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Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth coefficient, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles

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ABSTRACT

Background: Giant gourami (*Osphronemus goramy* Lacepede, 1801) is a freshwater species of Indonesia's most important commercial fish, and most of the giant gourami is produced by aquaculture. The first purpose of this investigation is to determine the effect of various newly formulated products on the amino acid composition of the diet and whole-body carcass. The second aim is to analysed the impact of newly formulated products on the growth performance, body indices, and gut micromorphology.

Methods: A total of 1.0 litres of palm sap sugar solution and 2.0 litres of mature coconut water were formulated. Each 1.0-litre product formulated was added to 2 g of *Aspergillus niger* (labeled CP2), 2 g of *Rhizopus oligosporus* (labeled CP3), and 2 g of *Saccharomyces cerevisiae* (labeled CP4). Commercial aquafeed to which freshwater was added was labeled CP1 (placebo). Aquafeed was added to CP1 and supplemented with CP2, CP3, and CP4 (labeled KP1, KP2, KP3, and KP4 diets). Their dosage was 150 ml/kg of feed. Juvenile giant gourami (initial weight 50 ± 0.25 g and length 13.2 ± 0.07 cm) were reared in triplicate net frames (2 m \times 1 m \times 1 m; water volume 1.5 m³/frame nets) in a freshwater concrete pond. The juveniles were randomly distributed at a stocking density of 30 juveniles/net.

Results: Results support our hypothesis that different product formulations have a significant effect ($P < 0.05$) on aquafeed nutrition and the whole-body carcass, growth indices, fish feed efficiency, and gut micromorphology of giant gourami juveniles. The KP3 diet contains a higher concentration of amino acids, which increased the growth rate, feed efficiency, and carcass quality more than the other diets that we tested.

Conclusion: Diet KP3 contains higher total amino acids in diets and carcasses and gives the better growth of giant gourami.

Keywords: Giant gourami, amino acid profile, growth performance, feed efficiency, coconut water, gut micromorphology

1. Introduction

In this decade, the production of capture fisheries has decreased; meanwhile, the demand for fish products for human consumption is increasing. Therefore 60% of fisheries production in the future will come from aquaculture activities and will continue to rise¹. The utilization of a variety of fish for aquaculture has now increased the need for commercial feed²⁻⁵. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge^{6, 7, 2, 8}. On the other hand, commercial feed produced by factories still does not contain complete nutrition for fish growth and is acknowledged for its positive effects on food safety^{9, 10, 11}. In this context, enriching fish feed with cost-effective natural ingredient resources is key to increasing feed nutrient quality and feed efficiency in commercial fish farming and ensuring the sustainability of aquaculture operations^{2, 12, 13}.

The target is fish feed that is wealthy many important nutrients, including protein, fat, vitamins, and mineral that cultured fish can utilize to increase their growth rate and survival and that is beneficial for human health^{14, 15, 4, 16}. Therefore, novel approaches have been developed to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA¹⁷, iodine and selenium¹⁰, methionine¹⁸, fish oil^{19, 11}, and soybean oil²⁰. In addition, supplementing probiotics into the diet²¹ and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied²².

Coconut water has extraordinary nutritional value and contains sanity-friendly supplements like minerals, amino acids, fatty acids, vitamins, enzymes, organic acids, and several phenolic compositions^{23, 24, 25, 26}. Palm sap sugar also has health benefits due to its essential nutrient content, such as a low glycaemic index, and contains antioxidants, vitamins, and minerals^{27, 28, 29, 30}. Meanwhile, the fungus has been widely used in fermentation due to its ability to degrade antigenic proteins in fish feed ingredients^{7, 31, 32}. Zhang et al.³³ reported that coconut water is a valuable nutrient for the body to preserve the eye lens from diabetic cataracts in rats. Coconut water is also a treatment for burning pain during urination, dysuria, gastritis, incineration of the eyes, and indigestion³⁴. However, new formulations of products containing coconut water and palm sap sugar are fermented with various mushrooms to enrich fish feed nutrients. Their effect on fish growth rate, body indices, and gut micromorphology has not yet been analysed.

The giant gourami, *Osphronemus goramy* Lacèpdèd, 1801, is a freshwater herbivorous species and is one of Indonesia's most important commercial fish because of its nutritional value, taste, aroma, and overall quality and most of the giant gourami consumed by humans

is produced by aquaculture^{35, 36, 37, 38, 9}. For this reason, many fish farmers in inland waters in Indonesia have expanded their annual production by culturing local strains, including tambago, palapah, sago, galunggung, and blusafir^{39, 40, 41, 43}. These species have account for 6.96% of Indonesia's total freshwater aquaculture production⁴³. In recent decades, research on nutrition and feeds for giant gourami have garnered increasing interest^{9, 44, 45}. Previous studies on the use of diets formulated with fish meal and azolla flour for giant gourami focused on the effect of the ratio of fish meal and azola flour on fish growth⁴⁴. Juvenile giant gourami were fed diets supplemented with recombinant growth hormone at different protein levels⁴⁶. There was an effect of different feeding rates on the specific growth rate and feed use¹⁹. The addition of artificial feed combined with tubifex worm increased the growth performance of giant gourami juveniles⁴⁷. Until now, there has been no information on the use of newly formulated products consisting of mature coconut water and palm sap sugar solution fermented with different mushrooms to supplement fish feed. Feed nutrition includes protein, fat, carbohydrates, and minerals in the diet, and the effect of diet on the amino acid composition of the whole-body carcass of giant gourami, body indices, and gut micromorphology.

We hypothesized that commercial aquafeed supplemented with different newly formulated products could improve the nutritional quality of the aquafeed and whole body carcass, growth performance, body indices, feed efficiency, and gut micromorphology. Hence, the first purpose of this investigation is to determine the effect of various newly formulated products on the amino acid composition of the diet and whole-body carcass. The second aim is to analysed the impact of newly formulated products on the growth performance, nutrient utilization, survival rate, growth coefficient, body indices, and gut micromorphology in giant gourami.

2. Materials and methods

2.1. Study design

The Research and Community Service Ethics Committee at Universitas Bung Hatta approved this research. The Ministry of Education, Culture, Research and Technology of the Republic of Indonesia funded the research under grant No. 076/E5/PG.02.00. PT/2022 on March 16, 2022. Experiments were conducted under guidelines in the Standard Operating Procedure of Laboratory Aquaculture, Universitas Bung Hatta.

