responses require further investigation. Previous studies have reported that probiotic microorganisms can produce metabolites and extracellular compounds that contribute to digestive processes [9, 17]. Probiotic supplementation has also been associated with increased digestive enzyme activity and improved feed degradation efficiency in fish, resulting in greater nutrient availability for growth and tissue formation [18, 19]. Although these mechanisms have contributed to the improved growth observed in the present study, digestive enzyme activity was not measured; therefore, such interpretations should be considered speculative and require further validation.

Growth responses can also relate to intestinal absorption efficiency. Probiotic supplementation has been reported to improve intestinal structure and nutrient absorption in some fish species [20]. Greater villi development and improved gut morphology can increase nutrient uptake efficiency and support biomass accumulation [21]. Efficient nutrient absorption can contribute to higher GR and SGR values during the rearing period. Intestinal morphology was not evaluated in the present study.

The gradual increase in GR and SGR across treatments indicates a dose-dependent response under the tested supplementation range. Treatment C produced the highest growth values without a reduction in performance. The tested supplementation levels remained within a range that supported fish growth and physiological function during the experimental period.

Dietary kombucha supplementation reduced FCR in *Clarias* sp. Fish receiving 16 mL/kg supplementation produced the lowest FCR value. Lower FCR values indicate more efficient feed utilization and improved conversion of nutrients into body mass. The response suggests that kombucha supplementation affected digestive performance and nutrient use efficiency during the culture period.

Probiotic microorganisms in kombucha can improve digestion efficiency through microbial activity in the gastrointestinal tract. Beneficial microorganisms can produce digestive enzymes and support enzymatic processes involved in nutrient breakdown [22, 23]. Improved digestion can increase nutrient availability and reduce nutrient loss during feed utilization [24]. Higher digestive efficiency can improve feed conversion performance in cultured fish.

Kombucha fermentation produces organic acids such as acetic acid and other metabolites. Organic acids can reduce gastrointestinal pH and create favourable conditions for beneficial microbial populations in the digestive system [25]. Lower intestinal pH can limit the growth of undesirable microorganisms and support digestive activity in fish.

Beneficial microorganisms can also suppress pathogenic bacteria through microbial competition and production of antimicrobial compounds [26, 27]. Reduced abundance of harmful microorganisms can improve intestinal health and decrease metabolic costs associated with physiological stress responses [28]. Stable intestinal conditions can support more effective nutrient utilization and feed conversion.

Nutrient assimilation efficiency can influence FCR values during fish culture. Probiotic supplementation can increase nutrient uptake and retention in the digestive tract [29]. Greater nutrient assimilation can increase biomass production from a given amount of feed intake [30]. The progressive reduction in FCR across treatments indicates that higher supplementation levels supported feed utilization within the tested dosage range.

The reduction in FCR observed in kombucha-supplemented treatments has implications beyond growth performance. Lower FCR values indicate that less feed is required to produce a unit of fish biomass, potentially reducing feed resource consumption, production costs, and nutrient waste release into aquatic environments. Improved feed efficiency, therefore, contributes to more sustainable aquaculture practices.

AWG and ALG increased with dietary kombucha supplementation in *Clarias* sp. Fish receiving 16 mL/kg supplementation produced the highest values for both parameters. Absolute growth reflects cumulative nutrient utilization and tissue deposition during the culture period. Growth in body weight and length depends on the availability of nutrients for protein synthesis, cell proliferation, and somatic development [31].

Improved absolute growth can relate to enhanced digestive and metabolic performance in fish receiving probiotic supplementation. Efficient digestion can increase nutrient availability for growth processes and reduce nutrient loss through waste products [32]. Greater nutrient availability can support protein accretion and tissue formation during fish development. Increased nutrient retention can contribute to

body mass accumulation and structural growth in cultured fish.

Kombucha contains microbial populations and fermentation metabolites that support physiological processes related to growth. Metabolites produced by *Komagataeibacter*, lactic acid bacteria, and yeast can influence intestinal conditions and nutrient processing in the digestive tract. Improved nutrient utilization can support continuous body development during the culture period. Body length and body weight often increase simultaneously when nutrient allocation supports normal growth patterns.

SR did not differ significantly among treatments. Survival values remained above 94% throughout the experimental period. High survival values indicate that rearing conditions remained suitable for *Clarias* sp. culture. Water quality conditions and husbandry practices can influence fish survival during cultivation. Stable environmental conditions can reduce physiological stress and support fish health during rearing.

