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RESEARCH PAPER

Application of Phytogenic Compounds on Growth Performance and Gut Microbial Community of Giant Gourami (*Osphronemus goramy* Lac. 1801)

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Abstract

The giant gourami (*Osphronemus goramy*) is a freshwater fish with great potential for aquaculture production. This omnivorous fish tends to become herbivorous when it reaches adulthood. In this study, we initially conducted a preliminary study to determine which plants support the growth of giant gourami and choosing taro leaves (*Colocasia esculenta*) as additional phytogenic. The aim of this study was to determine the effect of adding taro leaves on the growth performance and gut microbial community of giant gouramis weighing 213±9.72 g. In this study, five groups of diets were formulated to replace 0%, 25%, 50%, 75% or 100% of commercial feed. The cultivation period lasted 16 weeks. Several parameters were observed, including absolute weight growth, specific growth rate, survival rate, haematology tests and gut bacterial community profiling using nanopore sequencing of the 16S rRNA gene. The results showed that replacing 25% of commercial feed with taro leaves produced the greatest weight gain in the fish (166.87±17.27 g), although there were no significant differences among the treatments. Microbiome analysis showed that the gut microbial community was dominated by the Firmicutes phylum, with a relative abundance of 91.25%. The most abundant genera were *Clostridium*, *Paeniclostridium* and *Cellulosilyticum*.

Introduction

Giant gourami (*Osphronemus goramy*) is one of the freshwater aquaculture commodities with its native habitat in Southeast Asian countries such as Indonesia, Thailand, Myanmar, Malaysia, Philippines, and Singapore. Indonesia is the main producer of giant gourami which production value reaches 98% of total world production (FAO, 2024). This species is widely distributed throughout the main islands of Indonesia, Sumatra, Java, and Borneo (Hardaningsih et al., 2025). The production chain of giant gourami in Indonesia is strongly segmented starting from hatchery, nursery, early and final rearing stages (Arifin et al., 2019; Kristanto et al., 2019), but there are no standardized production methods which can vary from each fish farmers (Kristanto et al., 2019). This segmentation can reasonably be due to the slow growth rate of this fish, which make farmers shorten the rearing period to gain cash rapidly (FAO, 2024). Even though this species has been cultivated for decades, the slow growth rate of this fish still remains the main obstacle to increasing production. In 2020 the production volume of giant gourami in Indonesia only reached 152.669 tons. This

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amount is smaller compared to the production volume of other freshwater commodities in Indonesia such as tilapia (*Oreochromis* sp.) (1.3 million tons) and catfish (*Clarias* sp.) (993.768 tons) in the same year (Ministry of Marine Affairs and Fisheries of Indonesia, 2022).

Giant gourami is an omnivorous fish but tends to be herbivorous when they growing adult. The type of giant gourami feed varies depending on each stage of the production cycle (Kristanto et al., 2019; Amriawati et al., 2020). In the initial phase after hatching, giant gourami larvae will consume their egg yolk, then they will be fed with Artemia nauplii followed by *Tubifex* worm. After entering juvenile stages, giant gourami juveniles started to be fed using commercial powder feed when their digestive system almost resembled that of an adult giant gourami (Kristanto et al., 2019; Amriawati et al., 2020). At the rearing stage, this fish is generally fed a combination of commercial and phytogenic-based feed. Phytogenics are feed additives derived from plant parts. They can be used in dry, solid or powder form, or as essential oil extracts (Onomu & Okute, 2024). Fish farmers tend use more raw-plants when the fish has nearly reached commercial size of 500 to 750 g (FAO, 2024).

Several plants can be used as alternative resource for giant gourami feed such as water spinach cassava leaves, taro leaves, papaya leaves and river tamarind leaves (Sefni et al., 2019; Malau et al., 2021; Silaban et al., 2021; Ananda et al., 2022; Usman et al., 2023). Many studies have reported the effectiveness of a feed combination between commercial feed and raw plants to provide nutrients during rearing stage. However, most studies relatively use small fish size (0.01 to 20 g) (Sefni et al., 2019; Afriyanti et al., 2020; Malau et al., 2021; Silaban et al., 2021), which are still in the nursery and early rearing stage.

Although the plant-based feed is more often given has nearly reached its commercial size of 500–800 g, detailed information regarding the effect of adding plant-based feed to the diet of adult giant gourami on their growth performance is still limited. This condition may be related to anatomical development of giant gourami as they become more herbivorous in adulthood. Young fish tend to have difficulty digesting plants due to the limited ability to process plants mechanically in the pharyngeal digestive tract (Day et al., 2010), and their enzyme activity is relatively lower than that of adult fish (Kolkovski, 2001). Additionally, the addition of raw plant matter can affect the microbial community in the digestive tract, which plays a role in nutrient absorption and improving the immune system (Talwar et al., 2018; Jiao et al. 2023). Therefore, the objective of the present study is focused to investigate the effect of taro leaves as plant-based additives on the growth performance of medium-sized giant gourami (200 to 250 g) in the final rearing stage. We also used Nanopore Sequencing (Oxford Nanopore Technology) on the 16S rRNA gene to determine gut microbial community of giant gourami.

