Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Article submission received

5 messages

editorial@f1000research.com <editorial@f1000research.com> Sun, Jul 31, 2022 at 4:28 PM
To: azrita31@bunghatta.ac.id

Dear Azrita

Thank you for submitting your manuscript:

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

Funders: you have stated during the submission process that this work has been funded by:
The Ministry of Education, Culture, Research and Technology of the Republic of Indonesia (076/E5/PG.02.00. PT/2022)

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Before doing anything else, we need you to suggest **at least 5** suitable reviewers to peer review your manuscript following publication (in accordance with our publishing model), should it be accepted. We ask that authors do not contact reviewers directly about the peer review process. We will need your reviewer suggestions before we can publish the article so we recommend identifying them now via your Suggest Reviewers page for this article. You can also access this page via the article's record at My Research >> Submissions.

Please quote the article number 124706 in any correspondence.

Kind regards

The F1000Research Team

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azrita ubh <azrita31@bunghatta.ac.id> Tue, Sep 6, 2022 at 11:29 AM
To: editorial@f1000research.com

Dear
F1000 Research Team

On July 31, 2022, we submitted a manuscript entitled: Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, Osphronemus goramy (Lacepède, 1801), juveniles.

The first author is Azrita Undefined, citing article number 124706 in correspondence. May we get information from the F1000 Research team about the manuscript process?

Best regards

Azrita

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>
>

F1000.Research <research@f1000.com> Tue, Sep 6, 2022 at 8:15 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

We sent a response to your email address on 19 August. It was from our 'editorial@f1000research.com' email address.

Could you kindly checked your spam mail and confirm whether it arrived ok? The reason I ask is that if it is there, this is the quickest way for you to have the email. (I will need to look into the technical aspect of getting our system to resend the email if you have not received it.)

I look forward to hearing back.

Thanks.

Regards

Jonathan

F1000 Research editorial team

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Tuesday, September 6, 2022 5:30 AM
To: F1000.Research.Editorial <editorial@F1000Research.com>
Subject: Re: Article submission received

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Information Classification: General

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azrita ubh <azrita31@bunghatta.ac.id> Mon, Sep 12, 2022 at 9:53 PM
To: "F1000.Research" <research@f1000.com>

Dear
Jonathan Haynes
The Editorial Team, F1000 Research

We received your email on August 19, 2022, regarding manuscript No. 124706. We state that manuscript No. 124706 analyzes focused on the proximate composition, amino acids in diets, and whole body carcass compositions of amino acids. On the other hand, we also analyzed growth coefficient, body indices, and gut micromorphology. The product dosage formulated for feed enrichment in manuscript No. 124706 is 150 ml/kg of feed.

While on an article published in No. 74092, we are focused on analyzing the proximate composition of the diet and carcass. Fatty acids composition on diets and body carcasses, the nutritional quality of lipids (atherogenic and thrombogenic index). Including the growth performance of giant gourami with different parameters analyzed compared to manuscript No. 124706. The product dosage formulated for feed enrichment in manuscript No. 74092 was 300 ml/kg of feed.

Furthermore, an article recently, in the IP Conference Series: Earth and Environment Science, entitled: “Enrichment of commercial feed with new formula products on the growth, mortality, and yield of the giant gourami Osphronemus goramy.” We emphasize that the parameters we analyzed are not the same as in an article published in No. 74092 and manuscript No. 124706.

Thus, our explanation can be considered so that manuscript No. 124706 can be processed by editor team F1000 Research.

Best Regards

Azrita

On Tue, Sep 6, 2022 at 8:16 PM F1000.Research <research@f1000.com> wrote:

> Hi Azrita
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> We sent a response to your email address on 19 August. It was from our ‘editorial@f1000research.com’ email address.

Could you kindly checked your spam mail and confirm whether it arrived ok? The reason I ask is that if it is there, this is the quickest way for you to have the email. (I will need to look into the technical aspect of getting our system to resend the email if you have not received it.)

I look forward to hearing back.

Thanks.

Regards

Jonathan

F1000 Research editorial team

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F1000.Research <research@f1000.com> Tue, Sep 13, 2022 at 10:08 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

Thanks for your email. We are duly considering what you say and I will come back to you very shortly.

Kind regards

Jonathan

F1000 Research editorial team

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Monday, September 12, 2022 3:54 PM
To: F1000.Research <research@f1000.com>
Subject: Re: Article submission received

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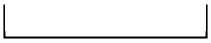

Information Classification: General

Information Classification: General

Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Your article submission 124706

4 messages

editorial@f1000research.com <editorial@f1000research.com> Fri, Aug 19, 2022 at 5:45 PM
To: azrita31@bunghatta.ac.id

Dear Azrita

Thank you for the article you have submitted to F1000, entitled, “Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, Osphronemus goramy (Lacepede 1801), juveniles”. We have read the piece with interest.

I am writing to you now about an enquiry relating to the article.

Of course, you published an earlier article through F1000, in November 2021, entitled: 'The utilisation of new products from water coconut, palm sap sugar, and fungus to increase nutritional feed quality, feed efficiency, growth, and carcass of gurami sago (Osphronemus goramy, Lacepede 1801) juvenile”.

We notice that you also published an article recently, in June this year, in the IP Conference Series: Earth and Environment Science, entitled: “Enrichment of commercial feed with new formula products on the growth, mortality and yield of the giant gourami Osphronemus goramy”.

We would be most grateful to you for confirming the main differences between the new F1000 submission and the other two articles. Also, can you confirm that the new article is not 'salami-slicing' done on the research?

I look forward to hearing back.

Thank you in advance.

Kind regards

Jonathan Haynes

The Editorial Team, F1000 Research

azrita ubh <azrita31@bunghatta.ac.id> Wed, Sep 7, 2022 at 10:05 AM
To: editorial@f1000research.com

Dear
Jonathan Haynes
The Editorial Team, F1000 Research

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While on an article published in No. 74092, we are focused on analyzing the proximate composition of the diet and carcass. Fatty acids composition on diets and body carcasses, the nutritional quality of lipids (atherogenic and thrombogenic index). Including the growth performance of giant gourami with different parameters analyzed compared to manuscript No. 124706. The product dosage formulated for feed enrichment in manuscript No. 74092 was 300 ml/kg of feed.

Furthermore, an article recently, in the IP Conference Series: Earth and Environment Science, entitled: "Enrichment of commercial feed with new formula products on the growth, mortality, and yield of the giant gourami Osphronemus goramy." We emphasize that the parameters we analyzed are not the same as in an article published in No. 74092 and manuscript No. 124706.

Thus, our explanation can be considered so that manuscript No. 124706 can be processed by editor team F1000 Research.

Best Regards

Azrita

[Quoted text hidden]

F1000.Research <research@f1000.com> Tue, Sep 13, 2022 at 11:36 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

We have discussed your reply in detail and so I am emailing you now with our response.

To avoid confusion related to the earlier published work – including of course, the study published by F1000 Research - we would ask that when describing the new study that your article is about, you include a link to the earlier F1000 piece, saying the difference/s in aim this time; and that you include how the product dosage for feed enrichment was 300ml /kg in the earlier study/studies, not 150 ml /kg (the dosage this time). In this way, one will avoid the outcome of peer reviewers needing to do a 'deep dive' to establish the overall position.

We note that there are various references in the existing piece to 'newly formulated products' (which are the products you are testing on the gourami) – for example, you say that to date there is no information on the products you describe being used to supplement fish feed; and that their effect on factors like fish growth rate has not yet been analysed. These references are in the Introduction; and the paragraph starting 'In the present study, commercial fish feed was enriched with natural resources…' in the Discussion has a similar reference (the first sentence of the Conclusion is also framed in a similar way). Along with the adjustment suggested in the first paragraph above, these statements and references would also need some alteration, to reflect the way some work in this area has recently been done by yourselves, with certain outcomes.

We will be able to go ahead with further processing of the manuscript, if hopefully you are able to make suitable alterations.

I would ask that you kindly make the adjustments within a copy of the existing manuscript, and highlight in yellow (or by a similar means), the sentences that have been tweaked. We can then take in the changes to the manuscript here. (Alternatively, if you wanted to send in altered versions of the relevant paragraphs or sentences, indicating where they occur in the manuscript, this would also work.)

We hope that you find the suggested small adjustments agreeable.

I much look forward to hearing back.

With best wishes

Jonathan

F1000 Research editorial team

[Quoted text hidden]
[Quoted text hidden]

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> The Editorial Team, F1000 Research

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azrita ubh <azrita31@bunghatta.ac.id> Thu, Oct 6, 2022 at 12:27 PM
To: "F1000.Research" <research@f1000.com>

Dear
Jonathan Haynes
The Editorial Team, F1000 Research

Thank you for your email on September 13, 2022; we have made changes to manuscript No. 124706. The changes we made are:

1. In the introduction, Research on the use of a new formula product containing coconut water and palm sugar fermented with various fungi at a dose of 300 ml/kg of feed has been reported by Azrita et al. (2021). Their newly formulated product can increase fatty acid levels in food and whole body carcasses. It also improves the growth performance and feeds efficiency of giant gourami (Osphronemus gourami) (highlighted in yellow).
2. The effect of the new formulation product at a dose of 150 ml/kg of feed on the diet and the body meat's proximate and amino acid composition has not been analyzed. Correspondingly, the relationship between thermal growth coefficient and condition factors, daily growth coefficient, feed utilization coefficient, including body index parameters, and gut micromorphology of giant gourami, have also not been analyzed (highlighted in yellow).
3. In the method, we also state that the dose used is 150 ml/kg of feed, including the analyzed parameters, namely the amino acid composition of the feed and the fish carcass. At the same time, we also analyzed the growth coefficient and feed utilization, the relationship between thermal growth coefficient and condition factor, daily growth coefficient, feed intake and protein efficiency ratio, body indices, and gut micromorphology (highlighted in yellow). In manuscript No. 74092, Azrita et al. (2021) did not report this parameter.
4. We have also written the comments in the discussion according to the parameters analyzed in this study.
5. The conclusions we presented has revised and are not the same as those reported in article No. 74092, published by F1000 Research.
6. We state that manuscript No. 124706 is not similar to manuscript No.74092.
7. Manuscript No. 124706 has a revised attached

Best regards

Azrita

[Quoted text hidden]

Revised_ Manusript No 124706_ Oct 6, 2022_ Effect of feed enriched by products formulated.doc
9150K

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

Azrita undefined[a*], Hafrijal Syandri[b], Netti Aryani[c], Ainul Mardiah[d]

[a]Department of Biology Education, Faculty of Education Universitas Bung Hatta, West Sumatera, 25133 Indonesia
[b]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Bung Hatta Padang, West Sumatera, Indonesia-25133.
[c]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, 28293, Indonesia.
[d]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Nahdlatul Ulama Sumatera Barat, 28293, Indonesia.
Corresponding author: Azrita31@bunghatta.ac.id

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 on the growth coefficient, 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 × 1 m × 1 m; water volume 1.5 m^3/frame nets) in a freshwater concrete pond with 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 coefficient, feed utilization, body indices, and gut micromorphology of giant gourami juveniles. The thermal growth coefficient strongly correlates with the daily growth coefficient (r^2 = 91%). The KP3 diet contains a higher concentration of amino acids, which increased the growth coefficient, feed utilization, 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 rise[1] (FAO, 2018). The utilization of a variety of fish for aquaculture has now increased the need for commercial feed [2,3,4,5]. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge [2,6,7,8]. 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 [4,14,15,16]. Therefore, novel approaches have been developed to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA[17], iodine and selenium [10], methionine[18], fish oil[19,11], and soybean oil[20]. In addition, supplementing probiotics into the diet[21] and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied[22].

In recent decades, research on nutrition and feeds for giant gourami have garnered increasing interest [9,23,24]. 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 growth[23]. Juvenile giant gourami were fed diets supplemented with recombinant growth hormone at different protein levels[25]. There was an effect of different feeding rates on the specific growth rate and feed use[19]. The addition of artificial feed combined with tubifex worm increased the growth performance of giant gourami juveniles[26]. Whether using coconut water and palm sugar fermented with mushrooms has an effect on feed nutrition and body carcass is still not understood.

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[27,28,29,30]. 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[31,32,33,34]. Meanwhile, the fungus has been widely used in fermentation due to its ability to degrade antigenic proteins in fish feed ingredients[7,35,36]. Zhang et al. [37] 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 indigestion[38] .

On the other hand, Azrita et al.[9] have reported using new formulations of products containing coconut water and palm sap sugar that are fermented with various mushrooms with a dosage of 300 ml/kg feed. Their newly formulated products can increase fatty acid levels in the diet and whole body carcasses. Besides that, it also improves giant gourami (Osphronemus gourami) 's growth performance and feeds efficiency.

However, the effect of the new formulation products with a dosage of 150 ml/kg feed on the diet and body meat's proximate and amino acid composition has not yet been analyzed. In line with that, the relationships between thermal growth coefficient and condition factor, daily growth coefficient, and feed utilization coefficient, including body indices parameters, as well as gut micromorphology of giant gourami, have not yet been analyzed.

We hypothesized that commercial aquafeed added with different newly formulated products with the dosage of 150 ml/kg feed could improve the amino acids compositions of the aquafeed and whole body carcass, body indices, and gut micromorphology. Hence, this investigation's first purpose is to analyze the effect of various newly formulated products on the diet's proximate compositions, amino acid composition, and whole-body carcass. The second aim is to analyze the impact of newly formulated products on the growth coefficient and relation to thermal growth coefficient, body indices, and gut micromorphology in giant gourami juveniles.

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 meter 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 m^3 (2.0 × 1.0 × 1.0 m) PVC pipe (water volume of 1.5 m^3) were placed inside two freshwater concrete ponds with a size of 18 m^3 (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 proximate carcass composition were analyzed using standard AOAC methods[39]. The matter was dried to a constant weight at 105 °C. We used the standard Kjeldahl method to analyse crude protein (N × 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 Cohen[40].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. Nutrient utilization and body indices

The growth coefficients of animal experiments were measured by the thermal growth coefficient (TGC), daily growth coefficient (DGC), total feed intake (FI), and protein efficiency ratio (PER) of giant gourami was assessed using the following formulae:

TGC = [(final weight (g)) ⅓ – (initial weight (g)⅓] / (mean water temperature (°C) × duration of rearing period (day)] × 1000

DGC = (Wf⅓ – Wi⅓) / duration of rearing period (day) × 100

FI as feed (FIasfeed in g/fish/day) = Total feed fed / (n × t)

PER = wet weight gain / total protein intake

Three fish from each net frame were sacrificed and dissected immediately to determine the Condition factor (CF), Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) as given below:

CF = 100 × [weight of the juvenile (g) / Length of juvenile $(cm)^3$]

GSI = 100 × [viscera weight (g) / whole body weight (g)]

HSI = 100 × [liver weight (g) / whole body weight (g)]

VFSI = 100 × [visceral fat weight (g) / whole body weight (g)]

BSI = 100 × [Bile weight (g) / weight of liver]

2.7. 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 µm, 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 Figure 1.

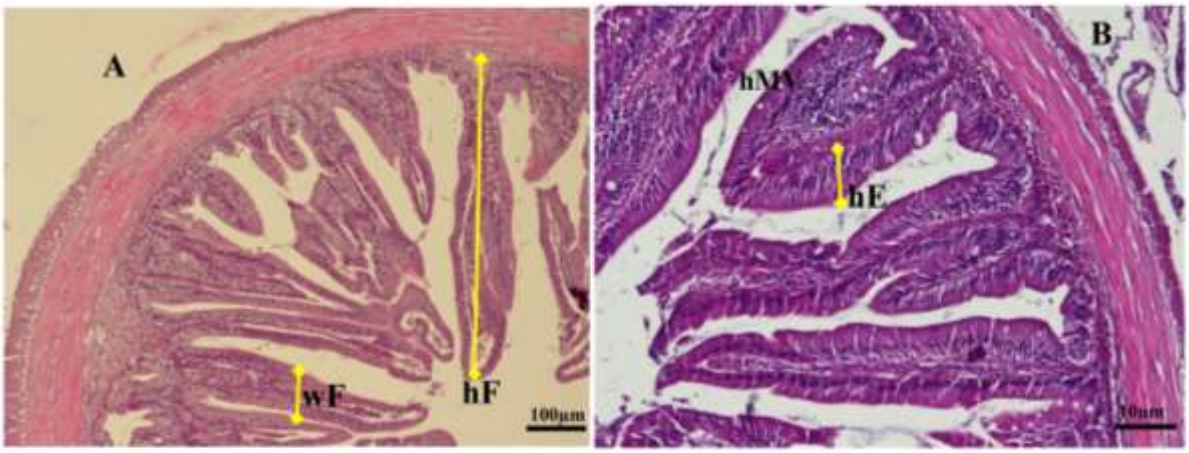

Figure 1. Transversal section photomicrographs of giant gourami juvenile foregut. Enteric section fish fed KP1 diet. (A) Fold height and fold width were analyzed in a lower magnification of objective lens of microscope (magnification × 100, (B) Enterocytes height and microvilli height were analyzed in a higher magnification of objective lens microscope (magnification × 200). hF = fold height, wF = fold width, hE = enterocyte height, hMV = microvillus height (hematoxylin and eosin).

2.8. 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 (Celcius scale) was used to measure water temperature. To measure water dissolved 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 (NO_3-N; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures[41].

2.9. Calculations and statistical method

The data from this study were reported as mean ± 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[42]. To create the figures was used Microsoft Office Professional Plus 2019.