Kombucha supplementation did not produce negative effects on fish viability during the study period. Probiotic supplementation supports physiological balance and maintains normal biological functions in fish. The absence of mortality differences among treatments suggests that supplementation levels remained within a tolerable range for *Clarias* sp. culture.

The growth response across treatments showed a gradual increase with increasing supplementation levels. Treatment C produced the highest growth performance and feed utilization values among the tested groups. The response pattern indicates a positive dose-dependent effect within the evaluated supplementation range. Higher supplementation levels did not reduce performance under the present experimental conditions. The tested dosage range supported growth and physiological function during the culture period.

Water quality parameters remained within acceptable ranges for *Clarias* sp. culture throughout the experimental period. Stable environmental conditions can support physiological processes, feeding activity, and normal fish metabolism. Suitable water conditions can reduce environmental stress and maintain favourable culture conditions during rearing.

Temperature, pH, DO, and ammonia concentrations can influence growth and feed utilization in fish culture systems. Temperature affects metabolic activity and nutrient utilization efficiency in fish. DO supports respiration and energy production required for growth processes. pH can influence physiological regulation and microbial conditions in aquatic environments. Ammonia accumulation can impair physiological performance and reduce fish growth when concentrations exceed tolerance limits.

The absence of significant mortality and the consistently high survival values suggest that environmental conditions remained suitable throughout the culture period. Water quality conditions likely minimized external stress factors during fish maintenance. Growth responses observed in the present study, therefore, relate primarily to dietary supplementation rather than environmental variation among treatments.

Growth and feed utilization responses showed a gradual increase across supplementation levels. Fish receiving 16 mL/kg kombucha supplementation produced the highest GR, SGR, absolute growth, and feed utilization values. The response pattern indicates a dose-dependent relationship within the tested supplementation range. Increasing supplementation levels has increased the availability of beneficial microorganisms and fermentation metabolites in the diet.

Dose-dependent responses can occur when microbial supplementation changes digestive and physiological activity in the gastrointestinal tract. Greater microbial abundance can influence nutrient processing, microbial balance, and digestive function in fish. Increased concentrations of probiotic microorganisms can support microbial colonization and prolong beneficial activity in the intestine.

Lower supplementation levels in treatments A and B produced intermediate responses. The amount of microbial supplementation did not reach a level sufficient to maximize physiological responses related to digestion and nutrient utilization. Biological responses to probiotic supplementation often depend on concentration, exposure duration, and host condition.

The present study focused primarily on growth performance and feed utilization responses. Measurements of digestive enzyme activity, intestinal microbiota composition, gut histomorphology, and immune indicators were not conducted. Consequently, the mechanisms underlying the observed growth enhancement remain speculative and require validation in future studies.

No reduction in growth performance was observed at the highest tested dosage. The absence of negative responses suggests that 16 mL/kg remained within a tolerable range for *Clarias* sp. culture under the present conditions. The evaluated supplementation range supported fish growth and feed utilization throughout the culture period. The present findings indicate an effective response range rather than an optimal dose because supplementation levels above 16 mL/kg were not evaluated.

4. CONCLUSION

Dietary kombucha supplementation significantly affected the growth performance and feed utilization of *Clarias* sp. Kombucha supplementation increased GR, SGR, AWG, and ALG while reducing FCR. Fish receiving 16 mL/kg supplementation produced the highest performance among the evaluated treatments, with GR of 0.412 ± 0.022 g day^{-1}, SGR of 3.141 ± 0.133 % day^{-1}, FCR of 1.447 ± 0.175, AWG of 11.530 ± 0.609 g, and ALG of 3.93 ± 0.252 cm. SR remained above 94% and did not differ significantly among treatments. The findings indicate that dietary kombucha supplementation improved the growth and feed utilization efficiency of *Clarias* sp. under the present experimental conditions. The 16 mL/kg treatment generated the best response among the supplementation levels evaluated. Further studies using higher supplementation levels and longer culture periods are required to determine the optimal dosage and long-term effects. A limitation of the present study is that the complete proximate and energy composition of the commercial feed was unavailable because only the crude protein content (31%) was provided by the manufacturer. Consequently, potential interactions between dietary nutrient composition and kombucha supplementation could not be evaluated. Future studies should employ fully characterized or formulated experimental diets to allow more precise assessment of nutritional and probiotic effects.

ACKNOWLEDGMENT

The authors acknowledge the support provided by the Off-Campus Study Program of Universitas Brawijaya in Kediri for laboratory facilities and institutional support during this study. This research did not receive external funding.