2.2. Preparation of formulated product

We prepared 100 g of palm sap sugar by traditional production and cooked it in 1.0 litre of fresh water for fifteen minutes at 60 °C. Then, it was cooled in an open space for twenty minutes. Furthermore, we also prepared 2.0 litres of mature coconut water (*Cocos nucifera* L.) and mixed it with 1.0 litres of palm sap sugar solution. The solution products were stored for ten minutes in a cool air-conditioned room. A total of 3.0 litres of the formulated product was divided into three parts of 1.0 litre each. We added 2 g of *Aspergillus niger* (labeled as CP2 product) to the first part of the formulated product solution, 2 g of *Rhizopus oligosporus* (labeled as CP3 product) to the second part, and 2 g of *Saccharomyces cerevisiae* (labeled as CP4 product) to the third portion. The CP2, CP3, and CP4 products were fermented for 48 h in a jerry can (2.0 litres) using an Aerasi PUJIMAC, MAC-40 K 40 L/min. The products of CP2, CP3, and CP4 were used to enrich the nutrition of commercial aquafeed: 781-2, PT. Japfa Comfeed Indonesia, Tbk (labeled as KP2, KP3, and KP4 diets). The aquafeed was supplemented with freshwater (labeled as the KP1 diet; placebo).

2.3. Preparation of experimental diets

Giant gourami juveniles were adapted for one month to standard feed, namely floating commercial aquafeed 781-2 (pellet size 2 mm) contained 10.66% water content, 30.10% crude protein, 4.09% crude fat, 45.35% total carbohydrates, 2.5% ash, and 9.18% crude fibre. Minerals of commercial feed were 280.08 mg/100 g Na, 1415.02 mg/100 g Ca, 1358.07 mg/100 g K, 1200.31 mg/100 g P, 292.03 mg/100 g Mg, 18.14 mg/100 g Fe, and 13.83 mg/100 g Zn. The aquafeed was added to CP1 and supplemented with CP2, CP3, and CP4 products at a dosage of 150 ml/kg of feed. The formulated product added to the aquafeed was mixed manually for three minutes to obtain maximum homogenization and then dried in the open air for thirty minutes. Furthermore, it was given to the trial animal.

2.4. Experimental procedures and sampling

In the present study, we measured fish weight using AD-600i scales with 0.001 g accuracy (ACIS model number AD-600i, Cina). At the same time, a metre ruler with 1 mm accuracy was used to estimate the body length. A total of 360 sago strain juveniles of giant gourami were counted; the initial mean weight was 50 ± 0.25 g, and the initial length was 13.2 ± 0.07 cm. For rearing juveniles, twelve net framed with 2 m³ (2.0 × 1.0 × 1.0 m) PVC pipe (water volume of 1.5 m³) were placed inside two freshwater concrete ponds with a size of 18 m³ (6.0 × 2.0 × 1.5 m). This experiment consisted of four treatments and three replicates, and each frame net was stocked with 30 juveniles. The giant gourami were fed the KP1, KP2, KP3, and KP4 diets three times a day (08:00, 12:00, and 17:00 h) during the 90-day feeding trial. Juveniles of giant gourami were fed at a 3% body weight rate per day based

on the percentage of stored biomass. Fish samples were collected every 30 days for body weight and length measurements. Ten fish per net frame were collected and anesthetized orally using clove oil. Then, their lengths and weights were measured. Prior to sampling, the fish fasted for 24 hours to empty their intestinal contents.

2.5. Proximate and amino acid composition

The diet samples and carcass proximate composition were analysed using standard AOAC methods⁴⁸. The matter was dried to a constant weight at 105 °C. We used the standard Kjeldahl method to analyse crude protein ($N \times 6.25$). We used the Soxhlet method with ether extraction to analyse crude lipids; the ash was incinerated at 550 °C for 16 h, whereas gross energy was measured in a bomb calorimeter. For amino acid analysis, the methods used were described by Cohen⁴⁹. The amino acid composition was determined by using a high-performance liquid chromatography (HPLC) system consisting of a water 1525 binary HPLC pump, 717 autosamplers (water ®), and water 2475 multi λ fluorescence detector optics (wavelengths: 250 nm for excitation and 395 nm for emission). It was hydrolysed in triplicate with 6 N hydrochloric acid for 24 h at 11 °C.

2.6. Histological examination of the gut

For histological analyses, each gut specimen of the animal was cut into the foregut, midgut, and hindgut. Moreover, the cells were cleaned in saline solution and fixed in Bouin's fixative solution for 24 hours. After sequential dehydration steps in alcohol, the gut samples were embedded in paraffin. The implanted tissue blocks were sectioned at 5 μ m, and sections were consistently stained with haematoxylin-eosin and observed under a light microscope (Olympus IX71) equipped with Image-Pro Plus 7.0 software. The digitalized analysis measures the micrometer length of various enteric structures of gut images. We determined the average fold height (hF), fold width (wF), and enterocyte height (hMV) of the gut per slice (5 fields per individual sample) according to procedures described by Li et al.¹⁸. The specific measurement method of gut samples is shown in Fig 1.

2.7. Pond water quality

The water quality values of the freshwater concrete ponds that were used to rear the giant gourami juveniles were recorded weekly. The water samples were collected at 10:00 AM at a depth of 20 cm from each concrete pond to determine the water temperature, dissolved oxygen, and pH. In addition, we also measured the total alkalinity, hardness, and nitrates of the water in the pond experiments. A thermometer (Celsius scale) was used for measured water temperature. To measure water oxygen (O_2 ; mg L^{-1}), use an oxygen meter (YSI Model 52, Yellow Instrument Co, Yellow Spring, OH USA). A digital pH meter (Mini 0–14 pH IQ,

Scientific Cemo Science Thailand) was used to determine the pH values of water in the pond experiments. The level of nitrate-nitrogen ($\text{NO}_3\text{-N}$; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures⁵⁰.

2.8. Calculations and statistical method

The data from this study were reported as mean \pm standard deviation for each treatment. Data were analysed by the SPSS 16.0 software package (SPSS; Chicago, IL). Normality was tested using the Kolmogorov–Smirnov statistic. Homogeneity was checked using absolute residuals according to Levine’s test. One-way ANOVA was used to determine the treatment effect, followed by a post hoc Duncan’s multiple range test (Duncan, 1955). To create the figures was used Microsoft Office Professional Plus 2019.

