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Enrichment of commercial feed with new formula products on the growth, yield, and mortality of the giant gourami *Osphronemus goramy*

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Enrichment of commercial feed with new formula products on the growth, yield, and mortality of the giant gourami *Osphronemus goramy*

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Abstract. Background and objective: The giant gurami (*Osphronemus goramy*) is considered the most desirable freshwater species in Indonesia, mainly for food security. This study evaluated the effectiveness of newly formulated products containing water, coconut, palm sap sugar, and fungus used for enriched commercial feed and their impact on gurami sago's (local strain) growth performance, yield, and mortality. Methods: A total of 100 g of palm sugar are cooked with 1,000 ml of freshwater for fifteen minutes at a temperature of 60 oC. Furthermore was added 2,000 ml of mature coconut water. For every 1,000 ml of a mixture of mature coconut water and palm sap sugar solution was added 2 g of *Aspergillus niger* (called product P1), 2 g of *Rhizopus oligosporus* (product P2), and 2 g of Saccharomyces cerevisiae (product P3). Commercial fish feed pellets enriched with P1, P2, and P3 were designated as P1, P2, and P3 diets. At the same time, the commercial feed added with freshwater is called P4 feed (placebo). The dosage of each product is 300 ml/kg of feed. Juvenile sago gourami (initial weight 50±2.5 g and total length 13.2±0.4 cm) were stocked in triplicate (0.5x0.5x0.5 m) in a freshwater concrete pond with a stocking density of 30 individuals. /net, an initial feeding rate of 3% per day for 90 days of the experiment.Results: The weight gain ranged from 172.43 to 215.6%, the specific growth rate increased from 0.60 and 0.75%/day. The coefficient of thermal growth increased from 27.26 to 32.83. At the same time, yield ranging from 14.88 to 21.03 g/L and mortality for 90 days of the experiment decreased from 22.22% to 6.66%. The coefficient of variation in weight was between 0.68% and 1.30%. Conclusion: Giant gourami juvenile survived and grew well in diet P2, moderate growth in diet P3, and diet P1; the lowest growth was recorded in diet P4.

1. Introduction

Approximately 70% of aquaculture-based animal production is fed with commercial feeds containing high protein [1,2]. Conversely, some aquaculture fish species' feed conversion ratio (FCR) is still higher [3]. In this background, fish feed ranging from 30 to 40% is released as a waste to the water environment [3-5]. On the other hand, the charge of aquafeed is still an important treatment for aquaculture activities [1,6-10] due to the continued reliance on fish meal, fish oil, and soybean meals as feed raw materials, most of which are imported products. The previous scientist has reported strategies used to improve aquafeed nutrition, like supplementing the aquafeed with fish oil [11,12], soybean oil [13], and the use of probiotics [14]. This method is carried out so that the feed given to cultured fish is wealthy in nutrients such as amino acids, fatty acids, minerals, and vitamins to meet their needs [10,15,16,17].

Several alternative plant-based protein sources as unconventional ingredients to replace the traditional feed ingredients have been studied in the last decades, aiming to reduce fish meal production and decrease the cost of fish meal [17,18]. Unconventional materials from sustainable natural resources are relatively economical such as sunflower, aquatic weed, and palm seeds. However, this material is limited to aquafeed in its pure form as it contains high fiber anti-nutrients and reduces feed digestibility [18-20].

In addition to the natural resources mentioned above, we hypothesize that coconut water and palm sap sugar by fungal fermentation could increase the nutritional value of commercial feeds. Coconut water contains vitamins, minerals, fatty acids, amino acids, enzymes, and a few phenolic compounds [21-23]. Coconut water has been effectively consumed to cure ailments in humans, such as throat infections, tapeworms, gonorrhea, digestive problems, influenza, lice, giardia, bronchitis, and cholera [24]. Moreover, palm sap sugar has health profit due to its low glycemic index and antioxidants, vitamins, and minerals [25]. Based on the previous, the current study evaluated the commercial feed enriched with different new formation products and their effects on growth rates, feed efficiency use, and mortality rate of gurami sago juveniles.