AUTHOR CONTRIBUTIONS

Conceptualization, R. Adharyan Islamy and Veryl Hasan; Methodology, R. Adharyan Islamy, Ahmad Bintang Berlian, Vania Aurellia Azarine, and Muhammad Fakhri; Validation, R. Adharyan Islamy, Taufik Budhi Pramono, and Veryl Hasan; Formal Analysis, Ahmad Bintang Berlian, Vania Aurellia Azarine, Muhammad Fakhri, and Muhamad Dwi Cahya; Investigation, Ahmad Bintang Berlian, Vania Aurellia Azarine, Muhammad Fakhri, Andi Masriah, and Muhamad Dwi Cahya; Resources, R. Adharyan Islamy, Taufik Budhi Pramono, and Veryl Hasan; Data Curation, Ahmad Bintang Berlian, Vania Aurellia Azarine, Muhammad Fakhri, and Muhamad Dwi Cahya; Writing – Original Draft Preparation, R. Adharyan Islamy, Ahmad Bintang Berlian, and Vania Aurellia Azarine; Writing – Review & Editing, R. Adharyan Islamy, Taufik Budhi Pramono, Fitri Sil Valen, Norshida Ismail, Ahmad Syazni Kamarudin, and Veryl Hasan; Visualization, Ahmad Bintang Berlian, Vania Aurellia Azarine, and Muhamad Dwi Cahya; Supervision, R. Adharyan Islamy, Taufik Budhi Pramono, and Veryl Hasan; Project Administration, R. Adharyan Islamy; Funding Acquisition, R. Adharyan Islamy and Veryl Hasan. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1] Srikulnath, K., Panthum, T., Singchat, W., et al. (2025). Addressing catfish (*Clarias* spp.) supply gap in Nigeria: A perspective on strategies for sustainable aquaculture growth. Sustainability, 17(21): 9645. https://doi.org/10.3390/su17219645

[2] Majhi, S.S., Singh, S.K., Biswas, P., et al. (2023). Effect of stocking density on growth, water quality changes and cost efficiency of butter catfish (Ompok bimaculatus) during Seed Rearing in a biofloc System. Fishes, 8(2): 61. https://doi.org/10.3390/fishes8020061

[3] De Verdal, H., Komen, H., Quillet, E., et al. (2017). Improving feed efficiency in fish using selective breeding: A review. Reviews in Aquaculture, 10(4): 833-851. https://doi.org/10.1111/raq.12202

[4] Serdiati, N., Safir, M., Rezkiyah, U., Islamy, R.A. (2023). Response of growth, albumin, and blood glucose of snakehead (Channa Striata) juvenile feed with the addition of different animal protein sources. Jurnal Penelitian Pendidikan IPA, 9(6): 4685-4692. https://doi.org/10.29303/jppipa.v9i6.3618

[5] Islamy, R.A., Aisyah, D., Ramadhani, A.W., et al. (2026). Biochemical transformation, functional properties, and digestibility of alligator weed fermented with Rhizopus oligosporus for Nile tilapia. Engineering Technology & Applied Science Research, 16(2): 33420-33425. https://doi.org/10.48084/etasr.17182

[6] Madhulika, Ngasotter, S., Meitei, M.M., et al. (2025). Multifaceted role of probiotics in enhancing health and growth of aquatic animals: Mechanisms, benefits, and applications in sustainable aquaculture—A review and bibliometric analysis. Aquaculture Nutrition, 2025(1): 5746972. https://doi.org/10.1155/anu/5746972

[7] Latif, A., Shehzad, A., Niazi, S., et al. (2023). Probiotics: Mechanism of action, health benefits and their application in food industries. Frontiers in Microbiology, 14: 1216674. https://doi.org/10.3389/fmicb.2023.1216674

[8] Elnakeeb, M.A., Ashry, A.M., El-Zayat, A.M., et al. (2025). Impact of effective probiotic microorganisms (EPMs) on growth performance, hematobiochemical panel, immuno-antioxidant status, and gut cultivable microbiota in striped catfish (Pangasianodon hypophthalmus). Fishes, 10(11): 573. https://doi.org/10.3390/fishes10110573

[9] Içen, H., Corbo, M.R., Sinigaglia, M., Korkmaz, B.I.O., Bevilacqua, A. (2023). Microbiology and antimicrobial effects of kombucha, a short overview. Food Bioscience, 56: 103270. https://doi.org/10.1016/j.fbio.2023.103270

[10] Ohwofasa, A., Dhami, M., Winefield, C., On, S.L.W. (2024). Elevated abundance of *Komagataeibacter* results in a lower pH in kombucha production; insights from microbiomic and chemical analyses. Current Research in Food Science, 8: 100694. https://doi.org/10.1016/j.crfs.2024.100694