The growth performance of animal experiments was measured by weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and daily growth coefficient (DGC). Feed utilization was analysed, and the total feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) of giant gourami was assessed using the following formulae:

$$\text{WG (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{DWG (g)} = \text{Final weight (g)} - \text{Initial weight (g)} / \text{Duration of rearing period (days)}$$

$$\text{FI as feed (FI}_{\text{asfeed}} \text{ in g/fish/day)} = \text{Total feed fed} / (n \times t)$$

$$\text{SGR (\%/day)} = (\text{Log}_e W_f - \text{Log}_e W_i / t) \times 100$$

$$\text{FCR} = \text{Feed supply in kg} / \text{Total harvest weight in kg}$$

$$\text{CF} = \text{Weight of the juvenile (g)} / \text{Length of juvenile (cm)}^3 \times 100$$

$$\text{TGC} = [(\text{Final weight (g)})^{1/3} - (\text{Initial weight (g)})^{1/3}] / (\text{Mean water temperature (}^\circ\text{C)} \times \text{Duration of rearing period (day)}) \times 100$$

$$\text{DGC} = (W_f^{1/3} - W_i^{1/3}) / \text{Duration of rearing period (day)} \times 100$$

$$\text{PER} = \text{Wet weight gain} / \text{Total protein intake}$$

Three fish from each net frame were sacrificed and dissected immediately to determine the condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI), liposomatic index (LSI), and bilesomatic index (BSI) as given below:

$$\text{HSI} = [\text{Liver weight (g)} / \text{weight of fish (g)}] \times 100$$

$$\text{VSI} = [\text{Viscera weight (g)} / \text{weight of fish (g)}] \times 100$$

$$\text{LSI} = [\text{Visceral fat weight (g)} / \text{weight of fish (g)}] \times 100$$

$$\text{BSI} = [\text{Bile weight (g)} / \text{weight of liver}] \times 100$$

3. Results

3.1. Effects of different products on the proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products has a significant effect on the proximate composition of diets. One-way ANOVA results showed a marginal interaction among treatments in the case of protein content ($F_{(3,8)} = 1.522$, $P = 0.282$), fat ($F_{(3,8)} = 5.663$, $P = 0.022$), carbohydrates ($F_{(3,8)} = 1.862$, $P = 0.214$), crude fibre ($F_{(3,8)} = 1.445$, $P = 0.300$), and ash ($F_{(3,8)} = 0.272$, $P = 0.844$), and the total energy content ($F_{(3,8)} = 1.112$, $P = 0.400$) differed considerably ($P < 0.05$) among the four diets (Table 1). Duncan's post-hoc test revealed that the protein content ($21.6967 \pm 0.17\%$) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate ($31.19 \pm 0.38\%$), crude fibre ($2.82 \pm 0.06\%$), and ash ($6.67 \pm 0.06\%$) contents were significantly higher ($P < 0.05$) in the KP3 diet than in the other diets. Conversely, the total energy content was 240.88 ± 0.74 (kg calories/100 g), which was significantly higher ($P < 0.05$) in the KP3 diets than in the KP1, KP2, and KP4 diets (Table 1).

The levels of free amino acids in the diets supplemented with different formulated products are presented in Table 1. All types of amino acids in the diets of KP1, KP2, KP3, and KP4 were significantly different ($P < 0.05$), except for tryptophan, and there was no significant difference ($P > 0.05$) between KP2, KP3, and KP4. Among the essential amino acids, leucine and arginine were found in the highest amounts in the KP1, KP2, KP3, and KP4 diets. There was no significant difference ($P > 0.05$) in the alanine content between KP2 and KP3 diets and the cystine level in KP1 and KP3 diets. Of the nonessential amino acids, glutamic and aspartic acid represented a major portion of all four diets.

The present study found significant differences in the overall free essential and nonessential amino acid pools in the KP1, KP2, KP3, and KP4 diets (Table 1). One-way ANOVA results exhibited a marginally significant interaction between experimental diets in terms of essential amino acids ($F_{(3,8)} = 11.371$, $P = 0.003$), nonessential amino acids ($F_{(3,8)} = 0.407$, $P = 0.752$), and overall amino acid pools (essential plus nonessential) ($F_{(3,8)} = 7.355$, $P = 0.011$). Duncan's post-hoc test revealed that the free essential amino acids ($9.10 \pm 0.011\%$), nonessential amino acids ($12.91 \pm 0.004 \pm 0.00\%$), and overall amino acid pools (22.02%) were significantly higher ($P < 0.05$) in feed supplemented with CP3 products, followed by CP2, CP4, and CP1 products (Table 1).

Table 1. The experimental diets' proximate and amino acid composition (% dry matter). Mean \pm SD *. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$)

| | KP1 | KP2 | KP3 | KP4 |
|---------------------------------|--------------------------------|--------------------------------|---------------------------------|--------------------------------|
| Proximate composition | % dry weight basis | | | |
| Dry matter | 38.42 \pm 0.25 | 38.27 \pm 0.01 | 37.59 \pm 0.16 | 38.41 \pm 0.10 |
| Crude protein | 19.68 \pm 0.41 ^a | 20.27 \pm 0.13 ^b | 21.70 \pm 0.18 ^c | 20.44 \pm 0.10 ^d |
| Crude lipid | 3.41 \pm 0.02 ^a | 3.67 \pm 0.13 ^b | 3.50 \pm 0.02 ^{ac} | 3.48 \pm 0.04 ^{ad} |
| Carbohydrate | 26.37 \pm 0.17 ^a | 29.50 \pm 0.54 ^b | 31.19 \pm 0.38 ^c | 30.57 \pm 0.06 ^d |
| Crude fibre | 2.23 \pm 0.05 ^a | 2.36 \pm 0.01 ^b | 2.82 \pm 0.06 ^c | 2.45 \pm 0.06 ^d |
| Ash | 2.75 \pm 0.03 ^a | 6.66 \pm 0.05 ^b | 6.57 \pm 0.04 ^c | 6.67 \pm 0.06 ^d |
| Energy total (kg calorie/100 g) | 240.87 \pm 0.38 ^a | 234.41 \pm 0.30 ^b | 240.88 \pm 0.74 ^{ac} | 237.11 \pm 0.43 ^d |
| Amino acid composition | | | | |
| EAA | | | | |
| Leucine | 1.36 \pm 0.01 ^a | 1.42 \pm 0.01 ^b | 1.46 \pm 0.01 ^c | 1.36 \pm 0.01 ^d |
| Isoleucine | 0.76 \pm 0.01 ^a | 0.79 \pm 0.01 ^b | 0.81 \pm 0.01 ^c | 0.76 \pm 0.01 ^d |
| Lysine | 0.95 \pm 0.01 ^a | 1.10 \pm 0.01 ^b | 0.98 \pm 0.01 ^c | 1.20 \pm 0.01 ^d |
| Valine | 0.86 \pm 0.01 ^a | 0.94 \pm 0.01 ^b | 0.96 \pm 0.01 ^c | 0.89 \pm 0.01 ^d |
| Threonine | 0.79 \pm 0.02 ^a | 0.92 \pm 0.01 ^b | 1.04 \pm 0.01 ^c | 0.83 \pm 0.01 ^d |
| Arginine | 1.02 \pm 0.01 ^a | 1.19 \pm 0.01 ^b | 1.30 \pm 0.01 ^c | 1.03 \pm 0.01 ^d |
| Phenylalanine | 0.67 \pm 0.01 ^a | 0.93 \pm 0.01 ^b | 1.05 \pm 0.01 ^c | 0.77 \pm 0.01 ^d |
| Tyrosine | 0.43 \pm 0.01 ^a | 0.53 \pm 0.00 ^b | 0.57 \pm 0.06 ^c | 0.45 \pm 0.01 ^d |
| Methionine | 0.18 \pm 0.01 ^a | 0.26 \pm 0.01 ^b | 0.30 \pm 0.01 ^c | 0.21 \pm 0.01 ^d |
| Histidine | 0.40 \pm 0.01 ^a | 0.50 \pm 0.01 ^b | 0.57 \pm 0.01 ^c | 0.43 \pm 0.01 ^d |
| Tryptophan | 0.06 \pm 0.01 ^a | 0.11 \pm 0.01 ^b | 0.07 \pm 0.00 ^{bc} | 0.09 \pm 0.01 ^{bd} |
| NEAA | | | | |
| Alanine | 0.85 \pm 0.01 ^a | 0.94 \pm 0.01 ^b | 0.87 \pm 0.06 ^c | 0.97 \pm 0.01 ^{bd} |
| Serine | 1.01 \pm 0.01 ^a | 1.12 \pm 0.01 ^b | 1.23 \pm 0.01 ^c | 1.01 \pm 0.01 ^d |
| Glycine | 1.15 \pm 0.01 ^a | 1.32 \pm 0.01 ^b | 1.29 \pm 0.01 ^c | 1.19 \pm 0.01 ^d |