Materials and Methods

Preliminary Study

We conducted preliminary research to determine the raw-plant which used as main plant-based feed mixture for giant gourami. A total of 90 of medium-sized fishes with initial weight 254.17±14.86 g were obtained from fish farmer from Bantul Regency, Special Region of Yogyakarta. Various plants used were water spinach, taro leaves, and cassava leaves as the main feed of giant gourami without adding any types of feed. The fish were acclimatized for 1 weeks prior to the start experiment, and then maintained in fish ponds (1m x 0.75m x 1m) located in semi-indoor pond for 10 weeks (70 days) with three replicates. The leaves were separated from the leaf petioles and skeletons prior to being consumed by the fish. The fish were provided with ad-libitum access to food. The parameters that were observed included the absolute body weight gain (AWG), specific growth rate (SGR) and survival rate (SR). The formula used to determine the growth parameters are:

$$\text{Absolute body weight gain (g)} = W_t - W_o$$

$$\text{Specific growth rate (\%/day)} = (\ln W_t - \ln W_o) / t * 100\%$$

$$\text{Survival rate (\%)} = N_t / N_o * 100\%$$

Where, W_t: final fish weight (g), W_o: initial fish weight (g), N_t: number of fishes in the end of cultivation, N_o: number of fishes in the early of cultivation, t: total days of cultivation

Effect The Addition of Taro Leaves as Giant Gourami Feed

According to the preliminary study results, we use taro leaves as plant-based feed additive for giant gourami, then combined with commercial feed with several ratios. Five treatment group consisted to 100% Commercial Feed (P1), 25% Taro Leaves+75% Commercial Feed (P2), 50% Taro leaves+50% Commercial Feed (P3), 75% Taro Leaves+25% Commercial Feed (P4), and 100% Taro Leaves (P5). respectively. The nutritional content of feed formulation was shown in Table 1. Feeding was done based on 3% of fish weight. The fish of 150 medium-sized giant gourami with initial weight 213±9.72 g were obtained from fish farmer in Bantul Regency, Special Region of Yogyakarta. The fishes were acclimatized to experimental condition 1 weeks prior to the start experiment, and then maintained in fish ponds (1m x 1m x 0.8m) for 112 days (16 weeks) with three replicates. Before being fed to the fish, the leaves were separated from leaf petioles and skeletons. The growth parameters we observed were the absolute body weight gain (AWG), specific growth rate (SGR), survival rate (SR), and haematological condition (haematocrit and total plasma protein), and gut bacterial community. Fish weight gain was observed every 2 weeks.

Haematological Test

Haematocrit

The haematocrit value was examined using microhaematocrit method (Anderson & Siwicki, 1995). Fishes were anesthetized using eugenol 200 ppm prior to the collection of blood. Blood samples were taken at the base of the tail using a syringe soaked with EDTA to prevent blood clots, then transferred into microtube. Fish blood sample were absorbed into a capillary tube until it fills approximately three quarters, then the bottom of the capillary tube was blocked by sticking it with wax plug. Then, it was centrifuged at speed of 600 rpm for 5 minutes. The haematocrit value was determined by measuring the ratio of the red blood cell (erythrocyte) volume from blood and stated as percentage (%).

Total Plasma Protein (TPP)

Total plasma protein was measured using Bradford method (Bradford, 1976). The principle of this method is to compared the plasma protein concentration to bovine serum albumin (BSA) standard curve. The protein concentration was calculated by the absorbance value with 600 nm wavelength. 2 µl of serum and 798 µl of distilled water were mixed with 200 µl of Bradford reagent then homogenized. The optical density was examined at 600 nm. To calculate the TPP values we used standard curve on bovine serum albumin at 0, 25, 50, 75, and 100 mg/ml.

DNA Extraction and 16S rRNA Gene Amplification

At the end of the experiment, three fish from P2 (25% Taro Leaves + 75% Commercial Feed) pond were randomly sampled and euthanized (eugenol; 500 ppm) to collect gut content. DNA from gut content samples was extracted with ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300) following the manufacturer's recommendations. DNA concentration and quality were measured using Qubit fluorometer and NanoDrop spectrophotometers respectively. Amplification of genomic DNA samples with 16S rRNA primers 27F and 1492R, and the sequenced using Oxford Nanopore Technology (V1 – V9 region).

Bioinformatics Analysis

Library preparations were conducted using Oxford Nanopore Technology Kit. GridION sequencing was operated by MinKNOW software version 20.06.09. Base calling was performed using Guppy version 4.0.11 with high accuration mode (Wick et al., 2019). The quality of FASTQ files was visualized using NanoPlot (de Coster et al., 2018). Filtered reads were classified using Centrifuge classifier (Kim et al., 2016). Bacteria and Archaea index was downloaded from centrifuge website (https://ccb.jhu.edu/software/centrifuge). Downstream analysis and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian) and Krona Tools (https://github.com/marbl/Krona).

Statistical Analysis

Data were shown as mean±standard error of mean (SEM). Before performing an ANOVA, data previously checked for normality and homoscedasticity. All analyses were performed using SPSS (version 24).