3. Results

3.1. Proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products with the dosage of 150 ml/ kg of feed significantly affects 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 ± 0.17%) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate (31.19 ± 0.38%), crude fibre (2.82 ± 0.06%), and ash (6.67 ± 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 with a dosage of 150 ml/kg of feed 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 significant 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 ±

0.011%), nonessential amino acids (12.91 ± 0.004 ± 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 ± 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 ± 0.25	38.27 ± 0.01	37.59 ±0.16	38.41± 0.10
Crude protein	19.68 ± 0.41^{a}	20.27 ± 0.13^{b}	21.70 ± 0.18^{c}	20.44 ± 0.10^{d}
Crude lipid	3.41 ± 0.02^{a}	3.67 ± 0.13^{b}	3.50 ± 0.02^{ac}	3.48 ± 0.04^{ad}
Carbohydrate	26.37 ± 0.17^{a}	29.50 ± 0.54^{b}	31.19 ± 0.38^{c}	30.57 ± 0.06^{d}
Crude fibre	2.23 ± 0.05^{a}	2.36 ± 0.01^{b}	2.82 ± 0.06^{c}	2.45 ± 0.06^{d}
Ash	2.75 ± 0.03^{a}	6.66 ± 0.05^{b}	6.57 ± 0.04^{c}	6.67 ± 0.06^{d}
Energy total (kg calorie/100 g)	240.87 ± 0.38^{a}	234.41 ± 0.30^{b}	240.88 ± 0.74^{ac}	237.11 ± 0.43^{d}
Amino acid composition				
EAA				
Leucine	1.36 ± 0.01^{a}	1.42 ± 0.01^{b}	1.46 ± 0.01^{c}	1.36 ±0 .01^{d}
Isoleucine	0.76 ± 0.01^{a}	0.79 ± 0.01^{b}	0.81 ± 0.01^{c}	0.76 ± 0.01^{d}
Lysine	0.95 ± 0.01^{a}	1.10 ± 0.01^{b}	0.98 ± 0.01^{c}	1.20 ± 0.01^{d}
Valine	0.86 ± 0.01^{a}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}	0.89 ± 0.01^{d}
Threonine	0.79 ± 0.02^{a}	0.92 ± 0.01^{b}	1.04 ± 0.01^{c}	0.83 ± 0.01^{d}
Arginine	1.02 ± 0.01^{a}	1.19 ± 0.01^{b}	1.30 ± 0.01^{c}	1.03 ± 0.01^{d}
Phenylalanine	0.67 ± 0.01^{a}	0.93 ± 0.01^{b}	1.05 ± 0.01^{c}	0.77 ± 0.01^{d}
Tyrosine	0.43 ± 0.01^{a}	0.53 ± 0.00^{b}	0.57 ± 0.06^{c}	0.45 ± 0.01^{d}
Methionine	0.18 ± 0.01^{a}	0.26 ± 0.01^{b}	0.30 ± 0.01^{c}	0.21 ± 0.01^{d}
Histidine	0.40 ± 0.01^{a}	0.50 ± 0.01^{b}	0.57 ± 0.01^{c}	0.43 ± 0.01^{d}
Tryptophan	0.06 ± 0.01^{a}	0.11 ± 0.01^{b}	0.07 ± 0.00^{bc}	0.09 ± 0.01^{bd}
NEAA				
Alanine	0.85 ± 0.01^{a}	0.94 ± 0.01^{b}	0.87 ± 0.06^{c}	0.97 ± 0.01^{bd}
Serine	1.01 ± 0.01^{a}	1.12 ± 0.01^{b}	1.23 ± 0.01^{c}	1.01 ± 0.01^{d}

Glycine	1.15 ± 0.01[a]	1.32 ± 0.01[b]	1.29 ± 0.01[c]	1.19 ± 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 commercial added with a new formulation product significantly affected 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 ± 0.17%), fat (3.47 ± 0.03%), carbohydrates (31.18 ± 0.37%), and crude fibre (2.81 ± 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 whole-body meat. 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 provided 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 ± 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 ±0.16	64.51 ± 0.34	64.14 ± 0.33	64.24 ± 0.12
Crude protein	28.64 ± 0.28^{a}	28.07 ± 0.79^{ab}	28.85 ± 0.45^{c}	28.66 ± 0.44^{ad}
Crude fat	2.79 ± 0.03^{a}	2.88 ± 0.02^{b}	2.67 ± 0.04^{c}	3.00 ± 0.02^{d}
Carbohydrate	1.38 ± 0.01^{a}	1.99 ± 0.06^{b}	1.97 ± 0.09c	1.31 ± 0.02^{d}
Crude fibre	0.97 ± 0.02^{a}	0.68 ± 0.01^{b}	0.83 ± 0.02^{c}	0.95 ± 0.04^{d}
Ash	1.63 ± 0.02^{a}	1.70 ± 0.02^{b}	1.54 ± 0.01^{c}	2.11 ± 0.04^{d}
Energy total (kg calorie/100 g)	144.77 ± 1.58^{a}	155.48 ± 1.26^{b}	157.90 ± 0.91^{c}	149.60 ± 0.29^{d}
Amino acid composition				
EAA				
Leucine	2.13 ± 0.01^{a}	2.37 ± 0.01^{b}	2.42 ± 0.01^{c}	2.26± 0.01^{d}
Isoleucine	1.13 ± 0.01^{a}	1.25 ± 0.01^{b}	1.38 ± 0.01^{c}	1.19± 0.01^{d}
Lysine	2.77 ± 0.01^{a}	3.16 ± 0.02^{b}	3.88 ± 0.01^{c}	2.86 ± 0.01^{d}
Valine	1.26 ± 0.01^{a}	1.40 ± 0.01^{b}	1.32 ± 0.01^{c}	1.35 ± 0.01^{d}
Threonine	1.38 ± 0.02^{a}	1.49 ± 0.01^{b}	1.43 ± 0.01^{d}	1.48 ± 0.01^{d}
Arginine	1.58 ± 0.01^{a}	1.71 ± 0.01^{b}	1.63 ± 0.01^{c}	1.70 ± 0.01^{d}
Phenylalanine	1.02 ± 0.01^{a}	1.11 ± 0.01^{b}	1.08 ± 0.01^{c}	1.11 ± 0.01^{d}
Tyrosine	0.80 ± 0.01^{a}	0.84 ± 0.00^{b}	0.83 ± 0.01^{c}	0.85 ± 0.06^{d}
Methionine	0.15 ± 0.01^{a}	0.21 ± 0.01^{b}	0.18 ± 0.01^{c}	0.16 ± 0.01^{d}
Histidine	0.55 ± 0.01^{a}	0.56 ± 0.01^{ab}	0.59 ± 0.01^{ac}	0.57 ± 0.01^{d}
Tryptophan	0.08 ± 0.01^{a}	1.02 ± 0.01^{b}	1.08 ± 0.01^{ac}	0.06 ± 0.00^{d}
NEAA				
Alanine	1.86 ± 0.01^{a}	2.08 ± 0.01^{b}	2.92± 0.01^{c}	1.97 ± 0.01^{d}
Serine	1.28 ± 0.01^{a}	1.31 ± 0.01^{b}	1.26± 0.01^{c}	1.31 ± 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]
∑EAA	12.68± 0.003[a]	15.13 ± 0.005[b]	15.82± 0.001[c]	13.61 ± 0.008[d]
∑NEAA	12.91± 0.007[a]	14.32 ± 0.01[b]	15.69 ± 0.002[c]	13.50 ± 0.001[d]
∑AA	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 coefficient and survival

The growth coefficient and feed utilization of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant different between experimental diets in the case of thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), and daily growth coefficient ($F_{(3,8)} = 59.88$, $P = 0.288$). While, total feed intake (% BW day-1) ($F_{(3,8)} = 14.938$, $P = 0.56$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Figure 2).

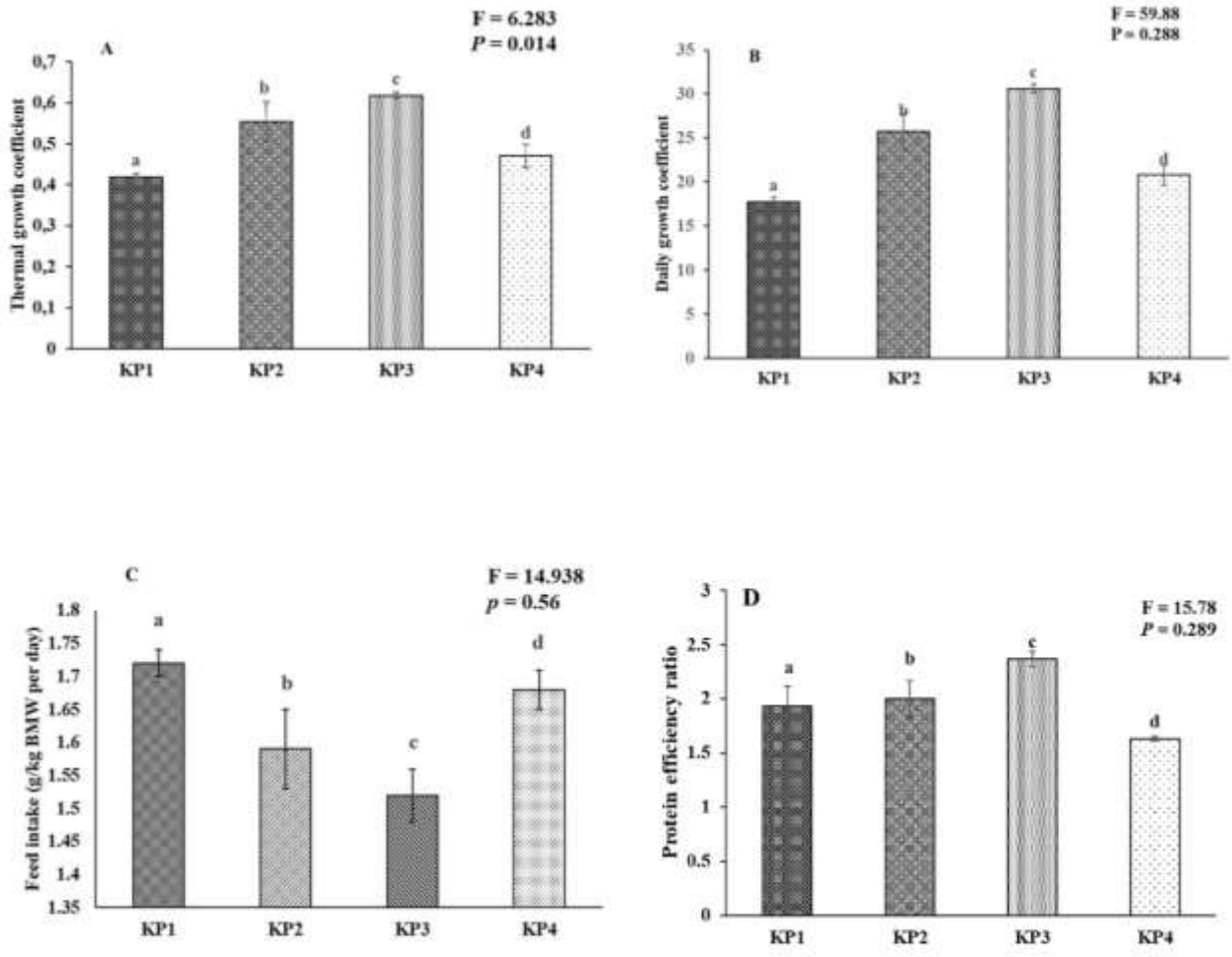

Figure 2. Growth coefficient and feed utilization of the giant gourami juveniles reared under different diets during 90 days of the experiment period, (A) thermal growth coefficient (TGC), (B) daily growth coefficient (DGC), (C) feed intake (FI), and (D) protein efficiency ratio (PER). The mean value and standard deviation (mean ± SD) are presented for giant gourami (n = 3). Different superscripts in the bar diagram of the giant gourami juvenile TGC, DGC, FI, and PER indicate significant differences among other diets ($P < 0.05$, One-way ANOVA Duncan Post-Hoc)

Furthermore, the thermal growth coefficient (TGC) has often been used to predict growth performance and fish farming production with fish-rearing water temperature. This study presents the relationship between thermal growth coefficient and condition factor, daily growth coefficient, and protein efficiency ratio (Figure 3). The thermal growth coefficient had strong relationships with the condition factor (r^2 = 0.777, figure 3A), daily growth coefficient (r^2 = 0.999, figure 3B), and protein efficiency ratio (r^2 = 0.749, figure 3D), while the thermal growth coefficient that had moderate relationship with the feed intake (r^2 = 0.699, figure 3C).

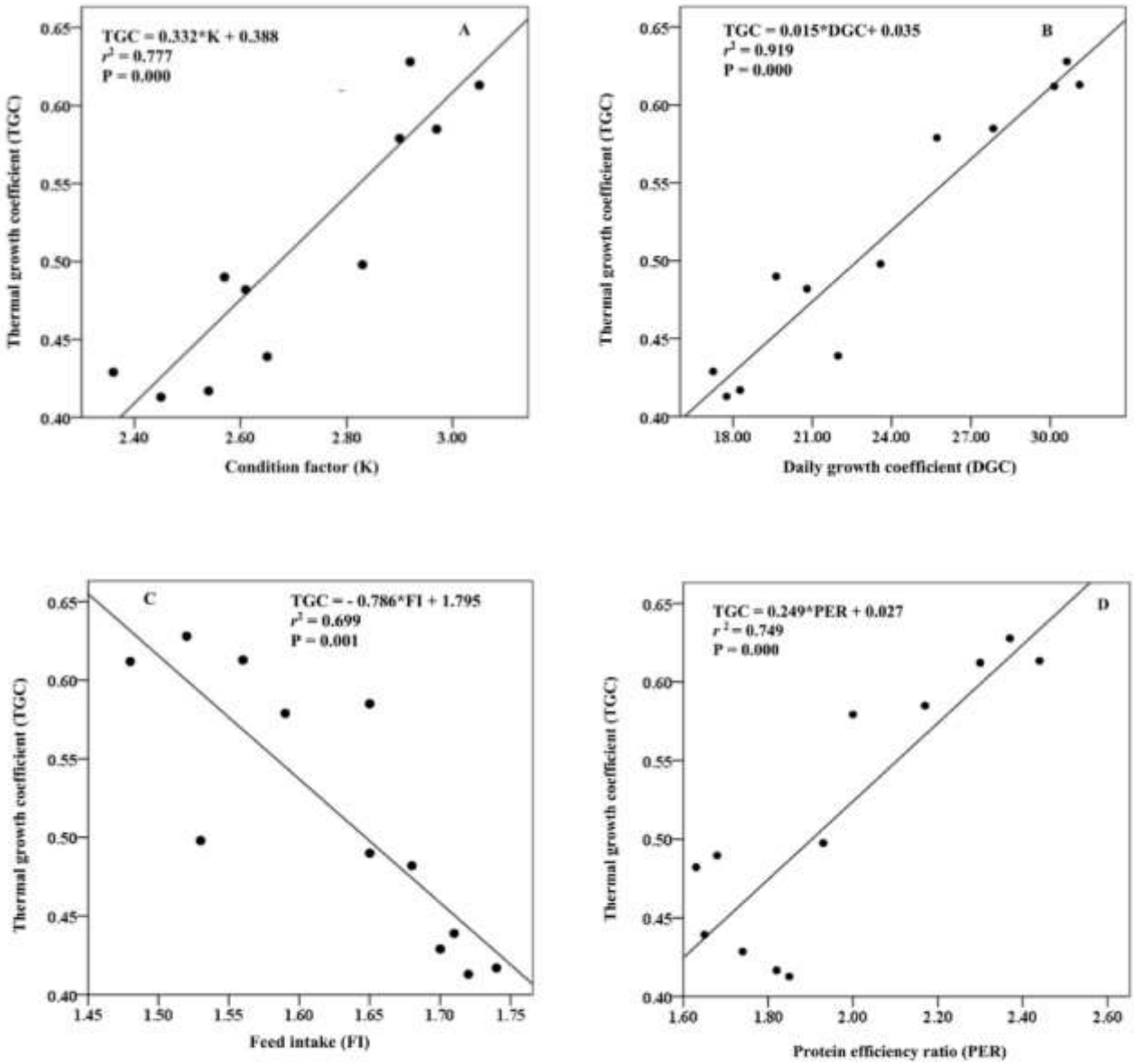

Figure 3. Relationship between thermal growth coefficient and condition factor (A), daily growth coefficient (B), feed consumption (C) and protein efficiency ratio (D) of giant gourami, fed diets supplemented with a new product formulated differently during 90 days.

3.4. Condition factor and body indices of giant gourami after 90 days of feeding

The condition factor was significantly different between diets ($F_{(3,8)}$ = 19.98, P = 0.566) in the present study. While the GSI, HIS, and VFSI displayed marginally significant differences between diets. The HIS 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). GSI was significantly ($F_{(3,8)}$ = 10.492, P = 0.243) in the KP3 diet and significantly among all different diets. The VFSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's Post-hoc test revealed that the HIS (1.30 ± 0.13%), GSI (4.15 ± 0.36%), and VFSI (2.75 ± 0.34%) were significantly higher (P < 0.05) in the KP3 diet than in the other diets. Meanwhile, BSI showed no significant difference (P > 0.05) among the treatment diets (Table 3).

Table 3. Mean (± SD) value condition factor 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}
Viscerosomatic index (GSI%)	3.20 ± 0.21^{a}	3.77 ± 0.09^{b}	4.15 ± 0.36^{c}	3.17 ± 0.02^{d}
Hepatosomatic (HIS%)	0.97 ± 0.05^{a}	1.06 ± 0.19^{ab}	1.30 ± 0.13^{c}	1.04 ± 0.12^{ad}
Visceral fat-somatic indexes (VFSI%)	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 ± 1.76 µm), fold width (53.23 ± 0.88 µm), enterocyte height (27.42 ± 0.42 µm), and microvillus height (2.79 ± 0.45 µm) 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 ± 1.43 µm), fold width (61.50 ± 1.02 µm), and enterocytes (32.82 ± 0.54 µm) 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			
	hF (µm)[a]	wF (µm)[b]	hE (µm)[c]	hMV (µm)[d]	hF (µm)	wF (µm)	hE (µm)	hMV (µm)	hF (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 (^{O}C)	45	28.01 ± 1.06	27 - 30	25-33	Prokoso et al.[43]
Dissolved Oxygen (mg L^{-1})	14	6.01 ± 0.14	5.80 – 6.20	3-5	Syandri et al.[44]
Total alkalinity (mg L^{-1} as $CaCo_3$)	14	58.09 ± 3.33	52.5 - 62.5	120	Boyd et al.[45]
Hardness (mg L^{-1} as $CaCo_3$)	14	66.34 ± 1.32	65 - 68.5	168	Boyd et al.[45]
pH	14	7.48 ± 0.19	7.2 – 7.8	6.5 – 9.0	Boyd et al.[45]
Nitrates (mg L^{-1})	14	0.04 ± 0.01	0.03 – 0.05	0.2 - 219	Boyd and Tucker[46]

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[47,48,49]. Giant gourami belongs to a group of herbivorous fish[50]. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish [51,49]. 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[2,52]. 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*[53], and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*[48] and for rearing Rohu, *Labeo rohita*[54]. 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, the 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), the dose used is 300 ml/kg of feed. This method is a new approach that has been developed by Azrita et al.[9] to improve feed nutrition and whole body carcasses, covering fatty acids, atherogenic index and thrombogenic, feed efficiency, and growth performance of giant gourami. We continue this study by reducing the feed dose to 150 ml/kg. This study found that supplementing feed with newly formulated products can increase feed nutrition, covering amino acids in diet and body meat, and the growth coefficient of giant gourami. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[12], fish oil [19, 11], and soybean oil[20]. In addition, the provision of feed has been supplemented with probiotics[21], glycine, and prebiotics[22]. 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 [55] and Vong et al. [56] 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[12,57] . *Saccharomyces cerevisiae* is one of the most 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[7,58].

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 diet[22]. Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid[59], and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein[60]. 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 feed[18]. 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 acids[60]. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu et al. [60] reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet[59] and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet[22]. 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[12,57]. The FAA profile differences could be related to different aspects, such as diet composition[61], dietary protein level[62], and methionine levels in the diet[18], including the water quality of the ponds[63]. 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[64,60]. The present study did not examine whether the 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 Jannatullah et al.[57] and Li et al.[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.[36] 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, the thermal growth coefficient (TGC) strongly correlated with the daily growth coefficient (DGC). Because faster daily fish growth requires a quality diet and

constant water temperature during the rearing period, in this study, water temperature ranged from 27 to 30 ºC, and dissolved oxygen was between 5.8 and 6.2 mg /L. According to Besson et al.[65], higher daily energy availability in the diet can lead to faster-growing fish, which is supported by constant water temperature and higher daily oxygen levels. The thermal growth coefficient had an essential change in environmental value [66]. Therefore, very important to keep the water temperature and dissolved oxygen constant in the aquaculture locations. At the same time, 78% of TGC values were determined by the condition factor connected to whole body weight and the total fish length. TGC of Atlantic cod, Gadus morhua, is influenced by body size and condition factors[67].

In this study, a higher value of TGC was detected in fed fish KP3; the effect is the daily growth coefficient, and the protein efficiency ratio is better. Conversely, that decreasing TGC has two effects, i.e., the growth rate of fish slow and lowered daily feed intake. Many scientists state that in aquaculture operations, net yield (kg/m^3) depends upon TGC fluctuation, feed intake, and daily oxygen consumption[65,68,69].