| | | | | |
|---------------|----------------------------|----------------------------|---------------------------|----------------------------|
| Proline | 1.01 ± 0.01 ^a | 1.05 ± 0.01 ^b | 1.03 ± 0.01 ^c | 1.03 ± 0.02 ^d |
| Aspartic acid | 1.25 ± 0.01 ^a | 1.50 ± 0.01 ^b | 1.40 ± 0.01 ^c | 1.56 ± 0.01 ^d |
| Glutamic | 2.15 ± 0.03 ^a | 2.88 ± 0.03 ^b | 2.59 ± 0.01 ^c | 3.01 ± 0.03 ^d |
| Cystine | 0.09 ± 0.01 ^a | 0.07 ± 0.01 ^b | 0.04 ± 0.01 ^c | 0.09 ± 0.01 ^{ad} |
| ∑EAA | 7.56 ± 0.003 ^a | 8.70 ± 0.003 ^b | 9.03 ± 0.003 ^c | 8.04 ± 0.003 ^d |
| ∑NEAA | 7.51 ± 0.008 ^a | 8.88 ± 0.007 ^b | 8.88 ± 0.004 ^c | 8.84 ± 0.008 ^d |
| ∑AA | 15.07 ± 0.004 ^a | 17.58 ± 0.002 ^b | 17.91 ± 0.00 ^c | 16.88 ± 0.003 ^d |

* Values represent the means of triplicate samples.

3.2. Proximate and amino acid profile of the whole body of giant gourami

Feed had a significant effect on the proximate carcass composition of juvenile giant gourami. One-way ANOVA results showed a marginal interaction among group treatments in the case of protein contents ($F_{(3,8)} = 1.522$, $P = 0.282$), fat ($F_{(3,8)} = 5.663$, $P = 0.022$), carbohydrates ($F_{(3,8)} = 1.862$, $P = 0.214$), and crude fibre ($F_{(3,8)} = 1.445$, $P = 0.300$). Duncan's post-hoc test revealed that the protein content ($21.69 \pm 0.17\%$), fat ($3.47 \pm 0.03\%$), carbohydrates ($31.18 \pm 0.37\%$), and crude fibre ($2.81 \pm 0.05\%$) were significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments. Meanwhile, the carcass protein content of fish fed KP1, KP2, and KP4 was not significantly different ($P > 0.05$) between treatments. For the energy total, KP3 was significantly higher ($P < 0.05$) than the other treatments (Table 2). However, the moisture content of the carcass did not show any significant variation among the KP1, KP2, KP3, and KP4 diets.

The mean quantities of total amino acids in the carcasses of *O. goramy* fed different diets are given in Table 2. Lysine and leucine represented a significant portion of the essential amino acids of the whole body carcass, and methionine was present in small quantities in all of the carcasses. Of the nonessential amino acids, glutamic acid, aspartic acid, and alanine were the highest, and cystine was the lowest for all whole-body carcasses of giant gourami fed different diets. The levels of glutamic acid were significantly higher in carcasses of fish fed the KP3 diet than in those fed the KP1, KP2, and KP4 diets.

Table 2. Whole-body proximate and amino acid composition of giant gourami after a 90-day feeding trial. Mean \pm SD *. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$)