[11] Wang, Q., Qi, Z., Fu, W., et al. (2024). Research and prospects of enzymatic hydrolysis and microbial fermentation technologies in protein raw materials for aquatic feed. Fermentation, 10(12): 648. https://doi.org/10.3390/fermentation10120648

[12] Chomová, N., Pavloková, S., Sondorová, M., et al. (2023). Development and evaluation of a fish feed mixture containing the probiotic Lactiplantibacillus plantarum prepared using an innovative pellet coating method. Frontiers in Veterinary Science, 10: 1196884. https://doi.org/10.3389/fvets.2023.1196884

[13] Wang, A., Lin, J., Zhong, Q. (2021). Spray-coating as a novel strategy to supplement broiler feed pellets with probiotic Lactobacillus salivarius NRRL B-30514. LWT, 137: 110419. https://doi.org/10.1016/j.lwt.2020.110419

[14] Liaqat, R., Fatima, S., Komal, W., et al. (2024). Effects of bacillus subtilis as a single strain probiotic on growth, disease resistance and immune response of striped catfish (Pangasius hypophthalmus). Plos One, 19(1): e0294949. https://doi.org/10.1371/journal.pone.0294949

[15] Ecklu-Mensah, G., Miller, R., Maseng, M.G., et al. (2024). Modulating the human gut microbiome and health markers through kombucha consumption: A controlled clinical study. Scientific Reports, 14(1): 31647. https://doi.org/10.1038/s41598-024-80281-w

[16] Horrocks, V., King, O.G., Yip, A.Y., Marques, I.M., McDonald, J.A. (2023). Role of the gut microbiota in nutrient competition and protection against intestinal pathogen colonization. Microbiology, 169(8): 001377. https://doi.org/10.1099/mic.0.001377

[17] Tandhanskul, A., Krungkaew, S., Li, L., Kham, S.A., Yonekura, L. (2025). Kombucha as a sustainable source of metabiotics: Potential, applications, and future perspectives. Beverages, 11(6): 173. https://doi.org/10.3390/beverages11060173

[18] Amenyogbe, E., Droepenu, E.K., Ayisi, C.L., et al. (2024). Impact of probiotics, prebiotics, and synbiotics on digestive enzymes, oxidative stress, and antioxidant