In the present study, feed enrichment with different formulated products did not affect HIS or VFSI except in the KP3 diet. Whereas GSI 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 Arriaga-Hernandez et al.[70] for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, Hassan et al. [71] 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.[72] 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.[64] 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* [44]. 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[18,73]. 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 length[74]. 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 gut[75] . 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 grouper[18], turbot, *Scophthalmus maximus*[12], largemouth bass, *Micropterus salmoides*[22] , and common carp, *Cyprinus carpio*[75]. 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

The present investigation observed that feed was enriched with newly formulated products made from mature coconut water and palm sap sugar, which fermented with various mushrooms with a dose of 150 ml/kg substantially affected the amino acid composition of the diet and whole-body carcass of giant gourami juveniles. It also affected the growth coefficient, feed utilization, body indices, and gut micromorphology size. The thermal growth coefficient had a strong relationship with the daily growth coefficient (r^2 = 91%) and a moderate relationship with the feed intake (r^2 = 69%). The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass, growth coefficient, body indices, and the ability of the intestines for feed absorb. 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 of the experimental diets' proximate composition
- Table 2. Raw data of amino acid of feed experimental
- Table 3. Raw data of whole body carcass proximate composition
- Table 4. Raw data of amino acid of whole-body carcass
- Table 5. Daily growth coefficient, feed utilization and body indices of giant gourami after 90 days of feeding.
- Table 6. Raw data gut micromorphology of giant gourami juveniles fed different diets for 90 days
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- Declaration of Competing Interest
- We, as the authors of this article, disclosed no competing interests.
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We hope that you find the suggested small adjustments agreeable.

I much look forward to hearing back.

With best wishes

Jonathan

F1000 Research editorial team

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> Dear Azrita
>
> Thank you for the article you have submitted to F1000, entitled, “Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, Osphronemus goramy (Lacepede 1801), juveniles”. We have read the piece with interest.
>
> I am writing to you now about an enquiry relating to the article.
>
> Of course, you published an earlier article through F1000, in November 2021, entitled: 'The utilisation of new products from water coconut, palm sap sugar, and fungus to increase nutritional feed quality, feed efficiency, growth, and carcass of gurami sago (Osphronemus goramy, Lacepede 1801) juvenile”.
>
> We notice that you also published an article recently, in June this year, in the IP Conference Series: Earth and Environment Science, entitled: “Enrichment of commercial feed with new formula products on the growth, mortality and yield of the giant gourami Osphronemus goramy”.
>
> We would be most grateful to you for confirming the main differences between the new F1000 submission and the other two articles. Also, can you confirm that the new article is not 'salami-slicing' done on the research?
>
> I look forward to hearing back.
>
> Thank you in advance.
>
> Kind regards
>
> Jonathan Haynes
>
> The Editorial Team, F1000 Research

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azrita ubh <azrita31@bunghatta.ac.id> Thu, Oct 6, 2022 at 12:27 PM
To: "F1000.Research" <research@f1000.com>

Dear
Jonathan Haynes
The Editorial Team, F1000 Research

Thank you for your email on September 13, 2022; we have made changes to manuscript No. 124706. The changes we made are:

1. In the introduction, Research on the use of a new formula product containing coconut water and palm sugar fermented with various fungi at a dose of 300 ml/kg of feed has been reported by Azrita et al. (2021). Their newly formulated product can increase fatty acid levels in food and whole body carcasses. It also improves the growth performance and feeds efficiency of giant gourami (Osphronemus gourami) (highlighted in yellow).
2. The effect of the new formulation product at a dose of 150 ml/kg of feed on the diet and the body meat's proximate and amino acid composition has not been analyzed. Correspondingly, the relationship between thermal growth coefficient and condition factors, daily growth coefficient, feed utilization coefficient, including body index parameters, and gut micromorphology of giant gourami, have also not been analyzed (highlighted in yellow).
3. In the method, we also state that the dose used is 150 ml/kg of feed, including the analyzed parameters, namely the amino acid composition of the feed and the fish carcass. At the same time, we also analyzed the growth coefficient and feed utilization, the relationship between thermal growth coefficient and condition factor, daily growth coefficient, feed intake and protein efficiency ratio, body indices, and gut micromorphology (highlighted in yellow). In manuscript No. 74092, Azrita et al. (2021) did not report this parameter.
4. We have also written the comments in the discussion according to the parameters analyzed in this study.
5. The conclusions we presented has revised and are not the same as those reported in article No. 74092, published by F1000 Research.
6. We state that manuscript No. 124706 is not similar to manuscript No.74092.
7. Manuscript No. 124706 has a revised attached

Best regards

Azrita

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Revised_ Manusript No 124706_ Oct 6, 2022_ Effect of feed enriched by products formulated.doc
9150K

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azrita ubh <azrita31@bunghatta.ac.id>

Manuscript 124706 conditionally accepted for publication

14 messages

editorial@f1000research.com <editorial@f1000research.com> Wed, Oct 12, 2022 at 6:26 PM
To: azrita31@bunghatta.ac.id

Dear Azrita

'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, Osphronemus goramy (Lacepède, 1801), juveniles'
Undefined A, Syandri H, Aryani N and Mardiah A

Thank you for your submission to F1000Research. We have noted a few issues with your manuscript (below) – once these are addressed we will be pleased to accept your article for publication

Methods: In order to ensure a minimum level of reproducibility of your methods, we require adequate information about the techniques used in your study. Comments in the edited document will help to guide you but are not comprehensive. Please avoid the use of citation shortcuts, such as "[technique] was performed according to the methods of [reference]" without giving complete details of the methods used, including reagents used, time frames, etc. and any allowances for controlling bias and unwanted sources of variability. We encourage authors to deposit step-by-step descriptions of their protocols on protocols.io and include the persistent DOI in the methods section of the manuscript.

Reviewers: As you know, F1000Research operates an author-driven publication model. This means that you will be responsible for suggesting suitable reviewers, whom we invite on your behalf, giving you an opportunity to ensure that appropriate experts review your article. Our transparent peer review process means that the peer review reports, together with the reviewers' names, will be published alongside your article.

To avoid delay to the publication process, we need you to provide us with at least five potential reviewers who meet our reviewer criteria before we can publish your article - please be aware that it is likely we will need to request further reviewer suggestions after publication. Please go to your Suggest Reviewers page, where you will find a useful tool to help you find reviewers; use this page to track the progress of the peer review process for your article. You can access this page directly via the article's record under My Research >> Submissions. See also our reviewer criteria and tips for finding reviewers.

Please remember that suggested reviewers should have appropriate level of experience and the right expertise to judge your article; they must be able to provide an unbiased report (e.g. they must not be recent collaborators or colleagues in your institute). All reviewer suggestions are checked by the editorial team and will be rejected if they do not meet our criteria.

Payment: As F1000Research is open access, we will require payment of the Article Processing Charge (APC) to be able to complete the processing of your submission. The APC is $1350.00 (ex. VAT) after any discounts you are eligible for have been applied. **Please provide us with the details of the individual/organization taking responsibility for paying the fee as soon as possible.** Please sign in with the credentials you used to submit the article or you will not be able to access this page. Our Accounts department will be in touch regarding payment.

We have also lightly copyedited your article - please download the document and check you are happy with the amendments and **then address the queries detailed in the margin. Please return your revised manuscript to the e-mail address above**. Please note that this is your final opportunity to make any changes to the content of your manuscript. Once the typeset PDF of your manuscript has been created, we will send you a final PDF proof for checking prior to publication.

Please respond to this email within two weeks addressing any issues raised. After two weeks, we will send you a reminder email to complete your revisions. If we do not hear from you within seven weeks your submission will be withdrawn.

Best wishes,

Jonathan
The Editorial Team, F1000Research

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

Azrita undefined[a*], Hafrijal Syandri[b], Netti Aryani[c], Ainul Mardiah[d]

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Corresponding author: Azrita31@bunghatta.ac.id

ABSTRACT

Background: Giant gourami, *Osphronemus goramy* (Lacepede, 1801) is a freshwater species and Indonesia's most important commercial fish. Most giant gourami are produced by aquaculture. The first purpose of this study 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 analyse the growth coefficient, 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. 1-litre of product was added in turn 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, to make diets labeled KP1, KP2, KP3, and KP4. The fish 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 (2×1×1 m; water volume 1.5 m^3/frame nets) in a freshwater concrete pond with a stocking density of 30 juveniles/net
Results: The results supported our hypothesis that different product formulations have a significant effect (P < 0.05) on aquafeed nutrition and the whole-body carcass, growth coefficient, feed utilization, body indices, and gut micromorphology of giant gourami juveniles. The thermal growth coefficient strongly correlated with the daily growth coefficient (r^2 = 91%). The KP3 diet contains a higher concentration of amino acids, which increased the growth coefficient, feed utilization, and carcass quality more than the other diets that we tested.
Conclusions: Diet KP3 contains higher total amino acids in diets and carcasses and leads to better growth for giant gourami.

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

Commented [HJ1]: Re: figures within the article.

1) Unfortunately, only one of the four attachments is at the resolution we need for reproduction (300 dpi); this is the one for Figure 3. Please therefore could you provide jpegs (or another suitable file format such as tiffs) at 300 dpi for the other figures to be used. Thanks.

2) Is it possible to edit the text of the Legends for the Figures? Ideally, if you could provide them separately, with the Legends removed from the jpgs, we can do this. (Just for example in Figure 1, for the sense it seems it should be: 'Enteric section of fish fed KP1 diet'; and there is a missing bracket at end of the details for picture A.)

Commented [HJ2]: Query re: this second entry ('b'). The details are somewhat different to the name/ address entered into our system for this institution. Please confirm the correct details that should be used.

On our system, the last part of the address that was entered currently reads: '…Universitas Bung Hatta, Padang, Sumatera Barat, 25113, Indonesia'.

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Commented [HJ3]: Small sense issue. It seems there is a repetition of 'aquafeed' being added to CP1 (after all, CP1 has already had aquafeed added to it, it is the placebo). Also, KP1 it seems, must be te same as CP1 (it is not created by adding cp2, cp3 or cp4 to cp1) -and so there is a second small sense issue.

The sense is tricky to follow for me but possibly you want this sentence to read: 'CP1 was labelled as the KP1 diet; CP2, CP3 and CP4 were added to it in turn to create diets labelled KP2, KP3 and KP4.' ? (this seems to match your meaning)

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Commented [HJ4]: A note re: Peer Reviewers. I see you have changed the style 1000 ml to 1.0 litre for this manuscript – this looks like it is in response to the Peer Reviewer of the last F1000 article, who queried the style on this point.

I see that they said that the Abstract should contain the detail that the coconut and palm sap formulation is a percentage solution. Therefore, suggest adding this in here (unless there is a reason for its omission…)

It seems best to ask if you can you kindly double-check whether there are any other points they raised that should be applied to this new article. (This is to be on the safe side, with regard to matching all the reporting guidelines for articles and meeting Peer Reviewer expectations.)

I can see that one of the points the reviewer made in the case of the previous article didn't seem to match F1000 style, incidentally.

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 this figure will continue to rise[1] (Food and Agricultural Organisation (FAO, 2018). The utilization of a variety of fish for aquaculture has now increased the need for commercial feed[2,3,4,5]. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge[2,6,7,8]. On the other hand, commercial feed produced by factories still does not contain complete nutrition for fish growth, while being 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 in many important nutrients, including protein, fat, vitamins, and minerals that cultured fish can utilize to increase their growth rate and survival and that is beneficial for human health[4,14,15,16]. Therefore, novel approaches have been developed by scientist to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[18], fish oil[19,11], and soybean oil[20]. In addition, supplementing probiotics into the diet[21] and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied[22].

The progress of aquaculture biotechnology has stimulated the interest of scientists to improve aquatic animal production, among others, increasing giant gourami production. One of the experimental techniques is to increase feed nutrition used for this purpose, i.e., the use of fish meal and Azolla flour as a feed ingredient for giant gourami[23] and the utilization of new products formulated from water coconut, palm sap sugar, and fungus for enriched of commercial feed[9]. Additionally, a diet supplemented using glutamine[24], fed feed supplemented with growth hormone[25], and substitute fish meal with chiken feather[26]. Whether using coconut water and palm sap sugar fermented with mushrooms affects the amino acid composition of the diet, body carcass, growth coefficient, and body indices is still not understood.

Coconut water has extraordinary nutritional value and contains supplements for health like minerals, amino acids, fatty acids, vitamins, enzymes, organic acids, and several phenolic compositions[27,28,29,30]. Palm sap sugar also has health benefits due to its essential nutrient content, such as a low glycaemic index, and it contains antioxidants, vitamins, and minerals[31,32,33,34]. Meanwhile, mushrooms have been widely used in fermentation due to their

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Commented [HJ5]: Re: addition of 'FAO, 2018', suggest cut as it appears in the References (so it is duplication). Unless you incorporated the detail differently, eg having 'the UN's Food and Agricultural Organization forecast' or similar, within the sentence.

Commented [HJ6]: Small query re Reference nos – are they correct please, as they jump from '4' to '14, 15, 16'? This sort of jump also occurs elsewhere.

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Commented [HJ7]: Query re sense. A) In these lines of the paragraph down to the sentence about artificial feed and tubifex worm, are you talking about a range of different studies, separate from one another, that have been undertaken by others? If so this isn't really clear at present.

b) Also, does this sentence that started 'There was an effect' describe the results of the study in the previous sentence (involving feeding the fish a recombinant growth hormone)?

Commented [HJ8]: Query re: sentence construction. The last phrase, 'and feed use' seems misplaced for the sense, how should it be included, please?

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Commented [HJ9]: Please confirm 'sanity' is the desired word here, it seems possibly wrong

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Commented [HJ10]: Query re word choice. I have changed 'fungus' to 'mushrooms' as I think this is what you mean and the phrase 'the fungus', mentioned like it was, seemed odd as a 'fungus' has not been mentioned before.

ability to degrade antigenic proteins in fish feed ingredients[7,35,36]. Additionally, coconut water is a functional food that can protect the lens from diabetic cataract in rats[37]. Coconut water is also a treatment for burning pain during urination, dysuria, gastritis, increasing semen, and indigestion[38].

On the other hand, Azrita *et al.*[9] have reported using new formulations of products containing coconut water and palm sap sugar that are fermented with various mushrooms involving a dosage of 300 ml/kg feed. Their newly formulated products can increase fatty acid levels in the diet and whole body carcasses. Besides that, they also improve giant gourami's growth performance and feed efficiency.

However, the effect of these new formulation products at a dosage of 150 ml/kg feed on the diet amino acid composition, and body meat's amino acid compositions has not yet been analyzed. In line with that, the relationships between thermal growth coefficient and condition factor, daily growth coefficient, and feed utilization coefficient, including body indices parameters, as well as the gut micromorphology of giant gourami, have not yet been analyzed.

We hypothesized that commercial aquafeed combined with different newly formulated products at the dosage of 150 ml/kg feed could improve the amino acids compositions of the aquafeed and whole body carcass, body indices, and gut micromorphology. Hence, this investigation's first purpose was to analyze the effect of various newly formulated products on the diet's proximate compositions, amino acid composition, and whole-body carcass. The second aim was to analyze the impact of newly formulated products on the growth coefficient and relation to thermal growth coefficient, body indices, and gut micromorphology in giant gourami juveniles.

Methods

Ethical approval

The Research and Community Service Ethics Committee at Universitas Bung Hatta approved this research (89/LPPM/Hatta/III-2022) which followed the ARRIVE guidelines. 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. Approval was given to collect and rear juvenile gurami sago in the aquaculture Laboratory, Faculty Fisheries and Marine Science Universitas Bung Hatta. All efforts have been made to relieve the suffering of experimental animals. Therefore, the animal did not suffer for this study, and they were still in good condition when they returned to the pond after research was

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completed. Where some fish were euthanized, this was carried out by piercing part of the fish's brain. Gurami sago fish were not classified as a protected animal according to Indonesian legislation..

Preparation of formulated product

We prepared 100 g (11%) 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 the 1.0 litre of palm sap sugar solution (equivalent 50% of palm sap sugar solution). The product was 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 hrs 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) and labeled as the KP2, KP3, and KP4 diets. The aquafeed was supplemented with freshwater (labeled as the KP1 diet; placebo).

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), which 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. The minerals in the 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 freshwater to create the KP1 diet as observed, and the formulated CP2, CP3, and CP4 products were added to the aquafeed at a dosage of 150 ml/kg of feed to create the enriched fish diets. The formulated product added to the aquafeed was mixed manually with it for three minutes to obtain maximum homogenization and then the blend was dried in the open air for thirty minutes. Thereafter, it was given to the trial animal.

Experimental procedures and sampling

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I see that in your earlier study published in F1000 Research, you include 5-6 lines about the ethical and humane treatment of the fish. I assume that the fish were treated in a similar way in this study? Suggest add similar details therefore, if they are applicable, to demonstrate the humane treatment in this new study.

On this subject, the sentence here about following the guidelines in the Standard Operating Procedure at Universitas Bung Hatta is (too) vague as the reader doesn't know what is being referred to.

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In the present study, we measured fish weight using AD-600i scales with 0.001 g accuracy (ACIS model number AD-600i, China). At the same time, a meter 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 nets framed with 2 m^3 (2×1×1 m) PVC pipe (water volume of 1.5 m^3) were placed inside two freshwater concrete ponds with a size of 18 m^3 (6×2×1.5 m). This experiment consisted of four treatments and three replicate, 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 17:00hrs) 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.

Proximate and amino acid composition

The diet samples and proximate carcass composition were analyzed using standard AOAC methods[39]. The matter was dried to a constant weight at 105–°C. We used the standard Kjeldahl method to analyse crude protein (N × 6.25). We used the Soxhlet method with ether extraction to analyse crude lipids; the ash was incinerated at 550–°C for 16 hrs, whereas gross energy was measured in a bomb calorimeter. 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 24hrs at 11 °C[40].

Nutrient utilization and body indices

The growth coefficients in the fish experiments were measured by using the thermal growth coefficient (TGC), daily growth coefficient (DGC), total feed intake (FI), and protein efficiency ratio (PER) of giant gourami, assessed using the following formulae:

TGC = [(final weight (g))⅓ – (initial weight (g))⅓] / (mean water temperature (°C)) × duration of rearing period (day)] × 1000

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DGC = (Wf⅓ – Wi⅓) / duration of rearing period (day) × 100

FI as feed (FI as feed in g/fish/day) = Total feed fed / (n × t)

PER = wet weight gain / total protein intake

Three fish from each net frame were sacrificed and dissected immediately to determine the Condition factor (CF), Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) as given below:

CF = 100 × [weight of the juvenile (g) / Length of juvenile (cm^3)]

GSI = 100 × [viscera weight (g) / whole body weight (g)]

HSI = 100 × [liver weight (g) / whole body weight (g)]

VFSI = 100 × [visceral fat weight (g) / whole body weight (g)]

BSI = 100 × [Bile weight (g) / weight of liver (g)]

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 24hrs. After sequential dehydration steps in alcohol, the gut samples were embedded in paraffin. The implanted tissue blocks were sectioned at 5 µm, 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 Figure 1.