| | KP1 | KP2 | KP3 | KP4 |
|------------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Proximate composition | %, dry wet basis | | | |
| Dry matter | 64.59 \pm 0.16 | 64.51 \pm 0.34 | 64.14 \pm 0.33 | 64.24 \pm 0.12 |
| Crude protein | 28.64 \pm 0.28 ^a | 28.07 \pm 0.79 ^{ab} | 28.85 \pm 0.45 ^c | 28.66 \pm 0.44 ^{ad} |
| Crude fat | 2.79 \pm 0.03 ^a | 2.88 \pm 0.02 ^b | 2.67 \pm 0.04 ^c | 3.00 \pm 0.02 ^d |
| Carbohydrate | 1.38 \pm 0.01 ^a | 1.99 \pm 0.06 ^b | 1.97 \pm 0.09 ^c | 1.31 \pm 0.02 ^d |
| Crude fibre | 0.97 \pm 0.02 ^a | 0.68 \pm 0.01 ^b | 0.83 \pm 0.02 ^c | 0.95 \pm 0.04 ^d |
| Ash | 1.63 \pm 0.02 ^a | 1.70 \pm 0.02 ^b | 1.54 \pm 0.01 ^c | 2.11 \pm 0.04 ^d |
| Energy total (kg calorie/100 g) | 144.77 \pm 1.58 ^a | 155.48 \pm 1.26 ^b | 157.90 \pm 0.91 ^c | 149.60 \pm 0.29 ^d |
| Amino acid composition | | | | |
| EAA | | | | |
| Leucine | 2.13 \pm 0.01 ^a | 2.37 \pm 0.01 ^b | 2.42 \pm 0.01 ^c | 2.26 \pm 0.01 ^d |
| Isoleucine | 1.13 \pm 0.01 ^a | 1.25 \pm 0.01 ^b | 1.38 \pm 0.01 ^c | 1.19 \pm 0.01 ^d |
| Lysine | 2.77 \pm 0.01 ^a | 3.16 \pm 0.02 ^b | 3.88 \pm 0.01 ^c | 2.86 \pm 0.01 ^d |
| Valine | 1.26 \pm 0.01 ^a | 1.40 \pm 0.01 ^b | 1.32 \pm 0.01 ^c | 1.35 \pm 0.01 ^d |
| Threonine | 1.38 \pm 0.02 ^a | 1.49 \pm 0.01 ^b | 1.43 \pm 0.01 ^d | 1.48 \pm 0.01 ^d |
| Arginine | 1.58 \pm 0.01 ^a | 1.71 \pm 0.01 ^b | 1.63 \pm 0.01 ^c | 1.70 \pm 0.01 ^d |
| Phenylalanine | 1.02 \pm 0.01 ^a | 1.11 \pm 0.01 ^b | 1.08 \pm 0.01 ^c | 1.11 \pm 0.01 ^d |
| Tyrosine | 0.80 \pm 0.01 ^a | 0.84 \pm 0.00 ^b | 0.83 \pm 0.01 ^c | 0.85 \pm 0.06 ^d |
| Methionine | 0.15 \pm 0.01 ^a | 0.21 \pm 0.01 ^b | 0.18 \pm 0.01 ^c | 0.16 \pm 0.01 ^d |
| Histidine | 0.55 \pm 0.01 ^a | 0.56 \pm 0.01 ^{ab} | 0.59 \pm 0.01 ^{ac} | 0.57 \pm 0.01 ^d |
| Tryptophan | 0.08 \pm 0.01 ^a | 1.02 \pm 0.01 ^b | 1.08 \pm 0.01 ^{ac} | 0.06 \pm 0.00 ^d |
| NEAA | | | | |
| Alanine | 1.86 \pm 0.01 ^a | 2.08 \pm 0.01 ^b | 2.92 \pm 0.01 ^c | 1.97 \pm 0.01 ^d |
| Serine | 1.28 \pm 0.01 ^a | 1.31 \pm 0.01 ^b | 1.26 \pm 0.01 ^c | 1.31 \pm 0.01 ^d |

| | | | | |
|---------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Glycine | 1.58 ± 0.01 ^a | 1.68 ± 0.01 ^b | 1.61 ± 0.01 ^c | 1.77 ± 0.01 ^d |
| Proline | 1.06 ± 0.01 ^a | 1.16 ± 0.01 ^b | 1.08 ± 0.01 ^c | 1.16 ± 0.01 ^d |
| Aspartic acid | 2.71 ± 0.01 ^a | 3.08 ± 0.01 ^b | 3.79 ± 0.01 ^c | 2.77 ± 0.01 ^d |
| Glutamic | 4.36 ± 0.03 ^a | 4.92 ± 0.01 ^b | 4.97 ± 0.01 ^c | 4.66 ± 0.01 ^d |
| Cystine | 0.06 ± 0.01 ^a | 0.09 ± 0.01 ^b | 0.06 ± 0.01 ^c | 0.05 ± 0.01 ^d |
| ΣEAA | 12.68 ± 0.003 ^a | 15.13 ± 0.005 ^b | 15.82 ± 0.001 ^c | 13.61 ± 0.008 ^d |
| ΣNEAA | 12.91 ± 0.007 ^a | 14.32 ± 0.01 ^b | 15.69 ± 0.002 ^c | 13.50 ± 0.001 ^d |
| ΣAA | 25.59 ± 0.003 ^a | 29.45 ± 0.04 ^b | 31.51 ± 0.001 ^c | 27.11 ± 0.004 ^d |

* Values represent the means of triplicate samples.

When the overall quantities of total essential and nonessential amino acids were compared, the whole-body carcass amino acid content was significantly lower ($P < 0.05$) in fish fed the KP1 diet than in those fed the KP2, KP3, and KP4 diets (Table 2). The number of amino acids (essential plus nonessential) in the carcasses of fish fed the KP3 diet was significantly higher than that in fish fed the KP1, KP2, and KP4 diets.

3.3. Growth performance, nutrient utilization, and survival

The growth performances of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant interaction between groups' experimental diets in the case of final weight ($F_{(3,8)} = 290.60$, $P = 0.43$), weight gain ($F_{(3,8)} = 67.63$, $P = 0.024$), and daily weight gain ($F_{(3,8)} = 65.05$, $P = 0.02$). Duncan's post-hoc test revealed that the final weight (147.74 ± 1.02 g), weight gain (97.49 ± 1.40 g), and daily weight gain (1.08 ± 0.02 g) were significantly higher ($P < 0.05$) in fish fed the KP3 diet than those fed the KP1, KP2, and KP4 diets (Fig. 2). Total feed intake (% BW day⁻¹) ($F_{(3,8)} = 14.938$, $P = 0.56$), feed conversion ratio ($F_{(3,8)} = 15.36$, $P = 0.11$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Fig. 3). Fig. 4 shows fish growth performance at 30, 60, and 90 days for all experimental groups. In the current study, no mortality was observed with any diets, so the survival rate was 100% during the 90 days of the trial.

3.4. Growth coefficient and body indices of giant gourami after 90 days of feeding

In the present study, the condition factor ($F_{(3,8)} = 19.98$, $P = 0.566$), thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), daily growth rate ($F_{(3,8)} = 15.849$, $P = 0.001$) and

daily growth coefficient ($F_{(3,8)} = 59.88, P = 0.288$). The Uji post hoc Duncan's test revealed that the condition factors (2.92 ± 0.13), thermal unit growth coefficient (42.56 ± 0.34), and daily growth coefficient (30.63 ± 0.48) were significantly higher ($P < 0.05$) in the KP3 diet than in the other diets. The daily growth rate ($F_{(3,8)} = 59.88, P = 0.001$) showed no significant difference ($P > 0.05$) among the treatment groups. Specific growth rate ($F_{(3,8)} = 16.02, P = 0.205$) displayed no significance ($P > 0.05$) between KP2 and KP3 (Table 3).