Results

Preliminary Study: Raw Plants Comparison for Giant Gourami Feed

To determine the most optimal raw-plants to support the growth of giant gourami during the rearing stage, we conducted preliminary study regarding the effect of several types of plants that potentially be employed as feed and support their growth. Based on the results shown in Figure 1A, the various types of plant (water spinach, taro leaves, and cassava leaves) did not provide significant results ($P>0,05$) on the absolute weight growth of the giant gourami, with a weight gain were 33.96±3.30, 39.8±4.6, and 32.76±5.64 g, respectively. The specific growth rate of fish on each treatment groups were ranged from 0.19±0.02 to 0.22±0.03 %/day ($P>0.05$) (Figure 1B). The survival rate of all treatments showed results greater than 80% ($P>0.05$) (Figure 1C). Even though, all treatments did not

Table 1. Nutritional content of feed formulation. CF: commercial feed, TL: taro leaves

Nutrients	Fish Feed				
	100 CF	25 TL+75 CF	50 TL+50 CF	75 TL+25 CF	100 TL
Carbohydrate (%)	32.68	25.39	18.10	10.80	3.51
Protein (%)	28.21	22.31	16.42	10.52	4.62
Fat (%)	6.44	4.96	3.49	2.01	0.53
Crude Fibre (%)	14.56	12.85	11.14	9.43	7.72
Ash (%)	9.09	7.39	5.68	3.98	2.27
Water (%)	9.02	27.10	45.18	63.26	81.34
Energy (cal/100g)	304.56	237.94	171.32	104.69	38.07

show the significant results, we saw that taro leaves feed showed the highest absolute weight growth results.

Effect of Taro leaves as Giant Gourami Feed

Fish Growth

In the experiment shown in Figure 2 and Table 2, we measured fish growth based on absolute weight gain for 16 weeks and samples were measured biweekly. The initial weight of the fish among treatments were ranged from 207.02±3.09 to 222.93±9.93 g (P>0.05), and the initial length were 22.82±0.28 to 23.5±0.77 cm (P>0.05). In the first 6 weeks of fish cultivation, P1 and P2 showed the highest fish weight gain among all treatments. P3 and P4 began to show increased weight gain in the weeks 8 and 10, while P5 still the lowest results of all treatments. At the end of cultivation (weeks 12 to 16), fish growth did not show significant results between commercial feed only (P1) and commercial feed-taro leaves additives treatments (P2, P3, P4) (P>0.05), except for P5 which only consists of taro leaves (P<0.05). At the beginning of cultivation, P2 showed a lower result than P1, but overtime P2 continued to increase and showed a higher result than P1 in the weeks 10 until the end of cultivation period. The same condition was showed by P3 but lower than P2. The specific growth rate of P1, P2, P3 and P4 did not shown significantly ranged from 0.46±0.03 to 0.52±0.02%/day (P>0.05). P5 have the lowest specific growth rate among all treatments 0.33±0,03%/day (P<0.05). The results of absolute fish weight gain in all treatments after cultivation were 157.27±10.19 g, 166.87±17.27 g, 161.6±7.85 g, 142.67±2.37 g, and 82.43±3.08 g (P<0.05), respectively. Final average fish mean weight from each group treatments were 366.9±9.15 g, 389.8±23.37 g, 368.8±7.29 g; 353.93±11.77 g, and 299.3±7.25 g