defense in fish farming: Current insights and future perspectives. Frontiers in Marine Science, 11: 1368436. https://doi.org/10.3389/fmars.2024.1368436
[19] Blum, J., Epstein, R., Watts, S., Thalacker-Mercer, A. (2021). Importance of nutrient availability and metabolism for skeletal muscle regeneration. Frontiers in Physiology, 12: 696018. https://doi.org/10.3389/fphys.2021.696018
[20] Hemarajata, P., Versalovic, J. (2012). Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic Advances in Gastroenterology, 6(1): 39-51. https://doi.org/10.1177/1756283x12459294
[21] Wang, J., Wu, Y., Zhou, T., Feng, Y., Li, L.A. (2025). Common factors and nutrients affecting intestinal villus height-A review. Animal Bioscience, 38(8): 1557-1569. https://doi.org/10.5713/ab.25.0002
[22] Jiang, Z., Mei, L., Li, Y., Guo, Y., Yang, B., Huang, Z., Li, Y. (2024). Enzymatic regulation of the gut microbiota: Mechanisms and implications for host health. Biomolecules, 14(12): 1638. https://doi.org/10.3390/biom14121638
[23] Sharma, R., Garg, P., Kumar, P., Bhatia, S.K., Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. Fermentation, 6(4): 106. https://doi.org/10.3390/fermentation6040106
[24] Khan, G.Q., Miladinovic, D.D., Niu, P., et al. (2025). Improving nutrient utilization in ruminants through extruded pelleted feeds. Animal Feed Science and Technology, 329: 116482. https://doi.org/10.1016/j.anifeedsci.2025.116482
[25] Dittoe, D.K., Ricke, S.C., Kiess, A.S. (2018). Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. Frontiers in Veterinary Science, 5: 216. https://doi.org/10.3389/fvets.2018.00216
[26] Ibrahim, S.A., Ayivi, R.D., Zimmerman, T., et al. (2021). Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods, 10(12): 29-32. https://doi.org/10.3390/foods10123131
[27] Sionek, B., Szydłowska, A., Kołożyn-Krajewska, D. (2024). The role of microorganisms and their antibacterial compounds in food biopreservation. Applied Sciences, 14(13): 5557. https://doi.org/10.3390/app14135557
[28] Ahmed, F., Alhodieb, F.S., Alsanie, S.A., Rasheed, M., Ndagire, C.T. (2025). Relationship between stress, diet, and gut microbiota: A cross-sectional study. Nutrition & Metabolism, 22(1): 122. https://doi.org/10.1186/s12986-025-01014-y
[29] Riaz, D., Hussain, S.M., Sarker, P.K., Ali, S., Naeem, A., Naeem, E., Nazish, N., Al-Anazi, K.M., Farah, M.A. (2024). Use of protexin as a probiotic-supplemented feed additive: Assessment of growth, digestibility, serum antioxidant enzyme activity, and blood profile in Cirrhinus mrigala. Frontiers in Sustainable Food Systems, 8: 1449325. https://doi.org/10.3389/fsufs.2024.1449325
[30] Elvy, J.E., Symonds, J.E., Hilton, Z., Walker, S.P., Tremblay, L.A., Casanovas, P., Herbert, N.A. (2022). The relationship of feed intake, growth, nutrient retention, and oxygen consumption to feed conversion ratio of farmed saltwater Chinook salmon (Oncorhynchus tshawytscha). Aquaculture, 554: 738184. https://doi.org/10.1016/j.aquaculture.2022.738184
[31] Yang, L., Yi, C., Mo, Y., He, Z., Xu, Z., He, Y., Ouyang, Y., Mao, Z., Qu, F., Tang, J., Liu, Z., Fang, Z., Cao, S. (2024). Effects of different protein sources on growth performance, muscle flavor substances and quality structure in triploid crucian carp. Fishes, 9(1): 23. https://doi.org/10.3390/fishes9010023
[32] Hidir, A., Aaqillah-Amr, M.A., Rahim, K.A.A., Azra, M.N., Ma, H., Shapawi, R., Noordin, N.M., Ikhwanuddin, M. (2026). Sustainable feed processing methods to enhance growth and digestibility in freshwater aquaculture: A review. Animal Nutrition, 25: 228-245. https://doi.org/10.1016/j.aninu.2025.07.011

NOMENCLATURE

ANOVA	Analysis of variance
AWG	Absolute weight gain, g
BW	Body weight, g
CFU	Colony-forming units
DO	Dissolved oxygen, mg L^{-1}
FCR	Feed conversion ratio
FW	Final weight, g
GR	Growth rate, g day^{-1}
LSD	Least Significant Difference
MR-VP	Methyl Red-Voges Proskauer test
SCOBY	Symbiotic Culture of Bacteria and Yeast
SD	Standard deviation
SGR	Specific growth rate, % day^{-1}
SIM	Sulfide-Indole-Motility test
SR	Survival rate, %
TPC	Total plate count
TSIA	Triple Sugar Iron Agar

Greek symbols

℃	Degree Celsius
±	Plus–minus variation
%	Percentage

APPENDIX

Table A1. Results of normality and homogeneity tests for response variables prior to one-way analysis of variance (ANOVA)

Response Variable	Shapiro–Wilk Test	Levene's Test (*p*-Value)	Statistical Analysis
Growth Rate (GR)	All treatment groups normally distributed (all $p > 0.05$)	0.087	One-way ANOVA + Fisher's LSD
Specific Growth Rate (SGR)	All treatment groups normally distributed (all $p > 0.05$)	0.041	One-way ANOVA + Fisher's LSD[1]
Feed Conversion Ratio	All treatment groups normally distributed (all	0.628	One-way ANOVA + Fisher's

(FCR)	$p > 0.05$)		LSD
Absolute Weight Gain (AWG)	All treatment groups normally distributed (all $p > 0.05$)	0.079	One-way ANOVA + Fisher's LSD
Absolute Length Gain (ALG)	All treatment groups normally distributed (all $p > 0.05$)	0.368	One-way ANOVA + Fisher's LSD
Survival Rate (SR)	Normality assumption not satisfied ($p < 0.001$)	1.000	Descriptive comparison[2]

Notes: [1] Although Levene's test indicated a slight deviation from homogeneity of variance for SGR ($p = 0.041$), one-way ANOVA was retained because the experimental design was balanced with equal sample sizes (n = 3 per treatment), making the analysis relatively robust to minor violations of homogeneity. [2] Survival rate values remained consistently high (>94%) with minimal variation among treatments; therefore, they were interpreted descriptively. No apparent treatment differences were observed. LSD = Least Significant Difference.