2. Methods

2.1. Preparation of product formulated

We prepared 2,000 ml of mature coconut water (*Cocos nucifera* L.). Then 100 g of palm sugar is cooked in 1,000 ml of fresh water at 60 ^{o}C for 15 minutes; after that, it was cooled for 20 minutes in an open space. Furthermore, mature coconut water (2,000 ml) and palm sap sugar (1,000 ml) were mixed until they became 3,000 ml. A total of 3,000 ml of the newly formulated product is divided into 1,000 ml. The first part of the new product formulation added 2 g of *Aspergillus niger* (called P1 product), the second part added 2 g of *Rhizopus oligosporus* (called P2 product), and the third part added 2 g of *Saccharomyces cerevisiae* (called P3 product). Each serving (1,000 ml) is fermented in a continuous aeration process for 48 hours in a jerry can (2,000 ml). The prepared solution was stored in a cool air-conditioned room for 10 minutes, away from direct sunlight. The products P1, P2, and P3 were used to supplement the nutrition of commercial feeds (781-2, PT. Japfa Comfeed Indonesia, Tbk) called P1, P2, and P3 diets. The aquafeed added fresh borehole water is called the P4 feed (placebo).

2.2. Preparation of experiment diets

They are floating commercial aquafeed 781-2 containing proximate composition (dry weight %), 10.66% water content, 30.10% crude protein, 4.09% crude fat, 45.35% total carbohydrates, 2.5% ash, and 9.18% crude fiber. The feed is enriched by-products P1, P2, P3, and P4, each of which is 300 ml/1 kg of feed. Each product is poured into each tube, then sprayed evenly into one kilogram of commercial aquafeed, then evaporated in the open air for thirty minutes. After that, the fish gave the aquafeed experimental.

2.3. Experimental procedures and sampling

We used 360 fish seeds of gurami sago for this study. The average weight was 35.5±0.25 g, and the length was 13.2±0.07 cm. The AD-600i scales with 0.001 g accuracy were used to measure weight fish, while a meter ruler with 1 mm accuracy was used for the body length. Twelve nets framed with size 0.5×0.5×0.5 m PVC pipe (75 L capacity) were placed inside two freshwater concrete ponds with the size 18-m3 (6×2×1.5 m). Each net was stocked with 30 fish, consist four treatments and three replicates. They were fed feed of P1, P2, P3, and P4 were conducted three times a day at 08.00 AM, 12.00, and 05.00 PM, at a proportion 3% body weight rate per day until the experiment was completed. Fish samples were collected every 30 days for length and weight measurements. Fish were put through a fasting phase for 24 hours before sampling to empty the intestinal contents. Thirty fish per net framed were collected and anesthetized orally using clove oil. Then, the length and weight were measured.

2.4. Measurements parameters

The following parameters were calculated to determine the animal experiment's growth performance: body weight gain (%), specific growth rate (SGR, %/day), and absolute growth rate. Thermal growth coefficient (TGC), Fulton's condition factor, yield (g/ L), Mortality Rate (%), coefficient of variation (CV) of weight (%), coefficient of variation (CV) of length (%), feed conversion ratio (FCR) and feed conversion efficiency (FCE). The parameters were analyzed according to formulas:

Parameter	**Formula**
Weight gain (%)	$\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$
Daily weight gain(mg)	$\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Duration of rearing period (days)}}$
Specific growth Rate (%/day)	$\frac{[\text{Ln}(\text{Final weight (g)}) - \text{Ln}(\text{Initial weight (g)})]}{\text{Duration of rearing period (days)}} \times 100$
Thermal growth coefficient (TGC)	$\frac{[(\text{Final weight (g)})^{1/3} - (\text{Initial weight (g)}^{1/3}]}{\text{Mean water temperature (°C)} \times \text{duration of rearing period (days)}} \times 1000$
Fulton s condition factor	$\frac{\text{Weight of the juvenile (g)}}{\text{Length of juvenile (cm)}^3} \times 100$
Yield (g/L)	$\frac{\text{Final weight (g)} - \text{Number of survived juvenile}}{\text{Water volume (liter)}}$
Mortality rate (%)	$\frac{\text{Number of juvenile stocked} - \text{Number of survived juvenile}}{\text{Number of juvenile stocked}} \times 100$
Survival rate (%)	$\frac{\text{Number of survive juvenile}}{\text{Number of juvenile stocked}} \times 100$
Coefficient of variation (CV) of weight (%)	$\frac{\text{Standart deviation of weight}}{\text{Mean weight (g)}} \times 100$
Coefficient of variation (CV) of length (%)	$\frac{\text{Standart deviation of length}}{\text{Mean lenght (cm)}} \times 100$
Feed conversion ratio (FCR)	$\frac{\text{Fed feed (g dry weight)}}{\text{Weight gained (g)}}$
Feed conversion efficiency (FCE)	$\frac{1}{\text{FCR}}$

2.5. Water quality parameters

This study monitored water quality parameters such as temperature, dissolved oxygen, and pH in an animal's reared net frame every week. The temperature was measured with a thermometer (Celsius scale). The pH values were determined with a pH meter (digital mini pH meter, 14pH, IQ Scientific, Chemo-science Thailand Co., Ltd, Thailand). The DO reading was measured using an oxygen meter (YSI model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total alkalinity, total hardness, and nitrate were monitored monthly for all the nets frame by standard methodology based on formulas [26].

2.6. Data analyses

Statistical data analysis was carried out with SPSS 16.0 software package (SPSS; Chicago, IL). Normality was subjected to Kolmogorov-Smirnov test. Homogeneity was checked using the absolute residuals according to Levene's test. Effect of treatment was carried out using one-way ANOVA, followed by post-hoc Duncans multiple range tests [27]. Differences were considered significant at the 95% confidence level (P<0.05). All mean are given with standard deviation (±SD).

3. Results

Biometric performance: Table 1 represents the growth rate, yield, mortality, and coefficient variation (CV) of length (%) and weight (%). The weight gain varied between 172.43 and 201.82%, with specific growth rates ranging from 0.60 and 0.75%. On the other hand, thermal growth coefficients were between 27.26 and 32.83. Juvenile mortality for 90 days experiment ranged between 6.66 and 22.22%, with the coefficient of weight variation varying from 0.60 to 1.31%. Several parameters from the data on survival, yield harvested, FCR, and FCE are shown in Figures 1 to 4.

Table 1. Growth performance and mortality rate of gurami sago (mean ± s.d.) for 90 days of the experimental period.

	Diet P1	**Diet P2**	**Diet P3**	**Diet P4**
Weight gain (%)	201.83 ± 3.20^{a}	215.36 ± 2.18^{b}	200.26 ± 1.11^{ac}	172.43 ± 2.90^{d}
Specific growth rate (%/ day)	0.71 ± 0.01^{a}	0.75 ± 0.01^{b}	0.70 ± 0.01^{c}	0.61 ± 0.01^{d}
Daily weight gain (mg)	0.80 ± 0.01^{a}	0.85 ± 0.01^{b}	0.77 ± 0.01^{c}	0.67 ± 0.01^{d}
Thermal growth coefficient (TGC)	31.10 ± 0.41^{a}	32.83 ± 0.24^{b}	30.23 ± 0.18^{c}	27.26 ± 0.41^{d}
Fulton's condition factor	4.68 ± 0.05^{a}	4.38 ± 0.10^{b}	4.39 ± 0.27^{c}	4.16 ± 0.07^{d}
Coefficient of variation (CV) of weight (%)	1.19 ± 0.01^{a}	0.69 ± 0.01^{b}	0.60 ± 0.00^{c}	1.31 ± 0.02^{c}
Coefficient of variation (CV) of length (%)	0.53 ± 0.00^{a}	0.80 ± 0.01^{b}	0.32 ± 0.02^{c}	0.66 ± 0.00^{d}
Mortality rate (%)	8.89 ± 3.85^{a}	6.67 ± 0.00^{b}	13.33 ± 6.67^{c}	22.22 ± 3.85^{d}