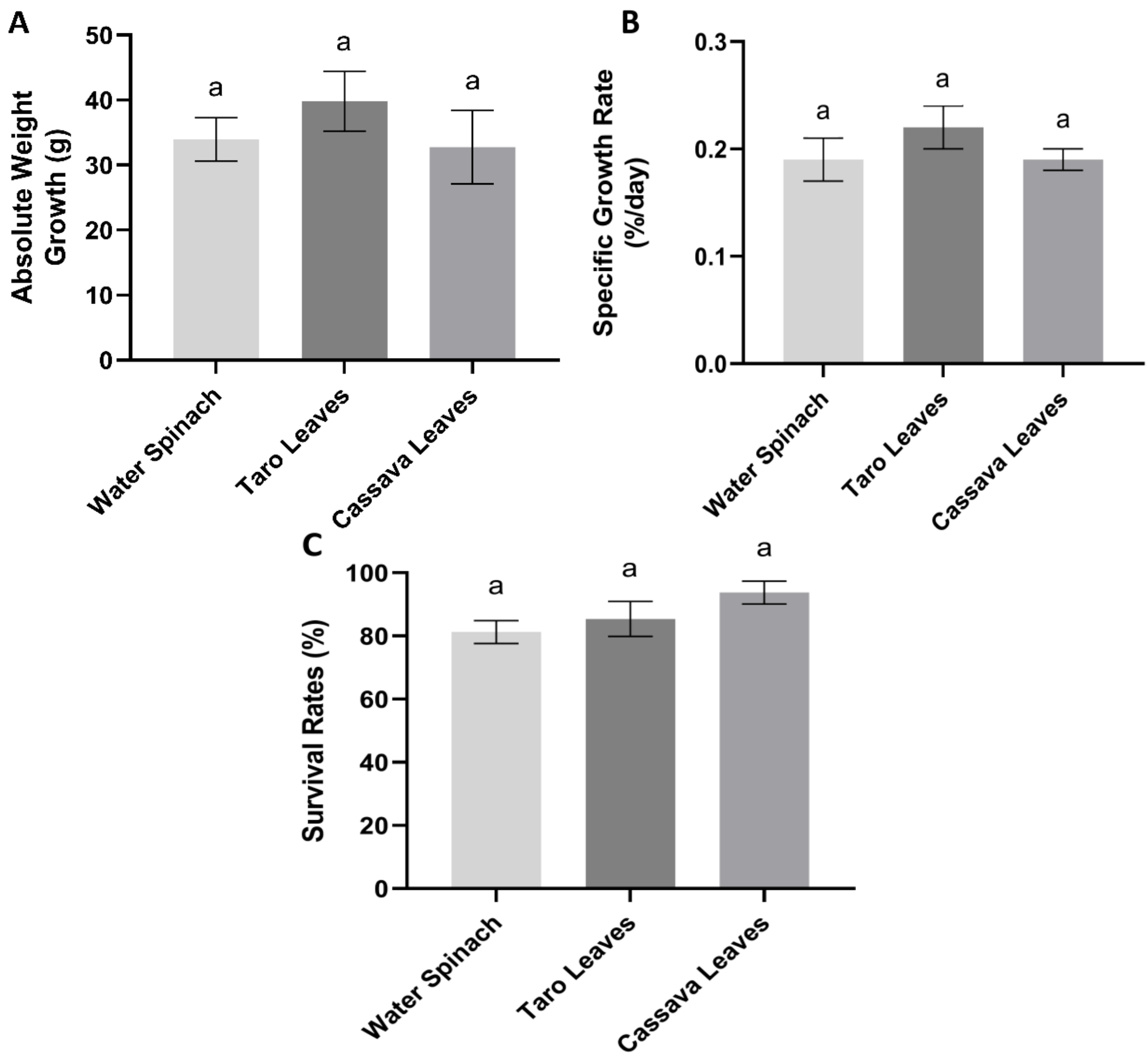

Figure 1. Absolute weight growth (A), specific growth rate (B) and survival rates (C) of giant gourami fed by three types of plants. The error bar indicated the standard error of mean (SEM). The same letters indicate no significant difference among treatments (P>0.05).

(P<0.05), respectively. The mean final length was ranged from 25.72±0.56 to 27.2±0.38 cm (P>0.05) (Table 2). According to linear regression, from other group treatments P2 have faster growth with regression equation y=11.019x+226.47 compared to P3 (y=10.623x +195.06); P4 (y=8.9097x+201.64); P1 (y=8.5772x+224.7 5) and P5 (y=5.3403x+215.4) (Figure 3).

Survival Rate and Haematologic Condition

According to the preliminary study, we decided to use taro leaves as a substitute feed in determining the feed composition ratio. During the cultivation period (16 weeks), all feed variations showed a survival rate of giant gourami reached 96.7±3.33 to 100% (P>0.05) (Table 2). To analyse haematological condition of giant gourami during cultivation, we measured the haematocrit content and total plasma protein content (Table 2). The percentage of haematocrit from giant gourami on all treatments ranged from 32.67±2.03 to 43.33±4.41% (P>0.05) and the concentration of total plasma protein ranged from 43.83±1.92 to 48.27±1.19 mg/ml (P>0.05).

Fish Gut Microbial Analysis

According to our findings, the combination of taro leaves and commercial feed could increase the giant gourami growth during cultivation. Based on this condition, we conducted a microbiome analysis to understand the microorganism community in giant gourami's gut. We analysed gut microbiome from pooled P2 sample which has the highest fish growth among the all treatments. In the experiment shown in