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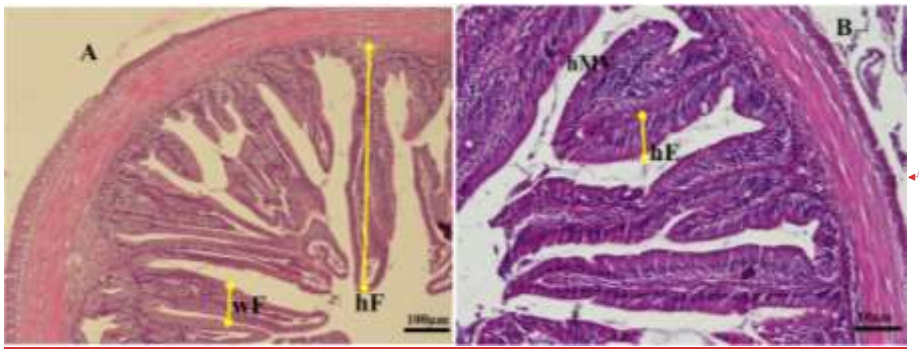

Figure 1. Transversal section photomicrographs of giant gourami juvenile foregut. (A) Fold height and fold width were analyzed in a lower magnification of objective lens of microscope (magnification × 100, (B) Enterocytes height and microvilli height were analyzed in a higher magnification of objective lens microscope (magnification × 200). hF = fold height, wF = fold width, hE = enterocyte height, hMV = microvillus height (hematoxylin and eosin).

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 value. In addition, we also measured the total alkalinity, hardness, and nitrates of the water in the pond experiments. A thermometer (Celsius scale) was used to measure water temperature. To measure water dissolved oxygen (O_2; mg L^{-1}), we used 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 experiments. The level of nitrate-nitrogen (NO_3-N; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures[41].

Calculations and statistical method

The data from this study were reported in the form of the mean ± standard deviation for each treatment. Data were analysed using 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[42]. To create the figures, Microsoft Office Professional Plus 2019 was used.

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Results

Proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products with the dosage of 150 ml/–kg of feed significantly affects 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±0.17%) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate (31.19 ± 0.38%), crude fibre (2.82±0.06%), and ash (6.67±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 with a dosage of 150 ml/kg of feed 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 significant 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 ± 0.011%), nonessential amino acids (12.91 ± 0.004 ± 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 ± 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 ± 0.25	38.27 ± 0.01	37.59 ± 0.16	38.41± 0.10
Crude protein	19.68 ± 0.41^{a}	20.27 ± 0.13^{b}	21.70 ± 0.18^{c}	20.44 ± 0.10^{d}
Crude lipid	3.41 ± 0.02^{a}	3.67 ± 0.13^{b}	3.50 ± 0.02^{ac}	3.48 ± 0.04^{a}
Carbohydrate	26.37 ± 0.17^{a}	29.50 ± 0.54^{b}	31.19 ± 0.38^{c}	30.57 ± 0.06
Crude fibre	2.23 ± 0.05^{a}	2.36 ± 0.01^{b}	2.82 ± 0.06^{c}	2.45 ± 0.06
Ash	2.75 ± 0.03^{a}	6.66 ± 0.05^{b}	6.57 ± 0.04^{c}	6.67 ± 0.06^{d}
Energy total (kg calorie/100 g)	240.87 ± 0.38^{a}	234.41 ± 0.30^{b}	240.88 ± 0.74^{ac}	237.11 ± 0.43^{d}
Amino acid composition				
EAA				
Leucine	1.36 ± 0.01^{a}	1.42 ± 0.01^{b}	1.46 ± 0.01^{c}	1.36 ±0 .01^{d}
Isoleucine	0.76 ± 0.01^{a}	0.79 ± 0.01^{b}	0.81 ± 0.01^{c}	0.76 ± 0.01^{d}
Lysine	0.95 ± 0.01^{a}	1.10 ± 0.01^{b}	0.98 ± 0.01^{c}	1.20 ± 0.01^{d}
Valine	0.86 ± 0.01^{a}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}	0.89 ± 0.01^{d}
Threonine	0.79 ± 0.02^{a}	0.92 ± 0.01^{b}	1.04 ± 0.01^{c}	0.83 ± 0.01^{d}
Arginine	1.02 ± 0.01^{a}	1.19 ± 0.01^{b}	1.30 ± 0.01^{c}	1.03 ± 0.01^{d}
Phenylalanine	0.67 ± 0.01^{a}	0.93 ± 0.01^{b}	1.05 ± 0.01^{c}	0.77 ± 0.01^{d}
Tyrosine	0.43 ± 0.01^{a}	0.53 ± 0.00^{b}	0.57 ± 0.06^{c}	0.45 ± 0.01^{d}
Methionine	0.18 ± 0.01^{a}	0.26 ± 0.01^{b}	0.30 ± 0.01^{c}	0.21 ± 0.01^{d}
Histidine	0.40 ± 0.01^{a}	0.50 ± 0.01^{b}	0.57 ± 0.01^{c}	0.43 ± 0.01^{d}
Tryptophan	0.06 ± 0.01^{a}	0.11 ± 0.01^{b}	0.07 ± 0.00^{bc}	0.09 ± 0.01^{bd}
NEAA				
Alanine	0.85 ± 0.01^{a}	0.94 ± 0.01^{b}	0.87 ± 0.06^{c}	0.97 ± 0.01^{bd}
Serine	1.01 ± 0.01^{a}	1.12 ± 0.01^{b}	1.23 ± 0.01^{c}	1.01 ± 0.01^{d}
Glycine	1.15 ± 0.01^{a}	1.32 ± 0.01^{b}	1.29 ± 0.01^{c}	1.19 ± 0.01^{d}

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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.

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

Commercial feed combined with a new formulation product significantly affected 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 ± 0.17%), fat (3.47 ± 0.03%), carbohydrates (31.18 ± 0.37%), and crude fibre (2.81 ± 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 whole-body meat. 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 provided 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 ± SD*. Mean values with different superscript letters in the same row are significantly different ($P < 0.05$).

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

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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]
∑EAA	12.68 ± 0.003[a]	15.13 ± 0.005[b]	15.82± 0.001[c]	13.61 ± 0.008[d]
∑NEAA	12.91 ± 0.007[a]	14.32 ± 0.01[b]	15.69 ± 0.002[c]	13.50 ± 0.001[d]
∑AA	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.

Growth coefficient and survival

The growth coefficient and feed utilization of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant difference between experimental diets in the case of the thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), and daily growth coefficient ($F_{(3,8)} = 59.88$, $P = 0.288$), while total feed intake (% BW day-1) ($F_{(3,8)} = 14.938$, $P = 0.56$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Figure 2).

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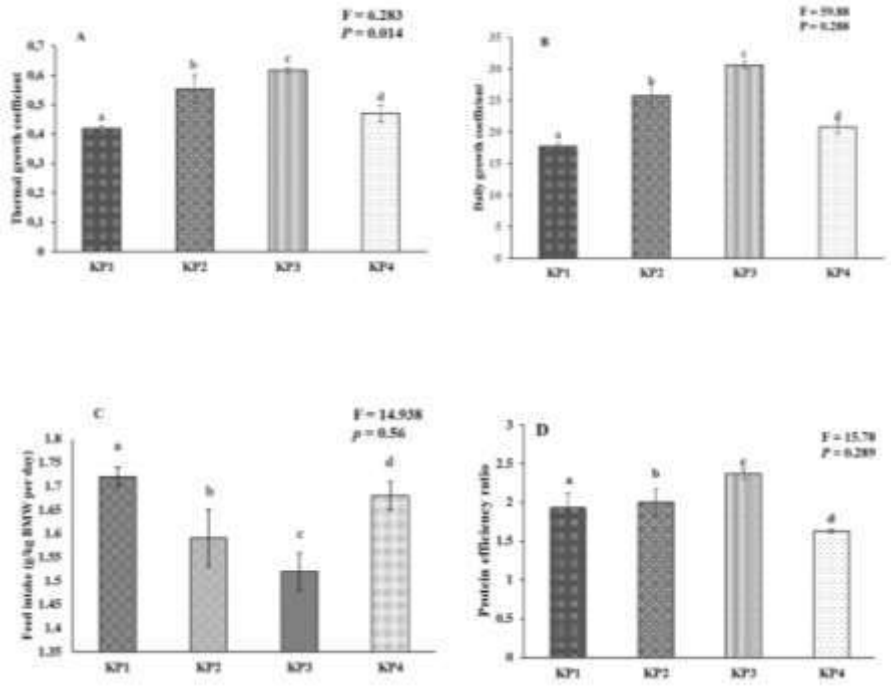

Figure 2. Growth coefficient and feed utilization of the giant gourami juveniles reared under different diets during 90 days of the experiment period, (A) thermal growth coefficient (TGC), (B) daily growth coefficient (DGC), (C) feed intake (FI), and (D) protein efficiency ratio (PER). The mean value and standard deviation (mean ± SD) are presented for giant gourami (n = 3). Different superscripts in the bar diagram of the giant gourami juvenile TGC, DGC, FI, and PER indicate significant differences among other diets ($P < 0.05$, One-way ANOVA Duncan Post-Hoc)

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Furthermore, the thermal growth coefficient (TGC) has often been used to predict growth performance and production performance of aquaculture with water temperature at fish rearing location. This study presents the relationship between thermal growth coefficient and condition factor, daily growth coefficient, and protein efficiency ratio (Figure 3). The thermal growth coefficient had strong relationships with the condition factor (r^2 = 0.777, figure 3A), daily growth coefficient (r^2 = 0.999, figure 3B), and protein efficiency ratio (r^2 = 0.749, figure 3D), while the thermal growth coefficient had a moderate relationship with the feed intake (r^2 = 0.699, figure 3C).

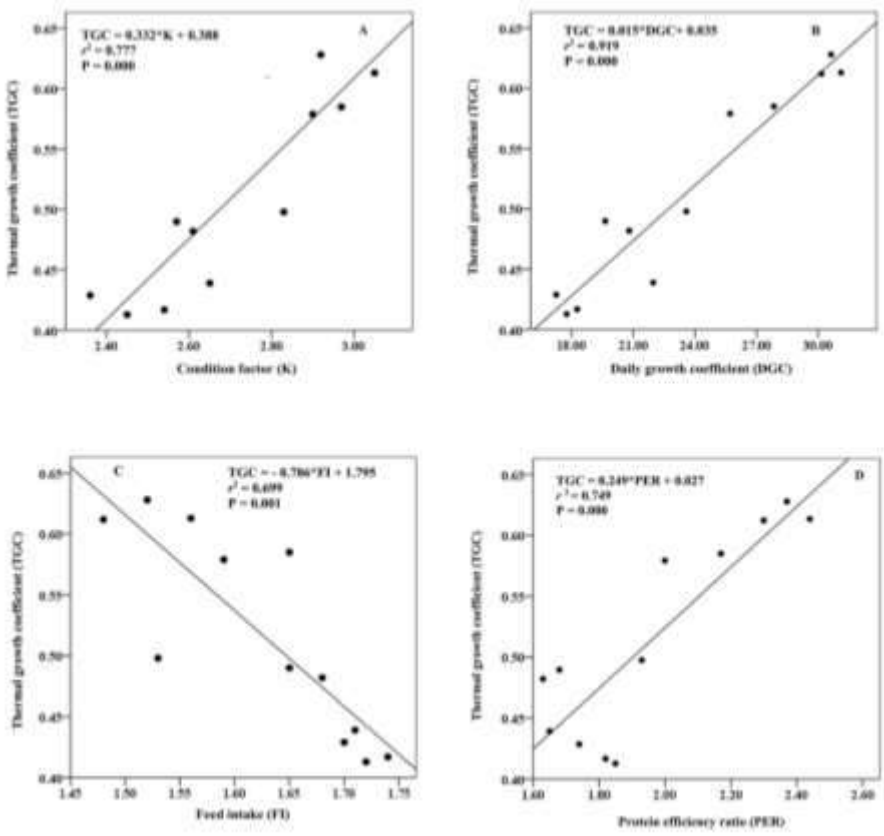

Figure 3. Relationships between thermal growth coefficient and condition factor (A), daily growth coefficient (B), feed intake (C) and protein efficiency ratio (D) for giant gourami (*O.gourami*) over 90 days.

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Condition factor and body indices of giant gourami after 90 days of feeding

The condition factor was significantly different between diets ($F_{(3,8)}$ = 19.98, P = 0.566) in the present study; while the GSI, HIS, and VFSI displayed marginally significant differences between diets. The HIS 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). GSI was significantly ($F_{(3,8)}$ = 10.492, P = 0.243) in the KP3 diet and significantly among all different diets. The VFSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's post-hoc test revealed that the HIS (1.30 ± 0.13%), GSI (4.15 ± 0.36%), and VFSI (2.75 ± 0.34%) were significantly higher (P < 0.05) in the KP3 diet than in the other diets. Meanwhile, BSI showed no significant difference (P > 0.05) among the treatment diets (Table 3).

Table 3. Mean (± SD) value condition factor 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}
Viscerosomatic index (GSI%)	3.20 ± 0.21^{a}	3.77 ± 0.09^{b}	4.15 ± 0.36^{c}	3.17 ± 0.02^{d}
Hepatosomatic (HIS%)	0.97 ± 0.05^{a}	1.06 ± 0.19 ^{ab}	1.30 ± 0.13^{c}	1.04 ± 0.12^{ad}
Visceral fat-somatic indexes (VFSI%)	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

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,

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P = 0.285). The Duncan's post-hoc test demonstrated that the foregut fold height (434.13 ± 1.76 µm), fold width (53.23 ± 0.88 µm), enterocyte height (27.42 ± 0.42 µm), and microvillus height (2.79 ± 0.45 µm) 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 ± 1.43 µm), fold width (61.50 ± 1.02 µm), and enterocytes (32.82 ± 0.54 µm) 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 ($F_{(3,8)} = 253.57$, $P = 0.316$).

Pond water quality

The pond water quality values of the giant gourami juvenile rearing freshwater concrete pond 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 in Table 5.

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

	Foregut				Midgut				Hindgut			
	hF (µm)[a]	wF (µm)[b]	hE (µm)[c]	hMV (µm)[d]	hF (µm)	wF (µm)	hE (µm)	hMV (µm)	hF (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

Table 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 (^{O}C)	45	28.01 ± 1.06	27 - 30	25-33	Prokoso *et al.*[43]
Dissolved oxygen (mg L^{-1})	14	6.01 ± 0.14	5.80 – 6.20	3-5	Syandri *et al.*[44]
Total alkalinity (mg L^{-1} as $CaCo_3$)	14	58.09 ± 3.33	52.5 - 62.5	120	Boyd *et al.*[45]
Hardness (mg L^{-1} as $CaCo_3$)	14	66.34 ± 1.32	65 - 68.5	168	Boyd *et al.*[45]
pH	14	7.48 ± 0.19	7.2 – 7.8	6.5 – 9.0	Boyd *et al.*[45]
Nitrates (mg L^{-1})	14	0.04 ± 0.01	0.03 – 0.05	0.2 – 219	Boyd and Tucker[46]

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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[47,48,49]. The giant gourami belongs to a trophic level of herbivorous fish[50]. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish[51,49]. 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[2,52]. 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*[53], and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*[48] and for rearing rohu, *Labeo rohita*[54]. 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, the 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*). In the recent past, the dose used was 300 ml/kg of feed.This method is a new approach that has been developed by Azrita *et al.*[9] to improve feed nutrition and whole body carcasses, covering fatty acids, the atherogenic index and thrombogenic, feed efficiency, and growth performance of giant gourami. Here, we continued the investigation by reducing the feed dose to 150 ml/kg. This study's results found that supplementing feed with newly formulated products can increase feed nutrition, covering amino acids in diet and body meat, and the growth coefficient of giant gourami. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[12], fish oil[19, 11], and soybean oil[20]. In addition, the provision of feed has been supplemented with probiotics[21], glycine, and prebiotics[22]. 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

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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[55] and Vong *et al.*[56] showed that *Rhizopus oligosporus* can produce various extracellular enzymes. *Aspergillus niger*, has a high capacity to degrade antigenic proteins, including carbohydrases, proteases, lipases, and phosphatases, when used for fermenting plant-sourced fish feed ingredients[12,57] . *Saccharomyces cerevisiae* is one of the most 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[7,58].

The composition of amino acids can be used to judge the quality of feed. In the present study, feed supplemented with different formulated products (CP1, CP2, CP3, and CP4), leucine, arginine, and glutamic acid were the most abundant free amino acids (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 diet[22]. Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid[59], and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein[60]. 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 feed[18]. 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. It was higher in the KP3 diet than the other diets. In contrast, the essential amino acids of fish feed for snubnose pompano were slightly higher than the nonessential amino acids content[60]. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu *et al.*[60] reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet[59] and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet[22]. In the present study, this difference in FAA content is caused by various mushrooms used in the formulated products.

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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 those fed 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[12,57]. The FAA profile differences could be related to different aspects, such as diet composition[61], dietary protein level[62], and methionine levels in the diet[18], including the water quality of the ponds[63]. 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[64,60]. The present study did not examine whether the 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 the insufficient KP1 diet were perhaps due 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 Jannatullah *et al.*[57] and Li *et al.*[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.*[36] 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.

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In the present study, the thermal growth coefficient (TGC) strongly correlated with the daily growth coefficient (DGC). Because faster daily fish growth requires a quality diet and constant water temperature during the rearing period, in this study, water temperature ranged from 27 to 30ºC, and dissolved oxygen was between 5.8 and 6.2 mg /L. According to Besson *et al.*[65], higher daily energy availability in the diet can lead to faster-growing fish, which is supported by constant water temperature and higher daily oxygen levels. The thermal growth coefficient had an essential change in environmental value[66]. Therefore, it was very important to keep the water temperature and dissolved oxygen constant in the aquaculture locations. At the same time, 78% of TGC values were determined by the condition factor connected to whole body weight and the total fish length. TGC of Atlantic cod, *Gadus morhua*, is influenced by body size and condition factors[67].

In this study, a higher value of TGC was detected in fish fed KP3; the effect is that the daily growth coefficient, and the protein efficiency ratio is better. Conversely, decreasing TGC has two effects, *i.e.*, a slow fish growth and lowered daily feed intake. Many scientists state that in aquaculture operations, net yield (kg/m^3) depends upon TGC fluctuation, feed intake, and daily oxygen consumption[65,68,69].

In the present study, feed enrichment with different formulated products did not affect HIS or VFSI except in the KP3 diet. Whereas GSI 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 Arriaga-Hernandez *et al.*[70] for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, Hassan *et al.*[71] 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.*[72] 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.*[64] 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*[44]. 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

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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[18,73]. 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 length[74]. In contrast, substituting as much as 12.5–25% soya protein concentrates with lupin (*Lupinus albus*) meal in carp (*Cyprinus carpio*) diets does not significantly affect the villi length and villi width of the gut[75]. 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 grouper[18], turbot, *Scophthalmus maximus*[12], largemouth bass, *Micropterus salmoides*[22], and common carp, *Cyprinus carpio*[75]. 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.

Conclusions

The present investigation observed that feed enriched with newly formulated products made from mature coconut water and palm sap sugar, and fermented with various mushrooms, given to fish in a dose of 150 ml/kg substantially affected the amino acid composition of the diet and whole-body carcass of giant gourami juveniles. It also affected the growth coefficient, feed utilization, body indices, and gut micromorphology size. The thermal growth coefficient had a strong relationship with the daily growth coefficient (r^2 =

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91%) and a moderate relationship with the feed intake (r^2 = 69%). The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass, growth coefficient, body indices, and the ability of the intestines for feed absorption. Thus, our study also informs fish farmers about culturing good quality giant gourami and fulfilling nutrition requirements for food security.