Furthermore, the present study also shows that the body index of giant gourami juveniles, such as the HSI, VSI, and LSI, displayed marginally significant differences between diets. The HSI was significantly ($F_{(3,8)} = 5.389, P = 0.500$) higher in the KP3 diet, but KP1, KP2, and KP4 diets had no significant differences among them (Table 3). VSI was significantly ($F_{(3,8)} = 10.492, P = 0.243$) in the KP3 diet and significantly among all different diets. The LSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's post-hoc test revealed that the HSI ($1.30 \pm 0.13\%$), VSI ($4.15 \pm 0.36\%$), and LSI ($2.75 \pm 0.34\%$) were significantly higher ($P < 0.05$) in the KP3 diet than in the other diets. Meanwhile, BLI showed no significant difference ($P > 0.05$) among the treatment diets (Table 3).

Table 3. Mean (\pm SD) value growth coefficients and body indices of giant gourami during the 90-day experimental period. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$)

| Growth coefficients | KP1 | KP2 | KP3 | KP4 |
|---------------------------------------|--------------------|----------------------|----------------------|----------------------|
| Condition factor (CF) | 2.45 ± 0.09^a | 2.90 ± 0.07^b | 2.92 ± 0.13^c | 2.61 ± 0.04^d |
| Thermal unit growth coefficient (TGC) | 29.86 ± 0.58^a | 37.47 ± 1.24^b | 42.60 ± 0.39^c | 32.87 ± 0.64^d |
| Daily growth coefficient (DGC) | 17.76 ± 0.51^a | 25.71 ± 2.13^b | 30.63 ± 0.48^c | 20.80 ± 1.17^d |
| Daily growth rate (DGR) | 0.65 ± 0.02^a | 0.92 ± 0.07^a | 1.08 ± 0.02^a | 0.76 ± 0.04^a |
| Specific growth rate (SGR) | 0.33 ± 0.01^a | 0.55 ± 0.10^b | 0.64 ± 0.02^{bc} | 0.42 ± 0.01^d |
| Hepatosomatic (HSI) | 0.97 ± 0.05^a | 1.06 ± 0.19^{ab} | 1.30 ± 0.13^c | 1.04 ± 0.12^{ad} |
| Viscerosomatic (VSI) | 3.20 ± 0.21^a | 3.77 ± 0.09^b | 4.15 ± 0.36^c | 3.17 ± 0.02^d |
| Liposomatic (LSI) | 2.15 ± 0.13^a | 2.29 ± 0.22^{ab} | 2.75 ± 0.34^c | 1.74 ± 0.21^{ad} |
| Bilesomatic (BSI) | 10.11 ± 0.76 | 10.58 ± 1.01 | 10.48 ± 1.28 | 10.29 ± 0.77 |

3.5. Gut micromorphology

The gut morphometric measurements of giant gourami juveniles are presented in Table 4. Fish gut micromorphology was significantly affected by different feeds. One-way ANOVA

results showed a significant effect of feed differences between groups in terms of foregut fold height ($F_{(3,8)} = 816.70, P = 0.135$), foregut fold width ($F_{(3,8)} = 129.34, P = 0.974$), height of the foregut ($F_{(3,8)} = 169.80, P = 0.882$), and microvillus height of the foregut ($F_{(3,8)} = 56.01, P = 0.285$). The Duncan's post hoc test demonstrated that the foregut fold height ($434.13 \pm 1.76 \mu\text{m}$), fold width ($53.23 \pm 0.88 \mu\text{m}$), enterocyte height ($27.42 \pm 0.42 \mu\text{m}$), and microvillus height ($2.79 \pm 0.45 \mu\text{m}$) were significantly higher ($P < 0.05$) in fish fed the KP3 diet than those fed the other diets. For the midgut, one-way ANOVA results showed a significant interaction among treatments in the case of fold height ($F_{(3,8)} = 5602.628, P = 0.055$), fold width ($F_{(3,8)} = 129.341, P = 0.974$), enterocyte height ($F_{(3,8)} = 169.809, P = 0.882$), and microvillus height ($F_{(3,8)} = 56.016, P = 0.285$). The Duncan's post hoc test showed that the fold height of the midgut ($324.96 \pm 1.43 \mu\text{m}$), fold width ($61.50 \pm 1.02 \mu\text{m}$), and enterocytes ($32.82 \pm 0.54 \mu\text{m}$) were significantly higher ($P < 0.05$) in fish fed the KP3 diet, whereas microvillus height was significantly higher in fish fed the KP2 diet (Table 4). Fish fed the KP3 diet showed a higher fold height of the hindgut ($F_{(3,8)} = 5459.01, P = 0.066$), fold width ($F_{(3,8)} = 271.94, P = 0.865$), enterocyte height ($F_{(3,8)} = 299.180, P = 0.821$), and microvillus height ($F_{(3,8)} = 253.57, P = 0.316$).

3.6. Pond water quality

The pond water quality values of the giant gourami juvenile rearing freshwater concrete ponds were recorded; water temperatures, dissolved oxygen (DO), total alkalinity, hardness, pH, and nitrates were in the range of typical values as given by WHO/FAO, as shown Table 5.

Table 4. Gut micromorphology of giant gourami juveniles fed different diets for 90 days. Mean values with different superscript letters in the same lane are significantly different ($P < 0.05$)

| | Foregut | | | | Midgut | | | | Hindgut | | | |
|-----|--|-----------------------------------|-----------------------------------|------------------------------------|-----------------------------|-------------------------|-------------------------|------------------------|-----------------------------|-------------------------|--------------------------|------------------------|
| | <i>hF</i> (μm) ^a | wF (μm) ^b | hE (μm) ^c | hMV (μm) ^d | <i>hF</i> (μm) | wF (μm) | hE (μm) | hMV (μm) | <i>hF</i> (μm) | wF (μm) | hE (μm) | hMV (μm) |
| KP1 | 336.17±5.59 ^a | 51.30±0.85 ^a | 26.21±0.43 ^a | 2.56±0.45 ^a | 227.50±0.16 ^a | 47.16±0.78 ^a | 24.31±0.31 ^a | 1.64±0.03 ^a | 213.92±0.19 ^a | 42.91±0.59 ^a | 20.22±0.25 ^a | 1.49±0.02 ^a |
| KP2 | 343.43±1.38 ^b | 52.14±0.86 ^b | 26.84±0.44 ^b | 2.77±0.45 ^b | 274.61±1.21 ^b | 58.12±0.97 ^b | 29.87±0.49 ^b | 1.85±0.01 ^b | 243.51±1.07 ^b | 53.01±0.88 ^b | 28.00±0.46 ^b | 1.64±0.01 ^b |
| KP3 | 434.13±1.76 ^c | 53.2±0.88 ^a | 27.42±0.42 ^c | 2.79±0.45 ^c | 324.96±1.43 ^c | 61.50±1.02 ^c | 32.82±0.54 ^c | 1.80±0.03 ^c | 305.60±1.35 ^c | 60.02±0.99 ^c | 29.54±0.49 ^c | 1.77±0.02 ^c |
| KP4 | 321.18±1.42 ^d | 50.20±0.83 ^{ad} | 25.62±0.79 ^d | 2.31±0.07 ^d | 228.45±1.01 ^{ad} | 56.95±0.95 ^d | 29.19±0.48 ^d | 1.69±0.01 ^d | 217.69±0.96 ^d | 61.64±1.03 ^d | 24.32±24.32 ^d | 1.40±0.01 ^d |