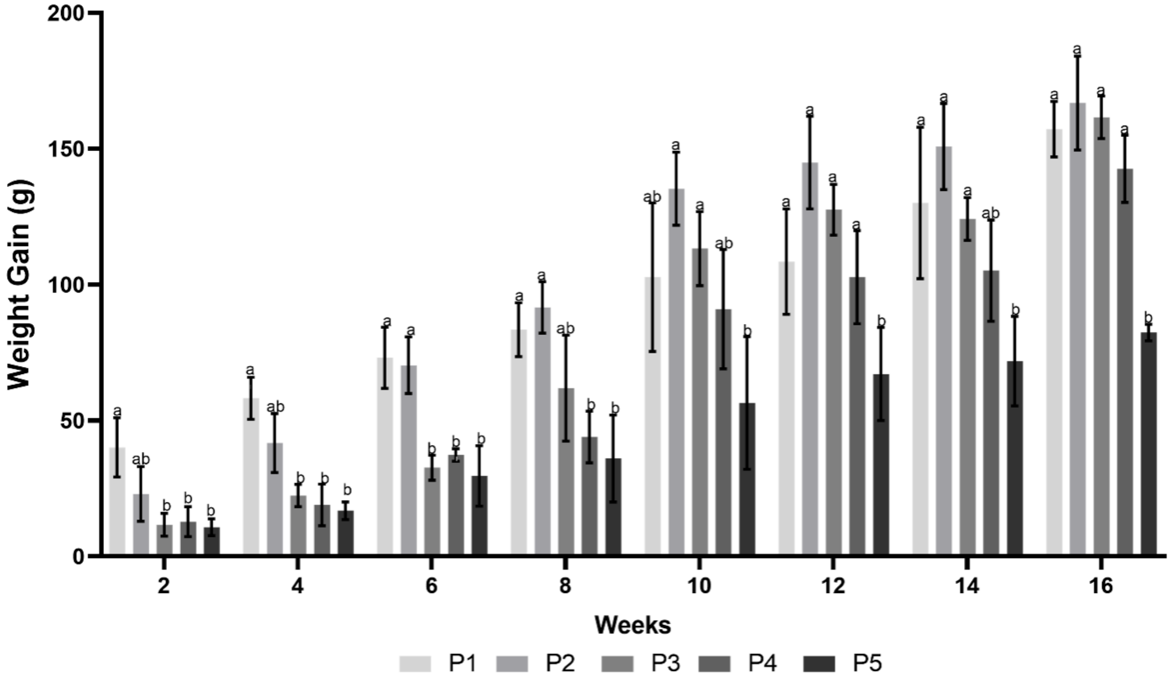

Figure 2. Weight gain of the giant gourami fed with various ratios of commercial feed and taro leaves. The statistical significance of the weight gains between the treatments group was tested by Duncan Test, only P5 that have significant differences (P<0,05). The error bar indicated the standard error of mean (SEM). P1: 100% CF, P2: 25% TL+75% CF, P3: 50% TL+50% CF, P4: 75% TL+25% CF, and P5: 100% TL. CF: Commercial Feed, TL: Taro Leaves. Different letters indicate significant difference (P<0.05).

Table 2. Fish growth, haematological condition, and survival rate parameters observed during cultivation. CF: commercial feed, TL: taro leaves

Growth Parameters	100 CF	25 TL+75 CF	50 TL+50 CF	75 TL+25 CF	100 TL	p-value
Mean initial weight (g)	209.63±3.02[a]	222.93±9.93[a]	207.02±3.09[a]	211.27±2.08[a]	216.87±5.91[a]	P>0.05
Mean final weight (g)	366.90±9.15[a]	389.8±23.37[a]	368.8±7.30[a]	353.93±11.77[a]	299.30±7.25[b]	P<0.05
Mean initial length (cm)	22.82±0,28[a]	23.5±0.77[a]	22.83±0.36[a]	23.42±0.30[a]	22.82±0.32[a]	P>0.05
Mean final length (cm)	26.69±0.41[a]	27.2±0.38[a]	26.81±0.62[a]	26.45±0.29[a]	25.72±0.56[a]	P>0.05
Specific growth rate (%/day)	0.50±0,03[a]	0,50±0,04[a]	0.52±0.02[a]	0.46±0.03[a]	0.33±0,03[b]	P<0.05
Absolute weight gain (g)	157.27±10.19[a]	166.87±17.27[a]	161.6±7.85[a]	142.67±2.37[a]	82.43±3.08[b]	P<0.05
Total plasma protein (mg/ml)	46.40±0.35[a]	44.63±1.64[a]	43.83±1.92[a]	48.27±1.19[a]	47.20±1.42[a]	P>0.05
Haematocrit (%)	42.67±1.86[a]	36.00±2.00[a]	32.67±2.03[a]	43.33±4.41[a]	42.67±2.40[a]	P>0.05
Survival rate (%)	96.67±3.33[a]	96.67±3.33[a]	96.67±3.33[a]	100±0.00[a]	100±0.00[a]	P>0.05