Data availability

Underlying data

Figshare: Underlying data for 'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles'.
https://doi.org/10.6084/m9.figshare.20407647[76]

This project contains the following underlying data:

- Table 1. Raw data of the experimental diets' proximate composition
- Table 2. Raw data of amino acid of feed experimental
- Table 3. Raw data of whole body carcass proximate composition
- Table 4. Raw data of amino acid of whole-body carcass
- Table 5. Daily growth coefficient, feed utilization and body indices of giant gourami after 90 days of feeding.
- Table 6. Raw data gut micromorphology of giant gourami juveniles fed different diets for 90 days

Competing interests

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

Grant information

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Acknowledgments

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a) What are the 'Publisher full text' phrases, throughout? We will delete them unless there is a specific reason they need to be included.
b) Re: use of bold for titles - this will be removed globally, for house style.
c) Re: article titles and Latin for fish names. For consistency of look/ house style, I have put them in italics.

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azrita ubh <azrita31@bunghatta.ac.id> Thu, Oct 13, 2022 at 6:50 AM
To: editorial@f1000research.com

Dear
Jonathan
Editorial Team F1000 Research,

Thank you for your email on October 12, 2022, and provide us with information about manuscript No. 124706, which F1000 Research has accepted. The person in charge of the Processing Charge article (APC) is Prof. Hafrijal Syandri (syandri_1960@bunghatta.ac.id), sourced from Research funded by the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia under grant No. 076/E5/PG.02.00. PT/2022 on March 16, 2022.

Best regards

Azrita

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azrita ubh <azrita31@bunghatta.ac.id> Fri, Oct 14, 2022 at 10:08 AM
To: editorial@f1000research.com

Dear
Jonathan
Editorial Team F1000 Research

Following up on your email on October 12, 2022, we have attached a revised manuscript No. 124706. The yellow highlight is the revision we have done.

Best regards

Azrita

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F1000.Research <research@f1000.com> Fri, Oct 21, 2022 at 10:51 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

I just wanted to acknowledge receipt of your email of last Friday at this stage, as I have been out of the office for much of this week. I have looked at the revised manuscript with interest, and will come back to you again shortly. I am aware that you have sent details of the person responsible for paying the APC also, for which, thanks.

Kind regards

Jonathan

F1000 Research editorial team

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Dear Azrita

'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, Osphronemus goramy (Lacepède, 1801), juveniles'
Undefined A, Syandri H, Aryani N and Mardiah A

Thank you for your submission to F1000Research. We have noted a few issues with your manuscript (below) – once these are addressed we will be pleased to accept your article for publication

Methods: In order to ensure a minimum level of reproducibility of your methods, we require adequate information about the techniques used in your study. Comments in the edited document will help to guide you but are not comprehensive. Please avoid the use of citation shortcuts, such as "[technique] was performed according to the methods of [reference]" without giving complete details of the methods used, including reagents used, time frames, etc. and any allowances for controlling bias and unwanted sources of variability. We encourage authors to deposit step-by-step descriptions of their protocols on protocols.io and include the persistent DOI in the methods section of the manuscript.

Reviewers: As you know, F1000Research operates an author-driven publication model. This means that you will be responsible for suggesting suitable reviewers, whom we invite on your behalf, giving you an opportunity to ensure that appropriate experts review your article. Our transparent peer review process means that the peer review reports, together with the reviewers' names, will be published alongside your article.

To avoid delay to the publication process, we need you to provide us with at least five potential reviewers who meet our reviewer criteria before we can publish your article - please be aware that it is likely we will need to request further reviewer suggestions after publication. Please go to your Suggest Reviewers page, where you will find a useful tool to help you find reviewers; use this page to track the progress of the peer review process for your article. You can access this page directly via the article's record under My Research >> Submissions. See also our reviewer criteria and tips for finding reviewers.

Please remember that suggested reviewers should have appropriate level of experience and the right expertise to judge your article; they must be able to provide an unbiased report (e.g. they must not be recent collaborators or colleagues in your institute). All reviewer suggestions are checked by the editorial team and will be rejected if they do not meet our criteria.

Payment: As F1000Research is open access, we will require payment of the Article Processing Charge (APC) to be able to complete the processing of your submission. The APC is $1350.00 (ex. VAT) after any discounts you are eligible for have been applied. **Please provide us with the details of the individual/organization taking responsibility for paying the fee as soon as possible.** Please sign in with the credentials you used to submit the article or you will not be able to access this page. Our Accounts department will be in touch regarding payment.

We have also lightly copyedited your article - please download the document and check you are happy with the amendments and **then address the queries detailed in the margin. Please return your revised manuscript to the e-mail address above**. Please note that this is your final opportunity to make any changes to the content of your manuscript. Once the typeset PDF of your manuscript has been created, we will send you a final PDF proof for checking prior to publication.

Please respond to this email within two weeks addressing any issues raised. After two weeks, we will send you a reminder email to complete your revisions. If we do not hear from you within seven weeks your submission will be withdrawn.

Best wishes,

Jonathan
The Editorial Team, F1000Research

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azrita ubh <azrita31@bunghatta.ac.id> Thu, Oct 27, 2022 at 9:56 AM
To: "F1000.Research" <research@f1000.com>

Dear

Jonathan

F1000 Research editorial team

Thank you for your email on October 21, 2022. We have revised manuscript No. 124706 and emailed it on October 14, 2022. Is there anything else we need to edit?

With best regards
Azrita

[Quoted text hidden]

F1000.Research <research@f1000.com> Fri, Oct 28, 2022 at 10:22 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

Thank you for this. I just wanted to acknowledge safe receipt. Hopefully you received my last email (of 21 Oct) ok. I have nearly completed going through the revised version of m/s 124706 and so, will come back to you by Monday morning about this.

Kind regards

Jonathan

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Information Classification: General

F1000.Research <research@f1000.com> Mon, Oct 31, 2022 at 8:58 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

Further to my last email, I have finished going through the revised m/s. I have a few last points to raise.

I attach my latest version of the manuscript here.

You had highlighted some changes in yellow when you returned the document. Please look at all yellow bits within the running text, as you will see that I have made some changes, using track changes.

Also, please see my comments in the margin. Please note that all *new comments* of mine in the margin start with '#2', and the first few words of the new comment are highlighted in yellow. Occasionally, I have left an earlier comment in the margin as it helps explain the position; but the points to look for are the latest ones that all start with '#2' (highlighted).

I look forward to receiving resolutions to the points back from you; and then, to putting the manuscript through for publication.

Kind regards

Jonathan

F1000 Research editorial team

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Thursday, October 27, 2022 3:57 AM
To: F1000.Research <research@f1000.com>

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azrita ubh <azrita31@bunghatta.ac.id> Wed, Nov 2, 2022 at 11:01 AM
To: "F1000.Research" <research@f1000.com>

Dear
Jonathan
F1000 Research editorial team

I have attached the latest version of manuscript No. 12470 here. We have revised the manuscript and highlighted some changes in the aqua color.

With best regards

Azrita

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F1000.Research <research@f1000.com> Wed, Nov 16, 2022 at 11:07 AM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

I have been through the revised m/s – thanks.

There are a handful of text points that I need to raise with you, about the new changes, before I can put the m/s into production. I am putting these below, after the end of this email, with a reference to the place in the manuscript that the points apply to. You will see that I have suggested solutions or am double-checking a possible solution, in each case.

Also, there is a point about the Figures and their resolution that I need to raise, as this is unfortunately somewhat unresolved. Hopefully this will be easy to fix…

I look forward to hearing back on these final points, so we can get this interesting manuscript to the typesetters for printing.

Best regards

Jonathan

F1000 Research editorial team

Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, 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 and Indonesia's most important commercial fish. Most giant gourami are produced by aquaculture. The first purpose of this study 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 analyse the growth coefficient, 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. 1 litre of product was added in turn 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. Aquafeed was added to CP1 and supplemented with CP2, CP3, and CP4, to make diets labeled KP1, KP2, KP3, and KP4. The fish 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 (2×1×1 m; water volume 1.5 m^3/frame nets) in a freshwater concrete pond with a stocking density of 30 juveniles/net.
Results: The results supported our hypothesis that different product formulations have a significant effect ($P < 0.05$) on aquafeed nutrition and the whole-body carcass, growth coefficient, feed utilization, body indices, and gut micromorphology of giant gourami juveniles. The thermal growth coefficient strongly correlated with the daily growth coefficient (r^2 = 91%). The KP3 diet contains a higher concentration of amino acids, which increased the growth coefficient, feed utilization, and carcass quality more than the other diets that we tested.
Conclusions: Diet KP3 contains higher total amino acids in diets and carcasses and leads to better growth for giant gourami.

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

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#2 - Unfortunately, only one of the four attachments is at the resolution we need for reproduction (300 dpi); this is the one for Figure 3. Please therefore could you provide jpegs (or another suitable file format such as tiffs) at 300 dpi for the other figures to be used. Thanks.

Commented [HJ2]: On our system, the last part of the address that was entered currently reads: '…Universitas Bung Hatta, Padang, Sumatera Barat, 25113, Indonesia'.

#2 - Which address is correct please? They are different.

Commented [HJ3]: #2 – you have made a change or two here. However the sentence starting 'Aquafeed was added…', which I queried, is still confusing, unfortunately. (It suggests that you add aquafeed to CP1 and then also add CP2, CP3, CP4 to the 'aquafeed plus CP1' mix.)

I think you must mean: 'Aquafeed was added to CP1, and then added to CP2, CP3, and CP4, to make diets labeled KP1, KP2, KP3, and KP4.' Please confirm.

Introduction

In this decade, the production of capture fisheries has decreased; meanwhile, the demand for fish products for human consumption is increasing. Therefore, according to the Food and Agriculture Organisation, 60% of fisheries production in the future will come from aquaculture activities and this figure will continue to rise[1] ~~(Food and Agricultural Organisation (FAO, 2018)~~. The utilization of a variety of fish for aquaculture has now increased the need for commercial feed[2,3,4,5]. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge[2,6,7,8]. On the other hand, commercial feed produced by factories still does not contain complete nutrition for fish growth, while being 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 in many important nutrients, including protein, fat, vitamins, and minerals that cultured fish can utilize to increase their growth rate and survival and that is beneficial for human health[4,14,15,16]. Therefore, novel approaches have been developed by scientists to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[18], fish oil[19,11], and soybean oil[20]. In addition, supplementing probiotics into the diet[21] and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied[22].

The progress of aquaculture biotechnology has stimulated the interest of scientists in ~~to~~ improving~~e~~ aquatic animal production, for example~~among others~~, to increas~~ing~~ giant gourami production. One of the experimental techniques is to increase feed nutrition used for this purpose, such as~~i.e.~~, the use of fish meal and Azolla flour ~~-~~as a feed ingredient for giant gourami[23]~~–~~, and the utilization of new products formulated from water coconut, palm sap sugar, and fungus for the enrichment~~ed~~ of commercial feed[9]. Additional research has involved~~ly,~~ a diet supplemented using glutamine[24], ~~fed -~~feed supplemented with a growth hormone[25], and substitute fish meal incorporating~~with~~ chicken feather[26]. Whether using coconut water and palm sap sugar fermented with mushrooms affects the amino acid composition of the diet, body carcass, growth coefficient, and body indices is still not understood.

Coconut water has extraordinary nutritional value and contains ~~-~~supplements for health like minerals, amino acids, fatty acids, vitamins, enzymes, organic acids, and several phenolic compositions[27,28,29,30]. Palm sap sugar also has health benefits due to its essential

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This seems to work well to me – if you agree?

nutrient content, such as a low glycaemic index, and it contains antioxidants, vitamins, and minerals[31,32,33,34]. Meanwhile, mushrooms have been widely used in fermentation due to their ability to degrade antigenic proteins in fish feed ingredients[7,35,36]. Additionally, coconut water is a functional food that can protect the lens from diabetic cataract development in rats[37]. Coconut water is also a treatment for burning pain during urination, dysuria, gastritis, increasing semen, and indigestion[38].

On the other hand, Azrita *et al.*[9] have reported using new formulations of products containing coconut water and palm sap sugar that are fermented with various mushrooms involving a dosage of 300 ml/kg feed. Their newly formulated products can increase fatty acid levels in the diet and whole body carcasses. Besides that, they also improve giant gourami's growth performance and feed efficiency.

However, the effect of these new formulation products at a dosage of 150 ml/kg feed on the diet amino acid composition, and body meat's amino acid compositions has not yet been analyzed. In line with that, the relationships between thermal growth coefficient and condition factor, daily growth coefficient, and feed utilization coefficient, including body indices parameters, as well as the gut micromorphology of giant gourami, have not yet been analyzed.

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We hypothesized that commercial aquafeed combined with different newly formulated products at the dosage of 150 ml/kg feed could improve the amino acids compositions of the aquafeed and whole body carcass, body indices, and gut micromorphology. Hence, this investigation's first purpose was to analyze the effect of various newly formulated products on the diet's proximate compositions, amino acid composition, and whole-body carcass. The second aim was to analyze the impact of newly formulated products on the growth coefficient and relation to thermal growth coefficient, body indices, and gut micromorphology in giant gourami juveniles.

Methods

Ethical approval

The Research and Community Service Ethics Committee at Universitas Bung Hatta, West Sumatera, Indonesia approved this research (89/LPPM/Hatta/III-2022) which followed the ARRIVE guidelines. 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. Approval was given by the ethics committee to collect and rear juvenile gurami sago in the aquaculture laboratory, Faculty of Fisheries and Marine Science at

Universitas Bung Hatta. All efforts werehave been made to relieve the suffering of experimental animals. Therefore, the animal did not suffer for this study, and they were still in good condition when they returned to the pond after research was completed. Where some fish were euthanized, this was carried out by piercing part of the fish's brain. Gurami sago fish arewere not classified as a protected animal according to Indonesian legislation.

Preparation of formulated product

We prepared 100 g (11%) 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 the 1.0 litre of palm sap sugar solution (equivalent 50% of palm sap sugar solution). The product was 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 48hrs 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) and labeled as the KP2, KP3, and KP4 diets. The aquafeed was supplemented with freshwater (labeled as the KP1 diet; placebo).

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), which 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. The minerals in the 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 freshwater to create the KP1 diet as observed, and the formulated CP2, CP3, and CP4 products were added to the aquafeed at a dosage of 150 ml/kg of feed to create the enriched fish diets. The formulated product added to the aquafeed was mixed manually with it for three minutes to obtain maximum homogenization and then the blend was dried in the open air for thirty minutes. Thereafter, it was given to the trial animal.

Commented [HJ6]: #2 – query. This '11%' applies to 'palm sap sugar' - I don't follow what this means. Are you saying that the palm sap sugar itself, at the start, was only 11%?

If so, suggest we have: 'We prepared 100 g of 11% palm sap sugar by traditional production and cooked it in…'.

If instead, you want to say that the overall preparation of 1.0 litres involved 11% palm sap sugar, you would need to have: 'We prepared 100g of palm sap sugar by traditional production and cooked it in 1.0 litre of fresh water for fifteen minutes at 60°C, to make an 11% palm sap sugar solution.'

Which is it please? Pls clarify.

Commented [HJ7]: #2 query – if it is 2 litres of the coconut water with 1 litre of the palm sap sugar production this would seem to make 33% of palm sap sugar solution – not 50%? Please clarify.

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, China). At the same time, a meter 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 nets framed with 2 m^3 (2×1×1 m) PVC pipe (water volume of 1.5 m^3) were placed inside two freshwater concrete ponds with a size of 18 m^3 (6×2×1.5 m). This experiment consisted of four treatments and three replicate, 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 17:00hrs) 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.

Commented [HJ8]: Sense of 'replicates' unclear…do you mean 'replications'?

#2 – I don't understand what 'replicate' means (or 'replicates'), I'm afraid. The word reads like an error. ('replicates' also reads like an error, it is incorrect usage.)

Do you mean you did the four treatments 3 times? (ie that you 'replicated' them 3 times? 'replicated' means 'repeated', as we know…)

I'd like to suggest that you phrase it a different way, so we can ensure the wording captures what you want…many thanks.

Proximate and amino acid composition

The diet samples and proximate carcass composition were analyzed using standard AOAC methods[39]. The matter was dried to a constant weight at 105°C. We used the standard Kjeldahl method to analyse crude protein (N × 6.25). We used the Soxhlet method with ether extraction to analyse crude lipids; the ash was incinerated at 550°C for 16 hrs, whereas gross energy was measured in a bomb calorimeter. 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 24hrs at 11-°C[40].

Nutrient utilization and body indices

The growth coefficients in the fish experiments were measured by using the thermal growth coefficient (TGC), daily growth coefficient (DGC), total feed intake (FI), and protein efficiency ratio (PER) of giant gourami, assessed using the following formulae:

TGC = [(final weight (g))⅓ – (initial weight (g))⅓] / (mean water temperature (°C)) × duration of rearing period (day)] × 1000

DGC = (Wf⅓ – Wi⅓) / duration of rearing period (day) × 100

FI as feed (FI as feed in g/fish/day) = Total feed fed / (n × t)

PER = wet weight gain / total protein intake

Three fish from each net frame were sacrificed and dissected immediately to determine the Condition factor (CF), Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) as given below:

CF = 100 × [weight of the juvenile (g) / Length of juvenile (cm^3)]

GSI = 100 × [viscera weight (g) / whole body weight (g)]

HSI = 100 × [liver weight (g) / whole body weight (g)]

VFSI = 100 × [visceral fat weight (g) / whole body weight (g)]

BSI = 100 × [Bile weight (g) / weight of liver (g)]

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 24hrs. After sequential dehydration steps in alcohol, the gut samples were embedded in paraffin. The implanted tissue blocks were sectioned at 5 µm, 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 Figure 1.

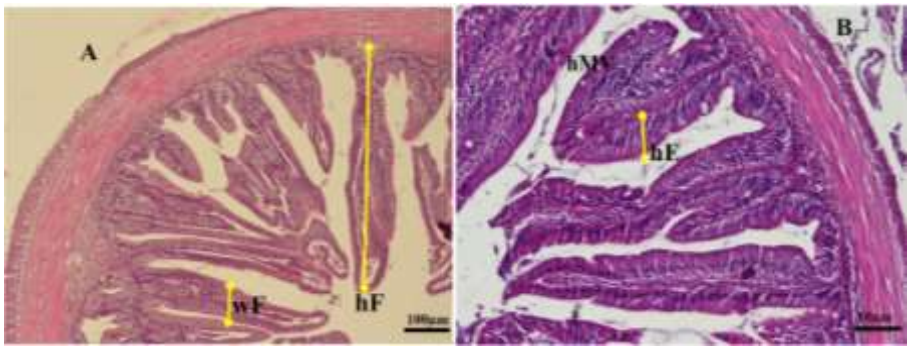

Figure 1. Transversal section photomicrographs of giant gourami juvenile foregut. (A) Fold height and fold width were analyzed in a lower magnification of objective lens of microscope (magnification × 100), (B) Enterocytes height and microvilli height were analyzed using a higher magnification of an objective lens microscope (magnification × 200). hF = fold height, wF = fold width, hE = enterocyte height, hMV = microvillus height (hematoxylin and eosin).

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 10:00am at a depth of 20 cm from each concrete pond to determine the water temperature, dissolved oxygen, and pH value. In addition, we also measured the total alkalinity, hardness, and nitrates of the water in the pond experiments. A thermometer (Celsius scale) was used to measure water temperature. To measure water dissolved oxygen (O_2; mg L^{-1}), we used 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 experiments. The level of nitrate-nitrogen (NO_3-N; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures[41].

Calculations and statistical method

The data from this study were reported in the form of the mean ± standard deviation for each treatment. Data were analysed using 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[42]. To create the figures, Microsoft Office Professional Plus 2019 was used.