^a *hF* = fold height

^b wF = fold width

^c hE = enterocyte height

^d hMV = microvillus height

Tabel 5. The average values and range of water quality parameters in the concrete pond during the 90-days of experiment

| Water quality parameters | n | Mean ± SD | Range | WHO/FAO limits | References |
|--|----|--------------|-------------|----------------|---------------------------------|
| Water temperatures ($^{\circ}\text{C}$) | 45 | 28.01 ± 1.06 | 27 - 30 | 24-30 | FAO, 2012; Prokoso et al., 2021 |
| Dissolved Oxygen (mg L^{-1}) | 14 | 6.01 ± 0.14 | 5.80 – 6.20 | 3-5 | Syandri et al., 2020 |
| Total alkalinity (mg L^{-1} as CaCO_3) | 14 | 58.09 ± 3.33 | 52.5 - 62.5 | 120 | Boyd et al., 2016 |
| Hardness (mg L^{-1} as CaCO_3) | 14 | 66.34 ± 1.32 | 65 - 68.5 | 168 | Boyd et al., 2016 |
| pH | 14 | 7.48 ± 0.19 | 7.2 – 7.8 | 6.5 – 9.0 | Boyd et al., 2016 |
| Nitrates (mg L^{-1}) | 14 | 0.04 ± 0.01 | 0.03 – 0.05 | 0.2 - 219 | Boyd and Tucker, 1998 |

4. Discussion

The chemical analysis of fish feed is essential because it provides valuable information to aquafeed nutritionists concerned with readily available sources of proximate and amino acid compositions, including minerals and vitamins. This study investigated the nutritional quality of fish feed enriched with three different formulation products and one as a placebo. Dietary protein levels for giant gourami ranged from 19.68 to 21.70%. Overall, the crude protein content in the feed of this study was within the ranges observed by other authors^{51, 52, 54}. Giant gourami belongs to a group of herbivorous fish³⁹. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish^{53, 54}. Reducing the protein content of aquafeed is one method to increase continuous fish farming by diminishing feed costs and reducing the impact on the aquatic environment^{2,55}. The fat content of the feed ranged from 3.41 to 3.67%, which is similar to the feed fat content for juvenile grass carp, *Ctenopharyngodon idella*⁵⁶, and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*⁵² and for rearing Rohu, *Labeo rohita*⁵⁷. At the same time, the carbohydrate content of all feed treatments ranged from 26.37 to 31.19%, and the energy total (kg calorie/100 g) was between 234.41 and 240.87. Although protein content as an energy source for the maintenance and growth of giant gourami is relatively low, energy can be acquired from either protein or nonprotein sources, i.e., fat and carbohydrates.

In the present study, commercial fish feed was enriched with natural sources, i.e., formulated products of mature coconut water and palm sap sugar fermented with various fungi (*Aspergillus niger*, *Rhizopus oligosporus*, and *Saccharomyces cerevisiae*). This method is a new approach that we have developed to improve the feed quality, feed efficiency, and growth rate of giant gourami. In this study, we found that supplementing feed with newly formulated products can increase feed nutrition. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA¹⁷, iodine and selenium¹⁰, methionine¹⁸, fish oil^{19,11}, and soybean oil²⁰. In addition, the provision of feed has been supplemented with probiotics²¹, glycine, and prebiotics²². In this study, mature coconut water and palm sap sugar solution fermented with various fungi were used to supplement fish feed. In addition to coconut water and palm sugar, mushrooms also play a role in increasing feed nutrition. However, it's better to use *Rhizopus oligosporus*. As in the present study, Varzakas (1998) and Vong et al. (2018) showed that *Rhizopus oligosporus* can produce various extracellular enzymes. *Aspergillus* sp. has a high capacity to degrade antigenic proteins, including

carbohydrases, proteases, lipases, and phosphatases, when used for fermenting plant-sourced fish feed ingredients^{12,58}. *Saccharomyces cerevisiae* is one of the acclaimed microorganisms. Its effectiveness is due to its useful composition, such as “ β -glucans, nucleic acids, mannan oligosaccharides and chitin,” which are used for fermented ingredients^{7,59}.

The composition of amino acids can be used to judge the quality of feed. In the present study, feed supplemented with different formulated products, leucine, arginine, and glutamic acid were the most abundant FAAs. Similarly, in other studies on fish feed, such as feed for largemouth bass, *Micropterus salmoides*, the feeds were supplemented with glycine, prebiotics, and nucleotides in a soybean meal-based diet²². Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid⁶⁰, and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein⁶¹. Apparently, supplementing feed with different ingredients is common, and in other species, leucine, arginine, and glutamic acid were the most abundant FAAs. Conversely, methionine levels were low in all experimental feeds. Methionine is one amino acid that must be available in fish feed because methionine is needed to protect body cells from stress. For optimal growth of juvenile hybrid grouper, 1.89% methionine is required in the feed¹⁸. The experimental feed contained 0.18–0.30% methionine, but whether this amount is sufficient for the needs of giant gourami is poorly understood.

In the current study, the nonessential amino acid compositions were slightly higher than the essential amino acid compositions in all the experimental diets. The KP3 diet was higher than the other diets. In contrast, the essential amino acids of fish feed for snubnose pompano were slightly higher than the nonessential amino acids⁶¹. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu et al.⁶¹ reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet⁶⁰ and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet²². In the present study, this difference in FAA content is caused by various mushrooms used in the formulated products.