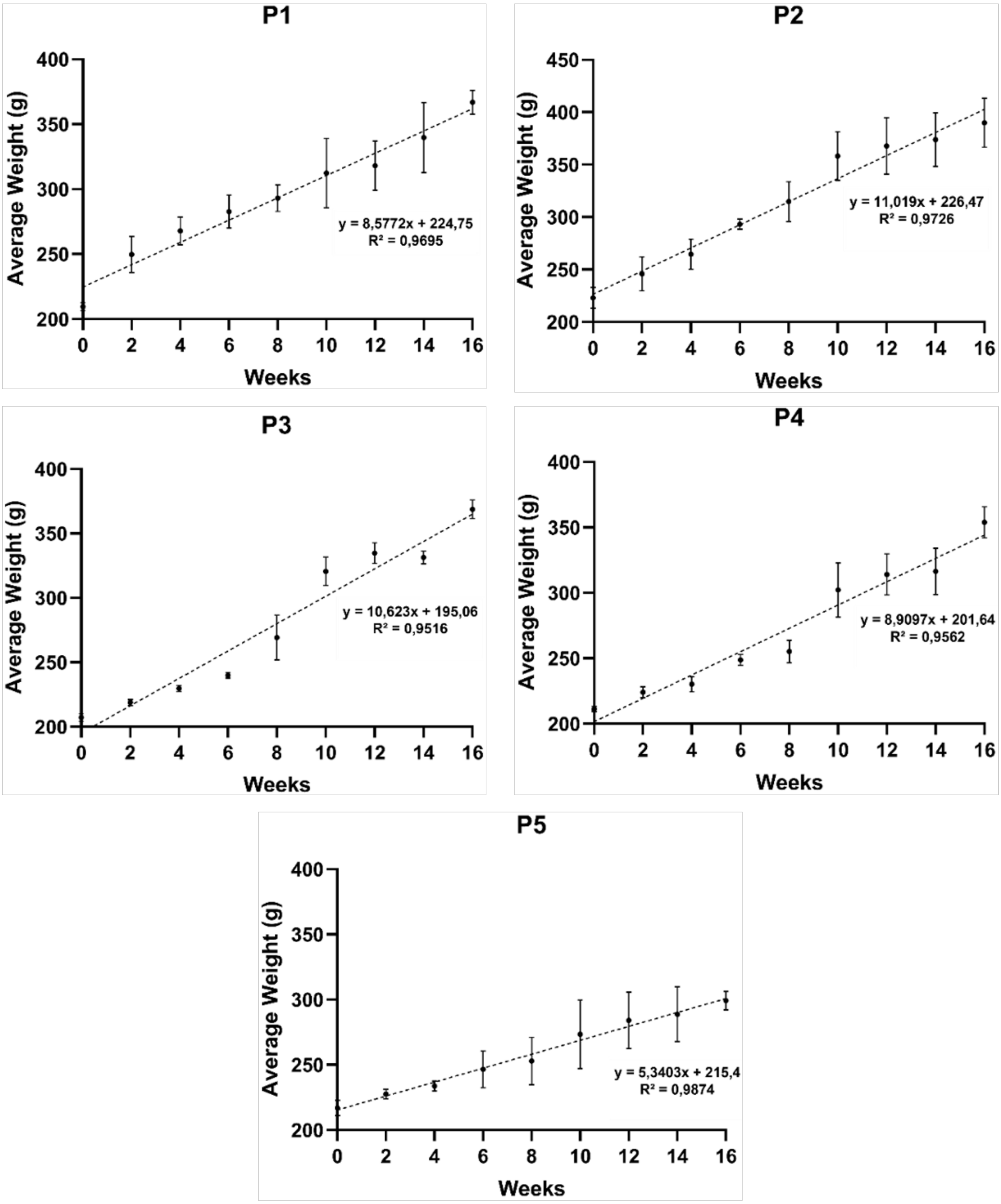

Figure 3. Correlation between cultivation time and average fish weight gain. P1: 100% CF, P2: 25% TL+75% CF, P3: 50% TL+50% CF, P4: 75% TL+25% CF, and P5: 100% TL. CF: Commercial Feed, TL: Taro Leaves.

Figure 4, the most abundant phyla were Firmicutes with 91.25% prevalence, followed by Proteobacteria (4.79%); Fusobacteria (1.13%); Cyanobacteria (1%); and Actinobacteria (1.13%). The most abundance genera were detected on giant gourami's gut consisting *Clostridium* (43.44%), *Paeniclostridium* (20.6%), and *Cellulosilyticum* (12.34%). We also find other bacteria with a lower abundance, namely Clostridioides (3.79%) Turicibacter (1.47%), Staphylococcus (1.32%), Fusobacterium (1.13%), Bacillus (0.99%) Klebsiella (0.99%); Alkaliphilus (0.96%); and Lachnoclostridium (0.76%) (Table 3).

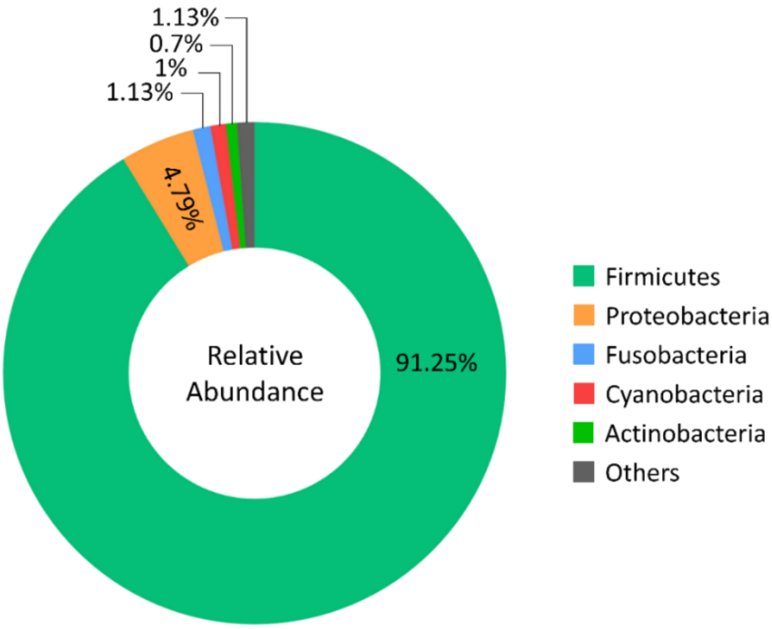

Figure 4. Relative abundance of bacterial phyla of giant gourami's gut feeding with 25% taro leaves+75% commercial feed.

Table 3. The relative abundance of gut microbial community of giant gourami feeding with 25% taro leaves+75% commercial feed

Phylum	Class	Order	Family	Genus	Relative Abundance (%)
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	43.44
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Paeniclostridium	20.6
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Cellulosilyticum	12.34
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Clostridioides	3.79
Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Turicibacter	1.47
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	1.32
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	1.13
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.99
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Klebsiella	0.99
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Alkaliphilus	0.96
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium	0.76

Discussion

Use of Various Raw-Plants on Growth Performance of Medium Sized Giant Gourami

In the early life stages, the giant gourami exhibits omnivorous tendencies, as demonstrated by its propensity to consume a diet comprising a significant proportion of protein, a preference that becomes more pronounced with age. The digestive tract of herbivorous fish has been shown to be more compatible with the digestion of complex compounds, such as dietary fibre and cellulose, which are more abundant in raw plants. As herbivorous fish, the giant gourami possesses notably elongated intestines, a trait that may facilitate the efficient digestion of fibre (Goodrich et al., 2020). In order to ensure the efficiency of food digestion and support growth in the giant gourami, it is necessary to determine the type of feed that suits the fish's anatomical condition, particularly if the fish are herbivorous.