Results

Proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products with the dosage of 150 ml/kg of feed significantly affects 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±0.17%) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate (31.19 ± 0.38%), crude fibre (2.82±0.06%), and ash (6.67±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 with a dosage of 150 ml/kg of feed 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 significant 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 ± 0.011%), nonessential amino acids (12.91 ± 0.004 ± 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 ± 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 ± 0.25	38.27 ± 0.01	37.59 ± 0.16	38.41± 0.10
Crude protein	19.68 ± 0.41^{a}	20.27 ± 0.13^{b}	21.70 ± 0.18^{c}	20.44 ± 0.10^{d}
Crude lipid	3.41 ± 0.02^{a}	3.67 ± 0.13^{b}	3.50 ± 0.02^{ac}	3.48 ± 0.04^{a}
Carbohydrate	26.37 ± 0.17^{a}	29.50 ± 0.54^{b}	31.19 ± 0.38^{c}	30.57 ± 0.06
Crude fibre	2.23 ± 0.05^{a}	2.36 ± 0.01^{b}	2.82 ± 0.06^{c}	2.45 ± 0.06
Ash	2.75 ± 0.03^{a}	6.66 ± 0.05^{b}	6.57 ± 0.04^{c}	6.67 ± 0.06
Energy total (kg calorie/100 g)	240.87 ± 0.38^{a}	234.41 ± 0.30^{b}	240.88 ± 0.74^{ac}	237.11 ± 0.43
Amino acid composition				
EAA				
Leucine	1.36 ± 0.01^{a}	1.42 ± 0.01^{b}	1.46 ± 0.01^{c}	1.36 ±0 .01^{d}
Isoleucine	0.76 ± 0.01^{a}	0.79 ± 0.01^{b}	0.81 ± 0.01^{c}	0.76 ± 0.01^{d}
Lysine	0.95 ± 0.01^{a}	1.10 ± 0.01^{b}	0.98 ± 0.01^{c}	1.20 ± 0.01^{d}
Valine	0.86 ± 0.01^{a}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}	0.89 ± 0.01^{d}
Threonine	0.79 ± 0.02^{a}	0.92 ± 0.01^{b}	1.04 ± 0.01^{c}	0.83 ± 0.01^{d}
Arginine	1.02 ± 0.01^{a}	1.19 ± 0.01^{b}	1.30 ± 0.01^{c}	1.03 ± 0.01^{d}
Phenylalanine	0.67 ± 0.01^{a}	0.93 ± 0.01^{b}	1.05 ± 0.01^{c}	0.77 ± 0.01^{d}
Tyrosine	0.43 ± 0.01^{a}	0.53 ± 0.00^{b}	0.57 ± 0.06^{c}	0.45 ± 0.01^{d}
Methionine	0.18 ± 0.01^{a}	0.26 ± 0.01^{b}	0.30 ± 0.01^{c}	0.21 ± 0.01^{d}
Histidine	0.40 ± 0.01^{a}	0.50 ± 0.01^{b}	0.57 ± 0.01^{c}	0.43 ± 0.01^{d}
Tryptophan	0.06 ± 0.01^{a}	0.11 ± 0.01^{b}	0.07 ± 0.00^{bc}	0.09 ± 0.01^{bd}
NEAA				
Alanine	0.85 ± 0.01^{a}	0.94 ± 0.01^{b}	0.87 ± 0.06^{c}	0.97 ± 0.01^{bd}
Serine	1.01 ± 0.01^{a}	1.12 ± 0.01^{b}	1.23 ± 0.01^{c}	1.01 ± 0.01^{d}
Glycine	1.15 ± 0.01^{a}	1.32 ± 0.01^{b}	1.29 ± 0.01^{c}	1.19 ± 0.01^{d}

Commented [HJ9]: Query – there is 'ac' and 'ad' in superscript in this line.

This sort of thing occurs occasionally elsewhere. Is it just a case of typos please, or is it deliberate? (If it is erroneous, please go through each table and delete the unwanted letters.)

#2 – Actually, there appears to be no explanation of what any of the superscript letters mean. Please add a note at the bottom of the table about this, therefore – and also, for any other table where this applies…

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.

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

Commercial feed combined with a new formulation product significantly affected 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 ± 0.17%), fat (3.47 ± 0.03%), carbohydrates (31.18 ± 0.37%), and crude fibre (2.81 ± 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 whole-body meat. 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 provided 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 ± 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 ± 0.16	64.51 ± 0.34	64.14 ± 0.33	64.24 ± 0.12
Crude protein	28.64 ± 0.28^{a}	28.07 ± 0.79^{ab}	28.85 ± 0.45^{c}	28.66 ± 0.44^{ad}
Crude fat	2.79 ± 0.03^{a}	2.88 ± 0.02^{b}	2.67 ± 0.04^{c}	3.00 ± 0.02^{d}
Carbohydrate	1.38 ± 0.01^{a}	1.99 ± 0.06^{b}	1.97 ± 0.09c	1.31 ± 0.02^{d}
Crude fibre	0.97 ± 0.02^{a}	0.68 ± 0.01^{b}	0.83 ± 0.02^{c}	0.95 ± 0.04^{d}
Ash	1.63 ± 0.02^{a}	1.70 ± 0.02^{b}	1.54 ± 0.01^{c}	2.11 ± 0.04^{d}
Energy total (kg calorie/100 g)	144.77 ± 1.58^{a}	155.48 ± 1.26^{b}	157.90 ± 0.91^{c}	149.60 ± 0.29^{d}
Amino acid composition				
EAA				
Leucine	2.13 ± 0.01^{a}	2.37 ± 0.01^{b}	2.42 ± 0.01^{c}	2.26 ± 0.01^{d}
Isoleucine	1.13 ± 0.01^{a}	1.25 ± 0.01^{b}	1.38 ± 0.01^{c}	1.19± 0.01^{d}
Lysine	2.77 ± 0.01^{a}	3.16 ± 0.02^{b}	3.88 ± 0.01^{c}	2.86 ± 0.01^{d}
Valine	1.26 ± 0.01^{a}	1.40 ± 0.01^{b}	1.32 ± 0.01^{c}	1.35 ± 0.01^{d}
Threonine	1.38 ± 0.02^{a}	1.49 ± 0.01^{b}	1.43 ± 0.01^{d}	1.48 ± 0.01^{d}
Arginine	1.58 ± 0.01^{a}	1.71 ± 0.01^{b}	1.63 ± 0.01^{c}	1.70 ± 0.01^{d}
Phenylalanine	1.02 ± 0.01^{a}	1.11 ± 0.01^{b}	1.08 ± 0.01^{c}	1.11 ± 0.01^{d}
Tyrosine	0.80 ± 0.01^{a}	0.84 ± 0.00^{b}	0.83 ± 0.01^{c}	0.85 ± 0.06^{d}
Methionine	0.15 ± 0.01^{a}	0.21 ± 0.01^{b}	0.18 ± 0.01^{c}	0.16 ± 0.01^{d}
Histidine	0.55 ± 0.01^{a}	0.56 ± 0.01^{ab}	0.59 ± 0.01^{ac}	0.57 ± 0.01^{d}
Tryptophan	0.08 ± 0.01^{a}	1.02 ± 0.01^{b}	1.08 ± 0.01^{ac}	0.06 ± 0.00^{d}
NEAA				
Alanine	1.86 ± 0.01^{a}	2.08 ± 0.01^{b}	2.92± 0.01^{c}	1.97 ± 0.01^{d}
Serine	1.28 ± 0.01^{a}	1.31 ± 0.01^{b}	1.26 ± 0.01^{c}	1.31 ± 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}

Commented [HJ10]: #2 – figures have changed in this table, without being highlighted. See column 3 for 'Serine' and 'Proline' here.
I am unclear why this is so….(inevitably, if changes aren't highlighted we are not aware of them and so cannot read them to check for typos etc…)
As a result of the changes, the numbers were closed up to the '±' symbol – which I had tweaked before. So I have re-corrected it here.

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]
∑EAA	12.68 ± 0.003[a]	15.13 ± 0.005[b]	15.82± 0.001[c]	13.61 ± 0.008[d]
∑NEAA	12.91 ± 0.007[a]	14.32 ± 0.01[b]	15.69 ± 0.002[c]	13.50 ± 0.001[d]
∑AA	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.

Growth coefficient and survival

The growth coefficient and feed utilization of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant difference between experimental diets in the case of the thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), and daily growth coefficient ($F_{(3,8)} = 59.88$, $P = 0.288$), while total feed intake (% BW day-1) ($F_{(3,8)} = 14.938$, $P = 0.56$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Figure 2).

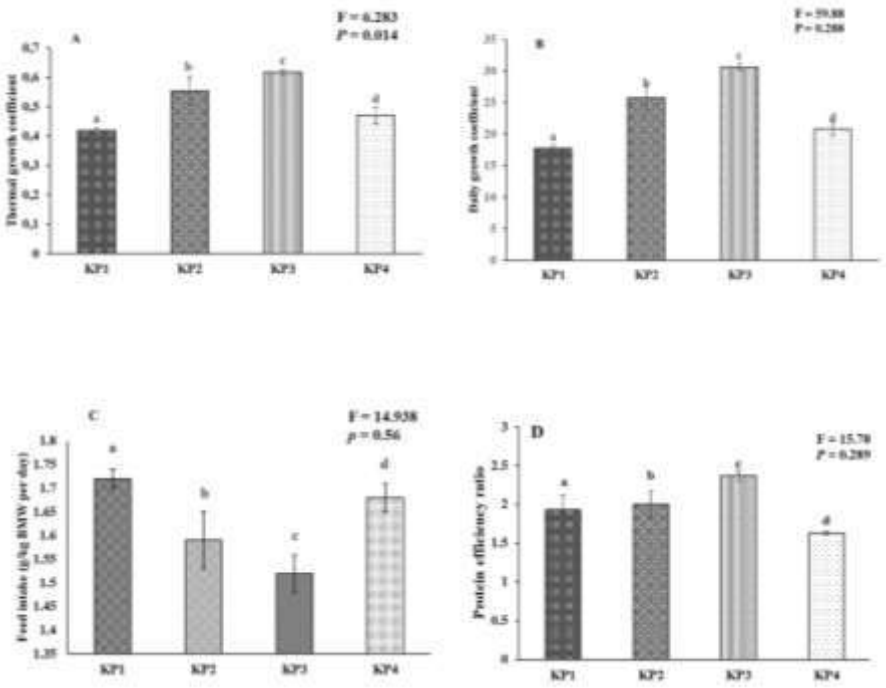

Figure 2. Growth coefficient and feed utilization of the giant gourami juveniles reared under different diets during 90 days of the experiment period.~~,~~ (A) T~~t~~hermal growth coefficient (TGC), (B) daily growth coefficient (DGC), (C) feed intake (FI), and (D) protein efficiency ratio (PER). ~~–~~The mean value and standard deviation (mean ± SD) are presented for giant gourami (n = 3). Different superscripts in the bar diagram of the giant gourami juvenile TGC, DGC, FI, and PER indicate significant differences among other diets (P < 0.05, One-way ANOVA Duncan Post-Hoc)

Furthermore, the thermal growth coefficient (TGC) has often been used to predict growth performance and ~~-~~production performance of aquaculture using~~with~~ ~~–~~water temperature at the

fish–rearing location. This study presents the relationship between thermal growth coefficient and condition factor, daily growth coefficient, and protein efficiency ratio (Figure 3). The thermal growth coefficient had strong relationships with the condition factor (r^2 = 0.777, figure 3A), daily growth coefficient (r^2 = 0.999, figure 3B), and protein efficiency ratio (r^2 = 0.749, figure 3D), while the thermal growth coefficient had a moderate relationship with the feed intake (r^2 = 0.699, figure 3C).

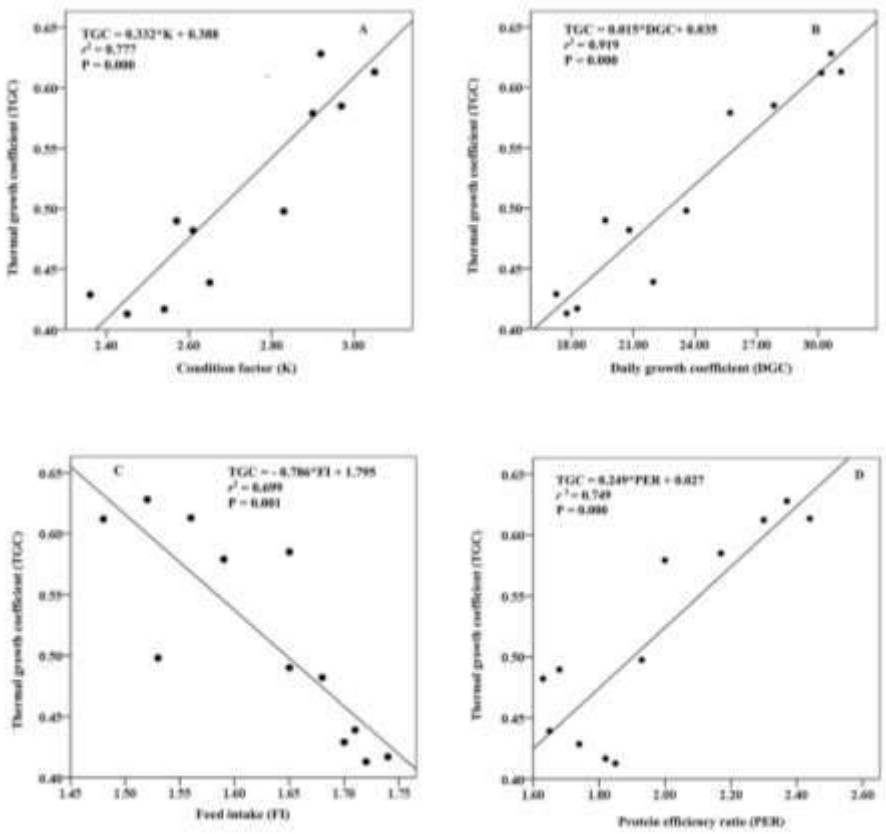

Figure 3. Relationships between thermal growth coefficient and condition factor (A), daily growth coefficient (B), feed intake (C) and -protein efficiency ratio (D) for giant gourami (*O. gourami*) over 90 days.

Condition factor and body indices of giant gourami after 90 days of feeding

The condition factor was significantly different between diets ($F_{(3,8)}$ = 19.98, P = 0.566) in the present study; while the GSI, HIS, and VFSI displayed marginally significant differences between diets. The HIS 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). GSI was significantly ($F_{(3,8)}$ = 10.492, P = 0.243) in the KP3 diet and significantly among all different diets. The VFSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's post-hoc test revealed that the HIS (1.30 ± 0.13%), GSI (4.15 ± 0.36%), and VFSI (2.75 ± 0.34%) were significantly higher (P < 0.05) in the KP3 diet than in the other diets. Meanwhile, BSI showed no significant difference (P > 0.05) among the treatment diets (Table 3).

Commented [HJ11]: #2 – an overlooked point…as mentioned, 'significantly' creates a small a sense problem (on both occasions). Perhaps it s a typo and you just mean 'significant' (on both occasions)? Please confirm.

Table 3. Mean (± SD) value condition factor 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}
Viscerosomatic index (GSI%)	3.20 ± 0.21^{a}	3.77 ± 0.09^{b}	4.15 ± 0.36^{c}	3.17 ± 0.02^{d}
Hepatosomatic (HIS%)	0.97 ± 0.05^{a}	1.06 ± 0.19 ^{ab}	1.30 ± 0.13^{c}	1.04 ± 0.12^{ad}
Visceral fat-somatic indexes (VFSI%)	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

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 ± 1.76 µm), fold width (53.23 ± 0.88 µm), enterocyte height (27.42 ± 0.42 µm), and microvillus height (2.79 ± 0.45 µm) 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 ± 1.43 µm), fold width (61.50 ± 1.02 µm), and enterocytes (32.82 ± 0.54 µm) 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).

Pond water quality

The pond water quality values of the giant gourami juvenile rearing freshwater concrete pond 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 in 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 line are significantly different ($P < 0.05$).

	Foregut				Midgut				Hindgut			
	hF (µm)[a]	wF (µm)[b]	hE (µm)[c]	hMV (µm)[d]	hF (µm)	wF (µm)	hE (µm)	hMV (µm)	hF (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

Table 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 (^{O}C)	45	28.01 ± 1.06	27 - 30	25-33	Prokoso *et al.*[43]
Dissolved oxygen (mg L^{-1})	14	6.01 ± 0.14	5.80 – 6.20	3-5	Syandri *et al.*[44]
Total alkalinity (mg L^{-1} as $CaCo_3$)	14	58.09 ± 3.33	52.5 - 62.5	120	Boyd *et al.*[45]
Hardness (mg L^{-1} as $CaCo_3$)	14	66.34 ± 1.32	65 - 68.5	168	Boyd *et al.*[45]
pH	14	7.48 ± 0.19	7.2 – 7.8	6.5 – 9.0	Boyd *et al.*[45]
Nitrates (mg L^{-1})	14	0.04 ± 0.01	0.03 – 0.05	0.2 – 219	Boyd and Tucker[46]

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[47,48,49]. The giant gourami belongs to thea trophic level of herbivorous fish[50]. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish[51,49]. 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[2,52]. 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*[53], and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*[48] and for rearing rohu, *Labeo rohita*[54]. 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, the 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*). In the recent past, the dose used was 300 ml/kg of feed.This method is a new approach that has been developed by Azrita *et al*.[9] to improve feed nutrition and whole body carcasses, covering fatty acids, the atherogenic index and thrombogenic, feed efficiency, and growth performance of giant gourami. Here, we continued the investigation by reducing the feed dose to 150 ml/kg. This study's results found that supplementing feed with newly formulated products can increase feed nutrition, covering amino acids in diet and body meat, and the growth coefficient of giant gourami. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[12], fish oil[19, 11], and soybean oil[20]. In addition, the provision of feed has been supplemented with probiotics[21], glycine, and prebiotics[22]. 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[55] and Vong *et al.*[56] showed that *Rhizopus oligosporus* can produce various extracellular enzymes. *Aspergillus niger*. has a high capacity to degrade antigenic proteins, including carbohydrases, proteases, lipases, and phosphatases, when used for fermenting plant-sourced fish feed ingredients[12,57] . *Saccharomyces cerevisiae* is one of the most 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[7,58].

The composition of amino acids can be used to judge the quality of feed. In the present study, in feed supplemented with different formulated products (CP1, CP2, CP3, and CP4), leucine, arginine, and glutamic acid were the most abundant free amino acids (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 diet[22]. Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid[59], and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein[60]. 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 feed[18]. 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. It was higher in the KP3 diet than the other diets. In contrast, the essential amino acids of fish feed for snubnose pompano were slightly higher than the nonessential amino acids content[60]. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu *et al.*[60] reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet[59] and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet[22]. In the present study, this difference in FAA content is caused by various mushrooms used in the formulated products.

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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 those fed 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[12,57]. The FAA profile differences could be related to different aspects, such as diet composition[61], dietary protein level[62], and methionine levels in the diet[18], including the water quality of the ponds[63]. 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[64,60]. The present study did not examine whether the 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 the insufficient KP1 diet were perhaps due 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 Jannatullah *et al.*[57] and Li *et al.*[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.*[36] 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, the thermal growth coefficient (TGC) strongly correlated with the daily growth coefficient (DGC). Because faster daily fish growth requires a quality diet and constant water temperature during the rearing period, in this study, water temperature ranged from 27 to 30ºC, and dissolved oxygen was between 5.8 and 6.2 mg /L. According to Besson *et al.*[65], higher daily energy availability in the diet can lead to faster-growing fish, which is supported by constant water temperature and higher daily oxygen levels. The thermal growth coefficient had an essential change in environmental value[66]. Therefore, it was very important to keep the water temperature and dissolved oxygen constant in the aquaculture locations. At the same time, 78% of TGC values were determined by the condition factor connected to whole body weight and the total fish length. TGC of Atlantic cod, *Gadus morhua*, is influenced by body size and condition factors[67].