a b c d - significant differences in rows, Analytical replicates n= 3

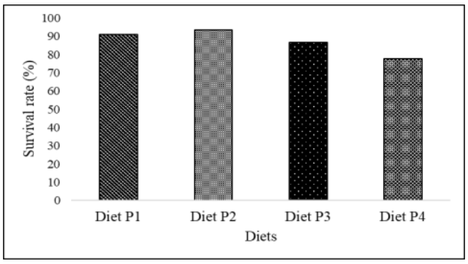

Figure 1. The survival rate of gurami sago under diets experiment for 90 days

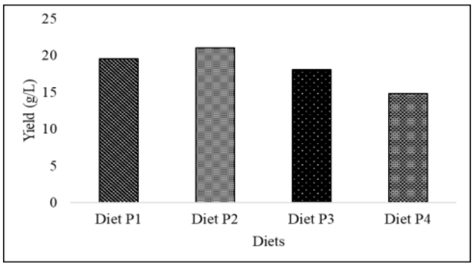

Figure 2. The yield of gurami sago under diets experiment for 90 days

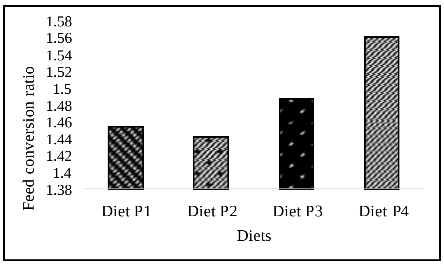

Figure 3. Feed conversion ratio (FCR) of gurami sago under diets experiment for 90 days

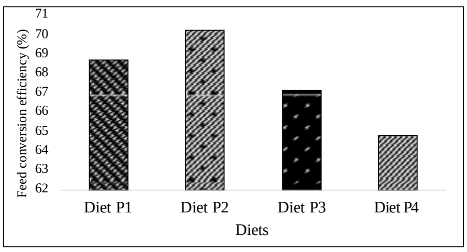

Figure 4. Feed conversion efficiency (FCE) of gurami sago under diets experiment for 90 days

Water quality parameters: We recorded the reading of the parameters in the net's frame during juvenile rearing. The water temperature in the juvenile nursed ranges from 28.0 ^oC to 30.0 ^oC. The dissolved oxygen fluctuated between 5.2 and 5.4 mg L^{-1}, and the pH was 7.2 and 7.4. Total alkalinity ranged from 51 mg L^{-1} to 56 mg L^{-1} as $CaCO_3$, hardness varies between 62 mg L^{-1} and 66 mg $^{-1}$ as $CaCO_3$, while Nitrite-Nitrogen (NO_2-N) was between 0.01 mg L^{-1} and 0.03 mg L^{-1} (Table 2).

Table 2. Water quality parameters in juvenile gurami sago reared

	Mean±SD	Range
Water temperature (^oC)	28.83±0.83	28-30
Dissolved oxygen (mg/L)	5.30±0.07	5.2-5.4
Alkalinity (mgL^{-1} as $CaCO_3$)	53.4±2.07	52- 56
Hardness (mg L^{-1} as CaCO3)	64.2±1.48	62-66
pH	7.28±0.07	7.2-7.4
Nitrite-Nitrogen (mg L^{-1})	0.018±0.0083	0.01-0.03