Giant gourami juveniles fed the KP3 diet showed higher levels of glutamic acid, aspartic acid, leucine, and lysine and lower levels of tyrosine, methionine, histidine, tryptophan, and cystine in their carcasses than other diets. The carcasses of giant gourami fed the KP3 diet showed the highest sum of FAAs compared to cultured fish fed the KP1, KP2, and KP4. The differences in the FAA profile in the whole-body carcasses of giant gourami could be related to the fungus type used in the formulated products for enriched feed. Each type of mushroom has a different function depending on the fermented fish feed ingredients and is correlated

with the whole-body carcass amino acids ^{12,58}. The FAA profile differences could be related to different aspects, such as diet composition ⁶², dietary protein level ⁶³, and methionine levels in the diet ¹⁸, including the water quality of the ponds ⁶⁴. This study does not analyse the relationship between growth performance and FAA profile or pond water quality. Several authors have reported that the physiological parameters of water quality and animal body composition are usually interrelated ^{61,65,66}. The present study did not examine whether difference in FAAs in the whole-body carcass is correlated to pond water quality.

The lower weight gain of fish fed the KP1 diet compared to fish fed the KP2, KP3, and KP4 diets shows that a deficiency of either fungus in the formulated product for the enriched diet could lower the protein content and related sum amino acids, leading to the inhibition of giant gourami growth. In addition, it also affects feed intake and feed conversion ratios. The low protein efficiency ratio and daily growth coefficient in fish provided insufficient KP1 diet were perhaps ascribed to an amino acid imbalance. The amino acid content of the KP2, KP3, and KP4 diets increased, ranging from 16.88% to 17.91% after fermentation. The increase may be due in part to the increased protein content in the KP2, KP3, and KP4 diets, which was in line with the results of ^{58,12}, who found that *Aspergillus niger* and *Aspergillus awamori* fermentation increased the amino acid content of soybean meal by 2.56% and 15.56%, respectively. In addition, [Dawood et al.](#)³² stated that the essential amino acid profile was changed after fermentation by *Saccharomyces cerevisiae*. This might result from the different fungi used having different utilization patterns for amino acids in this study. It influences the growth performance and nutrient utilization of giant gourami juveniles. We found that the methionine proportion was lower in the diets in the current study. In addition, methionine is an essential amino acid that plays a unique role in protein structure and metabolism ¹⁸. It is possible that *Aspergillus niger*, *Rhizopus oligosporus*, and *Saccharomyces cerevisiae* fermentation promoted the conversion of specific amino acids to methionine. However, the exact mechanisms need to be studied further.

In the present study, feed enrichment with different formulated products did not affect HSI or LSI except in the KP3 diet. Whereas VSI is influenced by differences in diet, it did not affect BSI. The condition factor of largemouth bass, *Micropterus salmoides* (1.49–1.52%), fed enriched 1–2% EPA + DHA ¹⁷ was different from the value (0.68) reported by ⁶⁷ for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, ⁶⁸ reported condition factor values ranging from 1.52 to 2.95 and an HSI between 1.4 and 1.5 for *Lates calcarifer* under different feeding rates (3–9% body weight d⁻¹). [Barbosa et al.](#)⁶⁹ reported VSI and LSI values of 2.24 and 3.86, respectively, for

Centropomus parallelus fed a commercial diet. On the other hand, Syed et al.⁶⁵ also reported HSI and VSI values of 3.41 and 4.90, respectively, for *Oreochromis niloticus* at different levels of aloe vera extract as feed additives. In our study, the VSI of *O. goramy* ranged from 3.17 to 4.15, and the LSIs were between 1.74 and 2.75, both higher than those recorded at different stocking densities of *O. goramy*⁴⁰. The high content of visceral fat observed in fish fed the KP3 diet might be explained by the diet having fat contents that exceed the needs of giant gourami juveniles and by the reduced energy expenditure of fish that are confined to rearing frame nets. Therefore, further analysis is necessary to determine the optimum dosage of the formulated product for the enrichment of feed to improve the growth performance of *O. goramy*.

For fish, the gut plays a significant role in absorbing nutrients, which is closely related to feed utilization^{18,70}. Rossi et al.,²² demonstrated that the development of enterocytes affected the nutrient-absorbing efficiency of the gut of *Micropterus salmoides*. Feeding *Lates calcarifer* juveniles with the same basal diet supplemented with 1% probiotic yeast, *Saccharomyces cerevisiae*, and lactic acid bacteria, *Lactobacillus casei*, revealed a higher number of gut mucosal goblet cells and increased microvillous length⁷¹. In contrast, substituting as much as 12.5–25% soya protein concentrate with lupin (*Lupinus albus*) meal in carp (*Cyprinus carpio*) diets does not significantly affect the villi length and villi width of the gut⁷². In the current study, enriched feed with products supplemented from coconut water, palm sap sugar, and fungus significantly affected the micromorphology and gut size. The fold height, fold width, enterocyte height, and microvilli of fish fed the KP3 diet were higher than those of fish fed the KP1, KP2, and KP4 diets. The KP3 diet is a relevant formulated product to enrich commercial feed to promote the development of the gut in animal experiments, which may somewhat describe the significant growth performance and feed efficiency used in this study.

Furthermore, the micromorphology gut size of giant gourami is smaller than that of juvenile hybrid grouper¹⁸, turbot, *Scophthalmus maximus*¹², largemouth bass, *Micropterus salmoides*²², and common carp, *Cyprinus carpio*⁷². The trophic food habits of fish may also affect the gut's hF, wF, hE, and hMV size because these habits are correlated with the digestibility coefficient. Under natural conditions, giant gourami is an herbivorous fish, while grouper, largemouth bass, and turbot are predatory fish, and common carp are omnivorous. Whether giving fish from different trophic levels the same diet affects the size of gut hF, wF, hE, and hMV is poorly understood.

5. Conclusion

This study concluded that feed enriched with newly various formulated products made from mature coconut water, palm sap sugar, and mushroom substantially affected the amino acid composition of the diet and whole-body carcass. It also affected giant gourami growth performance, feed utilization, body indices, and gut micromorphology. The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass and growth. Thus, our study also informs fish farmers about culturing good quality giant gourami and fulfilling nutrition requirements for food security.

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This project contains the following underlying data:

- Table 1. Raw data proximate composition of diets
- Table 2. Raw data amino acids composition in the diets enriched
- Table 3. Raw data proximate composition of whole body carcass
- Table 4. Raw data amino acids composition of whole body carcass
- Table 1a. Raw data growth for 0, 30, 60, and 90 days.
- Table 1b. Raw Data growth performance, FCR, FCE_90 days_giant gourami
- Table 2. Raw data proximate composition carcass of gurami sago after 90-days
- Table 3. Raw data proximate composition of diets
- Table 4. Raw data Composition of fatty acids and total lipid in the diets enriched
- Table 5. Raw data Composition of fatty acids of the carcass

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Declaration of Competing Interest

We, as the authors of this article, disclosed no competing interests.

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