In this study, a higher value of TGC was detected in fish fed KP3; the effect is that the daily growth coefficient, and the protein efficiency ratio is better. Conversely, decreasing TGC has two effects, *i.e.*, a slow fish growth and lowered daily feed intake. Many scientists state that in aquaculture operations, net yield (kg/m^3) depends upon TGC fluctuation, feed intake, and daily oxygen consumption[65,68,69].

In the present study, feed enrichment with different formulated products did not affect HIS or VFSI except in the KP3 diet. Whereas GSI 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 Arriaga-Hernandez *et al.*[70] for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, Hassan *et al.*[71] 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.*[72] 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.*[64] 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*[44]. 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[18,73]. 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 length[74]. In contrast, substituting as much as 12.5–25% soya protein concentrates with lupin (*Lupinus albus*) meal in carp (*Cyprinus carpio*) diets does not significantly affect the villi length and villi width of the gut[75]. 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 grouper[18], turbot, *Scophthalmus maximus*[12], largemouth bass, *Micropterus salmoides*[22], and common carp, *Cyprinus carpio*[75]. 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.

Conclusions

The present investigation observed that feed enriched with newly formulated products made from mature coconut water and palm sap sugar, and fermented with various mushrooms, given to fish in a dose of 150 ml/kg substantially affected the amino acid composition of the diet and whole-body carcass of giant gourami juveniles. It also affected the growth coefficient, feed utilization, body indices, and gut micromorphology size. The thermal growth coefficient had a strong relationship with the daily growth coefficient (r^2 =

91%) and a moderate relationship with the feed intake (r^2 = 69%). The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass, growth coefficient, body indices, and the ability of the intestines for feed absorption. Thus, our study also informs fish farmers about culturing good quality giant gourami and fulfilling nutrition requirements for food security.

Data availability

Underlying data

Figshare: Underlying data for 'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles'.
https://doi.org/10.6084/m9.figshare.20407647[76]

This project contains the following underlying data:

- Table 1. Raw data of the experimental diets' proximate composition
- Table 2. Raw data of amino acid of feed experimental
- Table 3. Raw data of whole body carcass proximate composition
- Table 4. Raw data of amino acid of whole-body carcass
- Table 5. Daily growth coefficient, feed utilization and body indices of giant gourami after 90 days of feeding.
- Table 6. Raw data gut micromorphology of giant gourami juveniles fed different diets for 90 days

Competing interests

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

Grant information

The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia funded this research under grant number: 076/E5/PG.02.00. PT/2022.

Acknowledgments

We are grateful to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for funding this research. ~~The ministry is pleased with the results achieved.~~

Commented [HJ13]: Please confirm you have checked that the body is happy for this acknowledgment to appear.

#2 – Suggest deleting the new line added, for convention, as it 'sounds odd'. The reason I asked if you had permission to thank the Ministry, is that F1000 always checks this; but it does not need to be stated or suggested in the Acknowledgements.

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***

Final points:

- re: *Figures query*. You say that Figures 1 and 2 are formatted at 300dpi. Perhaps the wrong versions have therefore been uploaded by mistake? 9or you have been passed the wrong version.) Please check this. According to our system (where the DPI shows up if you right-click on the picture file, scroll to Properties, and go to Details), the figures are showing as follows: Fig 1 - 150dpi; Fig 2 - 220dpi; Fig 3 - 300dpi; Fig 4 - 220dpi.

I'm afraid I cannot do anything about this as the '300dpi' specification comes from the typesetting department, who we need to follow as they are in charge of reproduction. Hopefully you have got the right versions already, or the person who has supplied the figures to you can let you have versions at 300dpi...many thanks in advance.

- Re: *percentage amount*. Under the crossheading 'Preparation of formulated product', you have made some adjustments, to create:

'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 60º C to make an 11% palm sap sugar solution. 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 the 1.0 litre of palm sap sugar solution (equivalent to 33% of palm sap sugar solution).'

Unfortunately, the detail about 33% is related to the earlier version of the text, before amounts and volumes were clarified. So it seems incorrect. If the palm sap sugar solution was 11%, then it seems that mixing a litre of it with 2 litres of another liquid (coconut water) will dilute the palm sap sugar content by 1/3rd; making 3 litres with 3.66% palm sap sugar solution per litre? NB, one potential solution here, rather than changing the percentage amount, would be to delete the phrase in brackets. What should be done please?

- Re: *small miscellaneous point*. In the paragraph after Figure 3, you adjusted the text to:

'GSI values in the KP3 diet were significantly (F (3,8) = 10.492, P = 0.243) higher than the KP1, KP2, and KP4 diets.'
- In fact there was unfortunately a small error at the start of the sentence, so I just wanted to double-check it is correct like this (with the plural 'GSI values...').

- Re: *overlooked point*: In the names and addresses of institutions, there is sometimes 'West Sumatra' and sometimes 'Sumatera Barat' - and there isn't full consistency between the m/s and the manuscript system on this. Which should it be? Suggest make consistent to 'West Sumatera' in both places. Ok?

- Tables 1-4. Re: *the point about superscript letters*. Unfortunately the wording didnt quite work, for correct sense. Suggest use the following: 'Note: If the numbers in a row have different superscript letters, this indicates there is a significant difference between them ($P < 0.05$). If the numbers in a row have the same superscript letter, this means they show no significant difference ($P > 0.05$).'

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> > > > Information Classification: General

azrita ubh <azrita31@bunghatta.ac.id> Wed, Nov 16, 2022 at 9:32 PM
To: "F1000.Research" <research@f1000.com>

Dear

Jonathan

F1000 Research editorial team

Thank you for your email on November 16, 2022. We have revised manuscript No. 124706 (attached). At the same time, we also send all images that have been formatted with Jpeg 300 pi and list correction of the manuscript.

With best regards

Azrita

[Quoted text hidden]

5 attachments

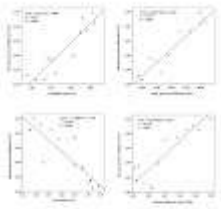

Figure 3_pooled_TGC_001.jpg
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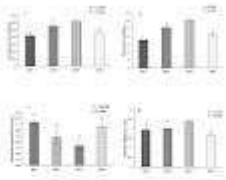

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List correction of manuscript No. 124706 .doc
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Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Thu, Nov 24, 2022 at 6:24 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

List correction of manuscript No. 124706 highlights magenta

1. All images have been formatted with Jpeg 300 pi
2. In Method (equivalent to 33% of palm sap sugar solution) has been deleted
3. In the paragraph after Figure 3, the text is revised to The GSI value of giant gourami was significantly different ($F_{(3.8)}$ = 10.492, P = 0.243) between diets, and the GSI of giant gourami fed KP3 ration was higher than ration KP1, KP2, and KP4
4. Names and addresses of institutions have been revised and have complete consistency
5. Universitas Nahdlatul Ulama Sumatera Barat is the name of the institution
6. About superscript letters for Tables 1,2 and 3 have been adjusted based on suggestions of a reviewer

Corresponding author: Azrita

Hi Azrita

Please see my email of last Weds below. If you could kindly come back to me about the points, we can hopefully get the m/s to the typesetters.

Best wishes

Jonathan

[Quoted text hidden]

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azrita ubh <azrita31@bunghatta.ac.id> Fri, Nov 25, 2022 at 7:42 PM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear

Jonathan

F1000 Research editorial team

Thank you for your email on November 24, 2022. We have revised Figures 1,2, and 3 (300 DPI) for manuscript No. 124706 (attached). In Figure 2, we modified the P value (P <0.001, ANOVA results). Figure 3 shows changes on the X and Y axes starting with 0.0.

With best regards

Azrita

[Quoted text hidden]

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3 attachments

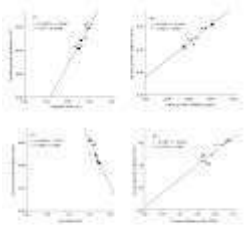

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Figure_1.jpg
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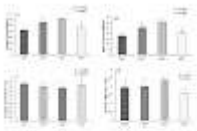

figure_2.jpg
751K

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Thu, Dec 1, 2022 at 7:24 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

Please see my email below, where I sent the miscellaneous queries left on your manuscript, including the Figures query which you have now resolved.

You'll see there were a few text queries in the same part of the email…

Best

Jonathan

F1000 Research editorial team

Information Classification: General
[Quoted text hidden]

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azrita ubh <azrita31@bunghatta.ac.id> Fri, Dec 2, 2022 at 11:05 AM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear
Jonathan
Editorial Team, F1000Research

I have read the email dated October 12, 2022. I have logged into the system to detail the individual/organization responsible for paying the publication fee. However, we did not find a format to fill in for payment for manuscript No. 124706.

Now I don't understand the process for manuscript payment No. 124706. I decided that the person responsible for the payment was Azrita (azrita31@bunghatta.ac.id). Please help me get an invoice as soon as possible so I can pay for the Article Processing Charge (APC). I'm waiting for good news from you.

With best regards

Azrita

[Quoted text hidden]

Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

RE: Manuscript 124706 (the final text points)

3 messages

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Wed, Dec 7, 2022 at 1:56 AM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

It's good that the payment query is resolved.

I am emailing you now about the outstanding *text* queries. You don't appear to have received these unfortunately, although I have sent them to you a couple of times… (Email can be very unreliable, I know…often not arriving at its intended destination…)

I am therefore resending them here. They were in my email to you of 16 November (resent on 24 November).

Please scroll down this page to the very end of my email of *16 November*, in the email string below; you'll see there is a section *after my name at the end of the email*. I put the queries under the heading 'Final Points' (which I have highlighted in yellow now for your ease of reference). Please note: you have already answered the query about Figures in the list of 'Final Points' – it is the other ones in the list that remain outstanding.

So please go through the 4 text points that remain. (Another way to find the points below is to scroll to the very end of this email string below, and you will find the list of 'Final Points' immediately above the end point – they are the last thing included below.)

You can ignore the first couple of short emails in the string below, as these were my covering notes when I resent you the outstanding points before.

I do hope you receive this email safely! Please acknowledge.

Very best wishes

Jonathan

F1000 Research editorial team

***

From: Haynes, Jonathan
Sent: Thursday, December 1, 2022 12:25 PM
To: azrita ubh <azrita31@bunghatta.ac.id>
Subject: FW: Manuscript 124706 conditionally accepted for publication

Dear Azrita

Please see my email below, where I sent the miscellaneous queries left on your manuscript, including the Figures query which you have now resolved.

You'll see there were a few text queries in the same part of the email…

Best

Jonathan

F1000 Research editorial team

**

From: Haynes, Jonathan
Sent: Thursday, November 24, 2022 11:25 AM
To: azrita ubh <azrita31@bunghatta.ac.id>
Subject: FW: Manuscript 124706 conditionally accepted for publication

Hi Azrita

Please see my email of last Weds below. If you could kindly come back to me about the points, we can hopefully get the m/s to the typesetters.

Best wishes

Jonathan

From: F1000.Research
Sent: Wednesday, November 16, 2022 4:08 AM

To: 'azrita ubh' <azrita31@bunghatta.ac.id>
Subject: RE: Manuscript 124706 conditionally accepted for publication

Hi Azrita

I have been through the revised m/s – thanks.

There are a handful of text points that I need to raise with you, about the new changes, before I can put the m/s into production. I am putting these below, after the end of this email, with a reference to the place in the manuscript that the points apply to. You will see that I have suggested solutions or am double-checking a possible solution, in each case.

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I look forward to hearing back on these final points, so we can get this interesting manuscript to the typesetters for printing.

Best regards

Jonathan

F1000 Research editorial team

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Unfortunately, the detail about 33% is related to the earlier version of the text, before amounts and volumes were clarified. So it seems incorrect. If the palm sap sugar solution was 11%, then it seems that mixing a litre of it with 2 litres of another liquid (coconut water) will dilute the palm sap sugar content by 1/3rd; making 3 litres with 3.66% palm

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---

azrita ubh <azrita31@bunghatta.ac.id> Fri, Dec 9, 2022 at 5:21 PM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear
Jonathan
F1000 Research editorial team

Thank you for your email on December 7, 2022. We found the email on November 16 and 24, 2022, and have already read the final points we must explain. We have revised manuscript No. 124706 (the manuscript and List correction are attached). We hope that this revision will be accepted for publication. Conversely, we have not received an invoice for payment settlement of manuscript No. 124706, and we are waiting for the invoice.

Best Regards

Azrita

On Wed, Dec 7, 2022 at 1:57 AM Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> wrote:

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highlighted in yellow now for your ease of reference). Please note: you have already answered the query about Figures in the list of 'Final Points' – it is the other ones in the list that remain outstanding.

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I do hope you receive this email safely! Please acknowledge.

Very best wishes

Jonathan

F1000 Research editorial team

***

Information Classification: General

From: Haynes, Jonathan
Sent: Thursday, December 1, 2022 12:25 PM
To: azrita ubh <azrita31@bunghatta.ac.id>
Subject: FW: Manuscript 124706 conditionally accepted for publication

Dear Azrita

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**

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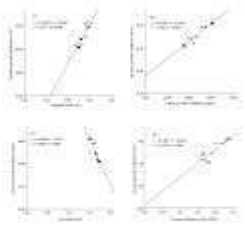
5 attachments



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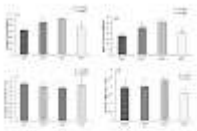

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List correction manuscript No. 124706.pdf
10K

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Fri, Dec 9, 2022 at 11:18 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

Thank you for the final changes. This is just a quick email to confirm that as I mentioned, our Accounts department will be in touch with you about payment when I have put the manuscript through for publication. So – assuming the last changes are fine (I will be looking at them later today) - this will probably be next week some time. If the changes are fine then the manuscript will go for typesetting on Monday morning, and so they will contact you after that.

Best / in haste, Jonathan

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Friday, December 9, 2022 10:22 AM
To: Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk>
Subject: Re: Manuscript 124706 (the final text points)

Dear

Jonathan

F1000 Research editorial team

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Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Final changes

5 messages

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Thu, Dec 15, 2022 at 8:52 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita

-

Re: 1 query – and 2 confirmations

Thank you for the resolutions to my text points.

1. Re: an outstanding point. Unfortunately, there is still one small area that does not seem quite right. This concerns the paragraph under the subheading, 'Preparation of formulated product'. This currently runs:

*'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 60º C to make an 11% palm sap sugar solution. 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 the 1.0 litre of palm sap sugar solution. The product was 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.'*

   a. Re: sentence 1. The figure of 11% in sentence 1 does not seem correct? (If 100g are cooked in 1 litre, the palm sap sugar makes 1/ 11^{th} of the product, or approximately 9%?)
   b. Re: sentence 3. If you created 1.1 litres of palm sap sugar solution in sentence 1 (not 1 litre…) then that is different to 'the 1.0 litre of palm sap sugar solution' you refer to here. Please note that in the Abstract (and possibly elsewhere), you also refer to mixing 2 litres of coconut water with 1.0 litre of palm sap sugar solution.

   Perhaps there is an explanation for point b) that has been left out? For example, perhaps you only got 1 litre of solution as a result of the process in sentence 1 due to some of it being 'boiled off' (?). If that was the case, you could add in such a detail and all would make sense. Alternatively, possibly you did obtain 1.1 litres of palm sap sugar solution, but only used 1 litre of it – again, if the text made this clear, then the details would add up. However, '11%' in sentence 1 does seem to be incorrect.

   Please clarify how the paragraph should run.

2. I have applied your other text changes. However, you actually sent versions of the manuscripts with slightly different corrections (perhaps they had not been updated?) in two cases. Therefore, please confirm the following text in the manuscript is correct on these two points:

   a. Under the heading, 'Condition factor and body indices of giant gourami after 90 days of feeding', after a number of lines you have got:

The GSI value of gourami was significantly ($F_{(3,8)} = 10.492$, P = 0.243) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 rations.

b. Under Acknowledgments, I have added your new sentence and left the existing one also. So it runs:

We are grateful to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for funding this research. We would also like to thank the students and fish farmers who helped with data collection in the field and in the laboratory.

Please confirm both these pieces of text in the manuscript are just as they should be.

I look forward to hearing back and sending the manuscript to be printed…

All the best

Jonathan

F1000 Research

PS By the way, I am the editor working on your manuscript, there is no-one else doing so (you sometimes sound like you are unclear on this). Best, Jonathan

---

azrita ubh <azrita31@bunghatta.ac.id> Sun, Dec 18, 2022 at 6:56 AM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear
Jonathan
F1000 Research Team

Thank you for your email on December 15, 2022; We have read the 1 query – and 2 confirmations about manuscript No. 124706. 1 query and two confirmations have been revised. Part revised Highlighted in red (the list correction is attached). Please help us complete this manuscript if there are small things we have not revised.

With best regards

Azrita
[Quoted text hidden]

2 attachments

124706 F1000 Research - Received JH2 - Des 16,22 .doc
3063K

List correction manuscript No. 124706 - Des 16, 2022.pdf
64K

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Sat, Dec 24, 2022 at 1:31 AM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

Just a quick email to say thanks for this. I have been a bit in and out of the office this week; I will be processing your manuscript next as it is top of the list. So hopefully I should be able to send the 'unconditional acceptance' for publication very shortly.

Best wishes

Jonathan

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Saturday, December 17, 2022 11:56 PM
To: Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk>
Subject: Re: Final changes

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> Dear Azrita

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

Azrita undefined[a*], Hafrijal Syandri[a], Netti Aryani[b], Ainul Mardiah[c]

[a]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Bung Hatta, Padang, West Sumatera, 25133 Indonesia
[b]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, 28293, Indonesia.
[c]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Nahdlatul Ulama Sumatera Barat, West Sumatera, 25118, Indonesia.
Corresponding author: Azrita31@bunghatta.ac.id

ABSTRACT

Background: Giant gourami, *Osphronemus goramy* (Lacepede, 1801) is the most important freshwater aquaculture farm in Indonesia's. The purpose of this study is to determine the effect of various newly formulated products on the amino acid composition of the diet and whole-body carcass, the growth coefficient, body indices, and gut micromorphology.
Methods: 100 g of palm sap sugar, cooked in 1.1 litre of fresh water for fifteen minutes, and only got 1 litre of solution due to some of it being boiled off to make an 11% palm sap sugar solution. Furthermore, 2 litres of coconut water and mixed in it with 1 litre of palm sugar solution. 1 litre of product was added in turn to 2 g of *Aspergillus niger* (CP2), 2 g of *Rhizopus oligosporus* (CP3), and 2 g of *Saccharomyces cerevisiae* (CP4), while, freshwater as a control (CP1). Aquafeed was added to CP1, CP2, CP3, and CP4, to make diets labeled KP1, KP2, KP3, and KP4. The 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 (2×1×1 m; water volume 1.5 m^3) in a freshwater concrete pond with a stocking density of 30 juveniles/net.
Results: The results supported our hypothesis that different product formulations have a significant effect ($P < 0.05$) on aquafeed nutrition and the whole-body carcass, growth coefficient, feed utilization, body indices, and gut micromorphology of giant gourami juveniles. The thermal growth coefficient strongly correlated with the daily growth coefficient (r^2 = 91%). The KP3 diet contains a higher concentration of amino acids, which increased the growth coefficient, feed utilization, and carcass quality more than the other diets that we tested.
Conclusions: Diet KP3 contains higher total amino acids in diets and carcasses and leads to better growth for giant gourami.