A variety of raw materials may be utilised in the production of a reference source for plant food intended for use with giant gourami. Practically, the determination of the most suitable raw plant materials for use as fish feed is contingent upon the farmer's subjective preferences and accumulated experiential knowledge. In this study, a preliminary investigation was conducted to ascertain the growth performance of giant gourami, utilising three distinct raw plants (water spinach, cassava leaves, and taro leaves) as the primary nutritional source, with no additional feed provided. These plants are readily identified in the wild and can be readily cultivated. The findings of this study indicate that there was no statistically significant difference in growth performance across all treatments (P>0.05). Conversely, this finding suggests that all plants in this study can be regarded as suitable plant food for giant gourami. The fish fed a diet consisting of taro leaves exhibited the greatest increase in weight compared to the other groups. According to Kristanto et al. (2019), taro leaves are the most frequently used plant food for adult gourami. It is estimated that most farmers utilise giant taro leaves and taro leaves as a plant food additive for giant gourami (Kristanto et al., 2019). Taro leaves have been found to contain essential nutrients such as vitamins A, C, B1, B2, and niacin (El Tawil et al., 2020). However, it has been demonstrated that the provision of raw plants alone is inadequate for sustaining the growth of giant gourami. In order to ensure the nutritional adequacy of these fish, a combination with a commercial feed is required.

Effect of Addition of Taro Leaves on Growth Performance and Gut Microbial Community of Medium Sized Giant Gourami

The addition of taro leaves to adult giant gourami has become a common behaviour carried out by many fish farmers in Indonesia (Kristanto et al., 2019). These fish habitually graze by nibbling vegetation in their living environment. To provide balanced nutrition and adaptation of digestive tract, adult giant gourami may need plant-based feed. Furthermore, the selection of an appropriate raw plant is a crucial aspect that requires consideration, especially if it contains toxic compounds that can interfere physiological condition of the fishes. During cultivation, the fishes were observed to be able to consume taro leaves and we did not find any negative effects caused by taro leaves on fish. According to haematological condition results in the end of cultivation period the fishes were in healthy condition. Haematological conditions and blood chemistry can be used to monitor fish welfare, health, immune system response, and nutritional status during cultivation (Esmaeili, 2021). In this study, giant gourami that have been cultivated showed haematocrit value ranged from 33% to 43%, and the total plasma protein ranged from 43.9 to 48.29 mg/ml. Haematocrit value indicates the erythrocyte turnover capacity of fish (Satriyo et al., 2017). The haematocrit value and total plasma protein levels of healthy fish ranged from 20 to 45% (Susandi et al., 2017) and 30 to 50 mg/ml (Tang et al., 2018), respectively. The haematocrit value of healthy giant gourami ranged from 30 to 39% (Wahyono et al., 2023), when the fish were fed plant-based diet.

In this study, the addition of taro leaves to fish diet (combination) namely P2, P3, and P4 did not result in a statistically significant improvement in growth performance when compared to a control group that was fed commercial diet (P1). This finding is quite interesting because even though taro leaves have less nutritional content than commercial feed, but the fish in both groups grew similarly. The lowest growth performance was shown in the group that were only fed taro leaves as main source of nutrients (P5). The growth performance of the taro leaves-addition group began to increase at the weeks 8 to 10, and showed not much different with the group that only fed by commercial feed alone at the end of cultivation. This reinforces the view that the inclusion of raw plant material, such as taro leaves, in the diet of adult giant gourami is essential for ensuring adequate nutritional intake. Furthermore, it has the potential to reduce the proportion of commercial feed used, which is associated with the highest operational costs. As omnivorous fish that tend to be herbivorous, longer intestine present in giant gourami facilitates enhanced exposure to the digestive processes that are necessary for effective digestion of a fibre-rich plant diet (Goodrich et al., 2020). Herbivorous fish tend to require lower protein requirements than carnivorous fish, and they have higher amylase activity which help cellulose digestion (Jauralde et al., 2021; Jiao et al., 2022). Specific growth rate of giant gourami was reported around 0.62 to 0.75%/day (Azrita et al., 2021), while other researcher reported ranged from 0.33 to 0.52%/day (Afriyanti et al., 2020).

At the early of cultivation period (weeks 2 to 6), the growth performance of the taro leaves-addition group still lower than the fishes that only fed by commercial feed alone. Taro leaves contain more complex compound which take longer to completely digest. Taro leaves are a rich source of proteins (4 gr/100 gr) and dietary fibre (3 gr/100 gr) which can act as prebiotic to improve gut health (Mitharwal et al., 2022). This condition is likely strongly related to the role of gut microbiome during the digestive process. The addition of taro leaves acts as a prebiotic which can increase the diversity of gut microbiome and improve the digestive process (Cui et al., 2021). To investigate this phenomenon, we conducted a metagenomics analysis using Oxford Nanopore Technology (ONT) to determine the diversity of microbiome in the digestive tract of giant gourami which fed with addition of taro leaves.