3.1. Discussion

The level of contrast between food and variation of aquaculture systems is an essential condition during juvenile rearing for better growth performance [5]. The growth performance is not only associated with cultivation conditions but also depends on cruising speed and active hours of the feed habits[4] but also diet composition [28] and feed nutrition [12,13,29]. In the present study, the growth performance of juvenile gurami sago was significantly influenced by commercial feed enriched with various formulation products. The fish reared with P2 feed showed better growth performance, i.e., specific growth rate (%/ day), daily weight gain (mg), and thermal growth coefficient (TGC) compared to other feeds. At the same time, Fulton's condition factor showed better in the diet P1. This factor may be due to differences in the nutritional content of the feed supplemented by the formulated product, which increases the visibility of the diet in the reared animals, resulting in better food consumption, lower food conversion ratios, and higher weight gain. Several researchers have been reported similar results in other species like Nile tilapia, *Oreochromis niloticus* [12-13], largemouth bass, *Micropterus salmoides* [29], and common carp, *Cyprinus carpio* [30].

The present study recorded moderate growth in diet P3, followed by diet P1, while the lowest growth was recorded in the P4 diet. These factors may be due to the deficient nutrition in diet P4, i.e., fatty acids levels in the FUFAs group, like EPA, DHA, and LA. According to [31], two essential fatty acids (EFA), linoleic acid (LA, 18:2 n-6) and linolenic acid (LNA, 18:3 n-3), must be included in the fish feed because this type of fatty acids is required for increased growth and disease resistance. The juvenile of giant gourami increased their weight more than three-fold over the 90-days feeding trial with very low mortality. Diet P1 had the second-highest growth after diet P2, and diet P2 shows the higher survival rate of 93.33%, followed by diet P1, P3, and P4, with 91.11%, 86.67%, and 77,78% survival rate, respectively (Figure 1). Thus, it can be concluded that differences in mortality rates are generally affected by inadequate diet.

Moreover, the mortality rate is probably one of the critical physiological parameters in aquaculture studies [32]. Juvenile growth of gurami sago was related to yield production of each diet; higher yield showed by diet P2 was 21.03 g L^{-1}, whereas the lowest was in diet P4 is 14.90 g L^{-1} (Figure 2). These data indicated that the P2 formulated product supplemented with *Rhizopus oligosporus* during fermentation, their feed was richer in nutrients compared to the P4 diet. However, diets P1 and P3 may also be rich in nutrition due to growth performance and better feed efficiency than diet P4 based on Specific growth rate (%/ day), daily weight gain (mg), and Fulton's condition factor. In this study, how the composition of fatty acids and amino acids in feed enriched with new formula products (coconut water, palm sugar water, and various mushrooms) is still poorly understood.

Furthermore, the fungus used in the new formula products enriched in commercial feeds has shown differences in feed conversion and efficiency ratios (Figures 3 and 4). Sago gourami fed with P1, P2, P3, and P4 for 90 experimental days showed FCR values were 1.45, 1.44, 1.48, and 1.56, respectively.

At the same time, FCE values were 68.76%, 69.40%, 67.22%, and 64.68%, respectively. Based on the FCE value of each experimental diet, we conclude that each 1 kg of feed yields harvested fish were between 0.64 and 0.68 kg; conversely, 0.32 to 0.36 kg of feed are released into water bodies as waste. The FCE values for gurami sago cultivated in earthen freshwater ponds, concrete ponds, and floating cages were 0.46, 0.69, and 0.77, respectively [5]. Several authors reported that feed conversion efficiency (FCE) is related to feeding nutrition, feed characteristics, and feeding rate [10-12], including different aquaculture systems [5], environmental factors, and husbandry factors [32]

4. Conclusion

This study revealed a significant effect of diet supplemented with the new formulation on juvenile gurami sago's growth and feed efficiency. The commercial feed supplemented with P2 products (coconut water and palm sugar solution fermented with *Rhizopus oligosporus*) are more suitable for the growth performance of juvenile giant gourami strain sago and increasing the feed's efficiency used and yield harvested. In addition, the use of diet P2 decreases mortality rates and reduces the load of waste released into the water body. Further studies are required to investigate the effect of feed enrichment with new formula products on time-related changes in the significant digestibility coefficients of gurami sago.

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