Firmicutes was the most dominant phyla found in the gut of giant gourami with a relative abundance of 91,25% and followed by Proteobacteria (4,79%), and Fusobacteria (1,13%). Firmicutes, Proteobacteria, and Fusobacteria were the most abundance phyla detected in the gut of grass carp *Ctenopharyngodon idella* (Ni et al., 2013). Firmicutes, and Proteobacteria are the most dominant phyla that commonly found in fish gut (Gao et al., 2020; Duan et al., 2021; Kim et al., 2021). Fusobacteria was more abundance in freshwater fish than sea water fish (Kim et al., 2021; Gallet et al., 2022). Gallet et al. (2020) reported that Firmicutes are most abundant phyla in the gut of omnivorous fish *Chrysichthys nigrodigidatus* with a relative abundance of 78%. Firmicutes is known to play an important role in short chain fatty acid (SCFA) synthesis and carbohydrate metabolism. Firmicutes are widely found and more abundant in the digestive tract of herbivorous fish than carnivorous fish (Egerton et al., 2018; Kim et al., 2021). These group bacteria are associated with their role as probiotics, one of which is the genus Clostridium which plays a role in the butyrate acid synthesis (Guo et al., 2020). Proteobacteria commonly have more abundance in animal-based diet fish (Egerton et al., 2018; Kim et al., 2021), and plays role in maintaining anaerobic homeostasis condition in the digestive tract environment which suitable for anaerobic bacteria like Firmicutes (Moon et al., 2018). The presence of Fusobacteria usually associated with the synthesis of vitamin B12 (cobalamin) (Qi et al., 2023). However, information regarding the role of fusobacteria in fish gut are still limited.

At the genus level, Clostridium (43.44%) was the most dominant genus in the digestive tract of giant gourami followed by Paeniclostridium (20.6%) and Cellulosilyticum (12.34%) which all of them belong to Firmicutes. Clostridium is commonly found in the freshwater fish and plays a role in providing fatty acids and vitamins for the host. Several species of Clostrdium such as *C. butyricum* play as probiotic which help the digestion of cellulose to produce SCFA that can optimize luminal environment and maintain intestinal health (Egerton et al.,2018: Guo et al., 2022). Some short chain fatty acid that produced by Clostridium are acetate, propionate, and butyrate. Acetate can be the co-substrate for other bacteria to produce butyrate. Propionate were used by liver for maintaining glucose and lipid metabolism. Butyrate is the most multifunctional SCFA for maintaining gut environment and improve immune health (Guo et al., 2022). Paeniclostridium also plays a role in helping the digestion of complex compounds such as bile acids, peptides, and phospholipids. These bacteria are commonly found in herbivorous fish. However, the presence of these bacteria is also a parameter in fish that have relatively slow growth (Chapagain et al., 2019). Cellullosilyticum plays a role in digesting cellulosic materials, and have variety of hemicelluloses such as cellobiohydrolase, feruloyl esterase, xylanase, and endoglucanase (Cai & Dong, 2010). Information related to the role of these bacteria in fish gut is still limited. In addition, several genera such as Turicibacter and Alkaliphilus are also found in the digestive tract of gourami fish.and play a role in the butyrate production (Vargas et al., 2023).

The microbial community that found in the digestive tract of giant gourami has an important role to support the digestion process, especially for cellulose metabolism. We hypothesed that the addition of taro leaves may increasing the amount of SCFA such as acetate, propionate, and butyrate. Acetate derivative, acetyl-coenzyme A (Ac-CoA), is a central molecule in a number of vital processes within the human body. These include energy production, lipid synthesis and protein acetylation. Propionate has been demonstrated to play a significant role in the process of gluconeogenesis within the liver, and increase resistance to hypoxic stress. Butyrate has been shown to serve as the primary energy source for ephitalial cell metabolism, thereby enhancing immune regulation (Facchin et al., 2024). Even though, we have not been able to find any significant results regarding the effect addition of taro leaves on giant gourami growth, but this finding can indicate that the addition of taro leaves can provide available nutrients for giant gourami and reduce the use of commercial feed. We assume that there is a possibility that larger giant gourami may show better growth performance when fed by taro leaves. We still curious whether the giant gourami become adult, it tends to consume raw-plants so that it can reduce the use of commercial feed, and we need to investigate further.

Conclusion

To summarise, the results of the study demonstrate that the growth performance of medium-sized giant gourami was significantly influenced by the nutritional regime. The group that consumed taro leaves as the primary nutrient exhibited the lowest growth performance, suggesting a potential correlation between nutrient source and growth outcome. The investigation revealed that the incorporation of taro leaves as phytogenic compounds did not yield substantial outcomes when compared to the utilisation of commercial feed treatments. This finding indicates that the incorporation of taro leaves plays a significant role in supplying essential nutrients to giant gourami, while concomitantly reducing the utilisation of commercial feed. Firmicutes represent the predominant bacterial phyla within the digestive system of the giant gourami, encompassing the genera *Clostridium* and *Cellulosilyticum*. These bacteria play a pivotal role in the metabolism of cellulose as a carbon source and the production of SCFA such as acetate, propionate, and butyrate which support the growth performance of the giant gourami.

Ethical Statement

All fish specimens were sourced from local farmers with the approval of the Universitas Gadjah Mada Ethical Clearance Commission (certificate No. 00038/VIII/UN1/LPPT /EC /2024). Researchers prioritized minimizing any discomfort experienced by the animals during the study.

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Author Contribution

Conceptualization: DWKS, IH and TBS; project administration and supervision: DWKS, IH and TBS; funding acquisition: DWKS; methodology; DWKS, IH, TBS and AS; resources: IR, FR, MAI and LKY; investigation; IR, FR, MAI and LKY; formal analysis: IR, FR, MAI and LKY; visualization: MAI and LKY; writing original draft: DWKS and MAI; writing review and editing: DWKS, IH, TBS and AS.

Conflict of Interest

No conflict of interest was reported by the authors.

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