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

Commented [HJ1]: Re: figures within the article.

#2 - Unfortunately, only one of the four attachments is at the resolution we need for reproduction (300 dpi); this is the one for Figure 3. Please therefore could you provide jpegs (or another suitable file format such as tiffs) at 300 dpi for the other figures to be used. Thanks.

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Commented [HJ2]: On our system, the last part of the address that was entered currently reads: '…Universitas Bung Hatta, Padang, Sumatera Barat, 25113, Indonesia'.

#2 - Which address is correct please? They are different.

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Commented [HJ3]: #2 – you have made a change or two here. However the sentence starting 'Aquafeed was added…', which I queried, is still confusing, unfortunately. (It suggests that you add aquafeed to CP1 and then also add CP2, CP3, CP4 to the 'aquafeed plus CP1' mix.)

I think you must mean: 'Aquafeed was added to CP1, and then added to CP2, CP3, and CP4, to make diets labeled KP1, KP2, KP3, and KP4.' Please confirm.

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Introduction

In this decade, the production of capture fisheries has decreased; meanwhile, the demand for fish products for human consumption is increasing. Therefore, according to the Food and Agriculture Organisation, 60% of fisheries production in the future will come from aquaculture activities and this figure will continue to rise[1]. The utilization of a variety of fish for aquaculture has now increased the need for commercial feed[2,3,4,5]. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge[2,6,7,8]. On the other hand, commercial feed produced by factories still does not contain complete nutrition for fish growth, while being 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 in many important nutrients, including protein, fat, vitamins, and minerals that cultured fish can utilize to increase their growth rate and survival and that is beneficial for human health[4,14,15,16]. Therefore, novel approaches have been developed by scientists to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[18], fish oil[19,11], and soybean oil[20]. In addition, supplementing probiotics into the diet[21] and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied[22].

The progress of aquaculture biotechnology has stimulated the interest of scientists in improving aquatic animal production, for example, to increase giant gourami production. One of the experimental techniques is to increase feed nutrition used for this purpose, such as, the use of fish meal and Azolla flour as a feed ingredient for giant gourami[23], and the utilization of new products formulated from water coconut, palm sap sugar, and fungus for the enrichment of commercial feed[9]. Additional research has involved a diet supplemented using glutamine[24], feed supplemented with a growth hormone[25], and substitute fish meal incorporating chicken feather[26]. Whether using coconut water and palm sap sugar fermented with mushrooms affects the amino acid composition of the diet, body carcass, growth coefficient, and body indices is still not understood.

Coconut water has extraordinary nutritional value and contains supplements for health like minerals, amino acids, fatty acids, vitamins, enzymes, organic acids, and several phenolic compositions[27,28,29,30]. Palm sap sugar also has health benefits due to its essential nutrient content, such as a low glycaemic index, and it contains antioxidants, vitamins, and minerals[31,32,33,34]. Meanwhile, mushrooms have been widely used in fermentation due to their

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ability to degrade antigenic proteins in fish feed ingredients[7,35,36]. Additionally, coconut water is a functional food that can protect the lens from diabetic cataract development in rats[37]. Coconut water is also a treatment for burning pain during urination, dysuria, gastritis, increasing semen, and indigestion[38].

On the other hand, Azrita *et al.*[9] have reported using new formulations of products containing coconut water and palm sap sugar that are fermented with various mushrooms involving a dosage of 300 ml/kg feed. Their newly formulated products can increase fatty acid levels in the diet and whole body carcasses. Besides that, they also improve giant gourami's growth performance and feed efficiency.

However, the effect of these new formulation products at a dosage of 150 ml/kg feed on the diet amino acid composition, and body meat's amino acid composition has not yet been analyzed. In line with that, the relationships between thermal growth coefficient and condition factor, daily growth coefficient, and feed utilization coefficient, including body indices parameters, as well as the gut micromorphology of giant gourami, have not yet been analyzed.

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We hypothesized that commercial aquafeed combined with different newly formulated products at the dosage of 150 ml/kg feed could improve the amino acids compositions of the aquafeed and whole body carcass, body indices, and gut micromorphology. Hence, this investigation's first purpose was to analyze the effect of various newly formulated products on the diet's proximate compositions, amino acid composition, and whole-body carcass. The second aim was to analyze the impact of newly formulated products on the growth coefficient and relation to thermal growth coefficient, body indices, and gut micromorphology in giant gourami juveniles.

Methods

Ethical approval

The Research and Community Service Ethics Committee at Universitas Bung Hatta, West Sumatera, Indonesia approved this research (89/LPPM/Hatta/III-2022) which followed the ARRIVE guidelines. 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. Approval was given by the ethics committee to collect and rear juvenile gurami sago in the aquaculture laboratory, Faculty of Fisheries and Marine Science at Universitas Bung Hatta. All efforts were made to relieve the suffering of experimental animals. Therefore, the animal did not suffer for this study, and they were still in good

condition when returned to the pond after research was completed. Where some fish were euthanized, this was carried out by piercing part of the fish's brain. Gurami sago fish are not classified as a protected animal according to Indonesian legislation.

Preparation of formulated product

We prepared 100 g of palm sap sugar by traditional production and cooked it in 1.1 litre of fresh water for fifteen minutes at 60º C, and only got 1 litre of solution due to some of it being boiled off to make an 11% palm sap sugar solution. 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 the 1.0 litre of palm sap sugar solution. The product was 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 48hrs 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) and labeled as the KP2, KP3, and KP4 diets. The aquafeed was supplemented with freshwater (labeled as the KP1 diet; placebo).

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), which 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. The minerals in the 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 freshwater to create the KP1 diet as observed, and the formulated CP2, CP3, and CP4 products were added to the aquafeed at a dosage of 150 ml/kg of feed to create the enriched fish diets. The formulated product added to the aquafeed was mixed manually with it for three minutes to obtain maximum homogenization and then the blend was dried in the open air for thirty minutes. Thereafter, it was given to the trial animal.

Experimental procedures and sampling

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If so, suggest we have: 'We prepared 100 g of 11% palm sap sugar by traditional production and cooked it in…'.

If instead, you want to say that the overall preparation of 1.0 litres involved 11% palm sap sugar, you would need to have: 'We prepared 100g of palm sap sugar by traditional production and cooked it in 1.0 litre of fresh water for fifteen minutes at 60°C, to make an 11% palm sap sugar solution.'

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In the present study, we measured fish weight using AD-600i scales with 0.001 g accuracy (ACIS model number AD-600i, China). At the same time, a meter 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 nets framed with 2 m^3 (2×1×1 m) PVC pipe (water volume of 1.5 m^3) were placed inside two freshwater concrete ponds with a size of 18 m^3 (6×2×1.5 m). This experiment consisted of four treatments and three replications, 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 17:00hrs) 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 24hrs to empty their intestinal contents.

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Do you mean you did the four treatments 3 times? (ie that you 'replicated' them 3 times? 'replicated' means 'repeated', as we know…)

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Proximate and amino acid composition

The diet samples and proximate carcass composition were analyzed using standard AOAC methods[39]. The matter was dried to a constant weight at 105°C. We used the standard Kjeldahl method to analyse crude protein (N × 6.25). We used the Soxhlet method with ether extraction to analyse crude lipids; the ash was incinerated at 550°C for 16 hrs, whereas gross energy was measured in a bomb calorimeter. 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 24hrs at 11°C[40].

Nutrient utilization and body indices

The growth coefficients in the fish experiments were measured by using the thermal growth coefficient (TGC), daily growth coefficient (DGC), total feed intake (FI), and protein efficiency ratio (PER) of giant gourami, assessed using the following formulae:

TGC = [(final weight (g))⅓ – (initial weight (g))⅓] / (mean water temperature (°C)) × duration of rearing period (day)] × 1000

DGC = (Wf⅓ – Wi⅓) / duration of rearing period (day) × 100

FI as feed (FI as feed in g/fish/day) = Total feed fed / (n × t)

PER = wet weight gain / total protein intake

Three fish from each net frame were sacrificed and dissected immediately to determine the Condition factor (CF), Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) as given below:

CF = 100 × [weight of the juvenile (g) / Length of juvenile (cm^3)]

GSI = 100 × [viscera weight (g) / whole body weight (g)]

HSI = 100 × [liver weight (g) / whole body weight (g)]

VFSI = 100 × [visceral fat weight (g) / whole body weight (g)]

BSI = 100 × [Bile weight (g) / weight of liver (g)]

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 24hrs. After sequential dehydration steps in alcohol, the gut samples were embedded in paraffin. The implanted tissue blocks were sectioned at 5 µm, 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 Figure 1.

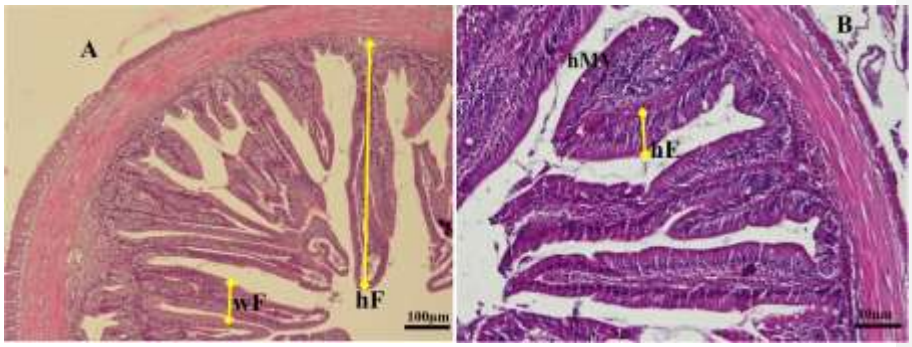

Figure 1. Transversal section photomicrographs of giant gourami juvenile foregut. (A) Fold height and fold width were analyzed in a lower magnification of objective lens of microscope (magnification × 100), (B) Enterocytes height and microvilli height were analyzed using a higher magnification of an objective lens microscope (magnification × 200). hF = fold height, wF = fold width, hE = enterocyte height, hMV = microvillus height (hematoxylin and eosin).

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 10:00am at a depth of 20 cm from each concrete pond to determine the water temperature, dissolved oxygen, and pH value. In addition, we also measured the total alkalinity, hardness, and nitrates of the water in the pond experiments. A thermometer (Celsius scale) was used to measure water temperature. To measure water dissolved oxygen (O_2; mg L^{-1}), we used 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 experiments. The level of nitrate-nitrogen (NO_3-N; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures[41].

Calculations and statistical method

The data from this study were reported in the form of the mean ± standard deviation for each treatment. Data were analysed using 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[42]. To create the figures, Microsoft Office Professional Plus 2019 was used.

Results

Proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products with the dosage of 150 ml/kg of feed significantly affects 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±0.17%) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate (31.19 ± 0.38%), crude fibre (2.82±0.06%), and ash (6.67±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 with a dosage of 150 ml/kg of feed 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 significant 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 ± 0.011%), nonessential amino acids (12.91 ± 0.004%), 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).

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Table 1. The experimental diets' proximate and amino acid composition (% dry matter). Mean ± SD*. Note: Numbers followed by different superscript of letters in the same row indicate a significant differences ($P < 0.05$). Numbers followed by superscript of the same letter in the same row showed no significant difference ($P > 0.05$).

	KP1	KP2	KP3	KP4
Proximate composition	%, dry weight basis			
Dry matter	38.42 ± 0.25^{a}	38.27 ± 0.01^{a}	37.59 ± 0.16^{a}	38.41± 0.10
Crude protein	19.68 ± 0.41^{a}	20.27 ± 0.13^{b}	21.70 ± 0.18^{c}	20.44 ± 0.10
Crude lipid	3.41 ± 0.02^{a}	3.67 ± 0.13^{b}	3.50 ± 0.02^{ac}	3.48 ± 0.04^{a}
Carbohydrate	26.37 ± 0.17^{a}	29.50 ± 0.54^{b}	31.19 ± 0.38^{c}	30.57 ± 0.06
Crude fibre	2.23 ± 0.05^{a}	2.36 ± 0.01^{b}	2.82 ± 0.06^{c}	2.45 ± 0.06
Ash	2.75 ± 0.03^{a}	6.66 ± 0.05^{b}	6.57 ± 0.04^{c}	6.67 ± 0.06
Energy total (kg calorie/100 g)	240.87 ± 0.38^{a}	234.41 ± 0.30^{b}	240.88 ± 0.74^{ac}	237.11 ± 0.43
Amino acid composition				
EAA				
Leucine	1.36 ± 0.01^{a}	1.42 ± 0.01^{b}	1.46 ± 0.01^{c}	1.36 ±0 .01
Isoleucine	0.76 ± 0.01^{a}	0.79 ± 0.01^{b}	0.81 ± 0.01^{c}	0.76 ± 0.01
Lysine	0.95 ± 0.01^{a}	1.10 ± 0.01^{b}	0.98 ± 0.01^{c}	1.20 ± 0.01^{d}
Valine	0.86 ± 0.01^{a}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}	0.89 ± 0.01^{d}
Threonine	0.79 ± 0.02^{a}	0.92 ± 0.01^{b}	1.04 ± 0.01^{c}	0.83 ± 0.01^{d}
Arginine	1.02 ± 0.01^{a}	1.19 ± 0.01^{b}	1.30 ± 0.01^{c}	1.03 ± 0.01^{d}
Phenylalanine	0.67 ± 0.01^{a}	0.93 ± 0.01^{b}	1.05 ± 0.01^{c}	0.77 ± 0.01^{d}
Tyrosine	0.43 ± 0.01^{a}	0.53 ± 0.00^{b}	0.57 ± 0.06^{c}	0.45 ± 0.01^{d}
Methionine	0.18 ± 0.01^{a}	0.26 ± 0.01^{b}	0.30 ± 0.01^{c}	0.21 ± 0.01^{d}
Histidine	0.40 ± 0.01^{a}	0.50 ± 0.01^{b}	0.57 ± 0.01^{c}	0.43 ± 0.01^{d}
Tryptophan	0.06 ± 0.01^{a}	0.11 ± 0.01^{b}	0.07 ± 0.00^{bc}	0.09 ± 0.01^{bd}
NEAA				
Alanine	0.85 ± 0.01^{a}	0.94 ± 0.01^{b}	0.87 ± 0.06^{c}	0.97 ± 0.01^{bd}
Serine	1.01 ± 0.01^{a}	1.12 ± 0.01^{b}	1.23 ± 0.01^{c}	1.01 ± 0.01^{d}
Glycine	1.15 ± 0.01^{a}	1.32 ± 0.01^{b}	1.29 ± 0.01^{c}	1.19 ± 0.01^{d}

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This sort of thing occurs occasionally elsewhere. Is it just a case of typos please, or is it deliberate? (If it is erroneous, please go through each table and delete the unwanted letters.)

#2 – Actually, there appears to be no explanation of what any of the superscript letters mean. Please add a note at the bottom of the table about this, therefore – and also, for any other table where this applies…

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.

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

Commercial feed combined with a new formulation product significantly affected 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 ± 0.17%), fat (3.47 ± 0.03%), carbohydrates (31.18 ± 0.37%), and crude fibre (2.81 ± 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 whole-body meat. 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 provided the KP1, KP2, and KP4 diets.

Table 2. Whole-body proximate and amino acid composition of giant gourami after a 90-day feeding trial. Note: Numbers followed by different superscript of letters in the same row

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indicate a significant differences ($P < 0.05$). Numbers followed by superscript of the same letter in the same row showed no significant difference ($P > 0.05$).

	KP1	KP2	KP3	KP4
Proximate composition		%, dry wet basis		
Dry matter	64.59 ± 0.16[a]	64.51 ± 0.34[a]	64.14 ± 0.33[a]	64.24 ± 0.12[a]
Crude protein	28.64 ± 0.28[a]	28.07 ± 0.79[ab]	28.85 ± 0.45[c]	28.66 ± 0.44[ad]
Crude fat	2.79 ± 0.03[a]	2.88 ± 0.02[b]	2.67 ± 0.04[c]	3.00 ± 0.02[d]
Carbohydrate	1.38 ± 0.01[a]	1.99 ± 0.06[b]	1.97 ± 0.09[c]	1.31 ± 0.02[d]
Crude fibre	0.97 ± 0.02[a]	0.68 ± 0.01[b]	0.83 ± 0.02[c]	0.95 ± 0.04[d]
Ash	1.63 ± 0.02[a]	1.70 ± 0.02[b]	1.54 ± 0.01[c]	2.11 ± 0.04[d]
Energy total (kg calorie/100 g)	144.77 ± 1.58[a]	155.48 ± 1.26[b]	157.90 ± 0.91[c]	149.60 ± 0.29[d]
Amino acid composition				
EAA				
Leucine	2.13 ± 0.01[a]	2.37 ± 0.01[b]	2.42 ± 0.01[c]	2.26 ± 0.01[d]
Isoleucine	1.13 ± 0.01[a]	1.25 ± 0.01[b]	1.38 ± 0.01[c]	1.19± 0.01[d]
Lysine	2.77 ± 0.01[a]	3.16 ± 0.02[b]	3.88 ± 0.01[c]	2.86 ± 0.01[d]
Valine	1.26 ± 0.01[a]	1.40 ± 0.01[b]	1.32 ± 0.01[c]	1.35 ± 0.01[d]
Threonine	1.38 ± 0.02[a]	1.49 ± 0.01[b]	1.43 ± 0.01[d]	1.48 ± 0.01[d]
Arginine	1.58 ± 0.01[a]	1.71 ± 0.01[b]	1.63 ± 0.01[c]	1.70 ± 0.01[d]
Phenylalanine	1.02 ± 0.01[a]	1.11 ± 0.01[b]	1.08 ± 0.01[c]	1.11 ± 0.01[d]
Tyrosine	0.80 ± 0.01[a]	0.84 ± 0.00[b]	0.83 ± 0.01[c]	0.85 ± 0.06[d]
Methionine	0.15 ± 0.01[a]	0.21 ± 0.01[b]	0.18 ± 0.01[c]	0.16 ± 0.01[d]
Histidine	0.55 ± 0.01[a]	0.56 ± 0.01[ab]	0.59 ± 0.01[c]	0.57 ± 0.01[d]
Tryptophan	0.08 ± 0.01[a]	1.02 ± 0.01[b]	1.08 ± 0.01[c]	0.06 ± 0.00[d]
NEAA				
Alanine	1.86 ± 0.01[a]	2.08 ± 0.01[b]	2.92± 0.01[c]	1.97 ± 0.01[d]
Serine	1.28 ± 0.01[a]	1.31 ± 0.01[b]	1.26 ± 0.01[c]	1.31 ± 0.01[d]
Glycine	1.58 ± 0.01[a]	1.68 ± 0.01[b]	1.61 ± 0.01[c]	1.77 ± 0.01[d]

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As a result of the changes, the numbers were closed up to the '±' symbol – which I had tweaked before. So I have re-corrected it here.

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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]
∑EAA	12.68 ± 0.003[a]	15.13 ± 0.005[b]	15.82± 0.001[c]	13.61 ± 0.008[d]
∑NEAA	12.91 ± 0.007[a]	14.32 ± 0.01[b]	15.69 ± 0.002[c]	13.50 ± 0.001[d]
∑AA	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.

Growth coefficient and survival

The growth coefficient and feed utilization of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant difference between experimental diets in the case of the thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), and daily growth coefficient ($F_{(3,8)} = 59.88$, $P = 0.288$), while total feed intake (% BW day-1) ($F_{(3,8)} = 14.938$, $P = 0.56$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Figure 2).

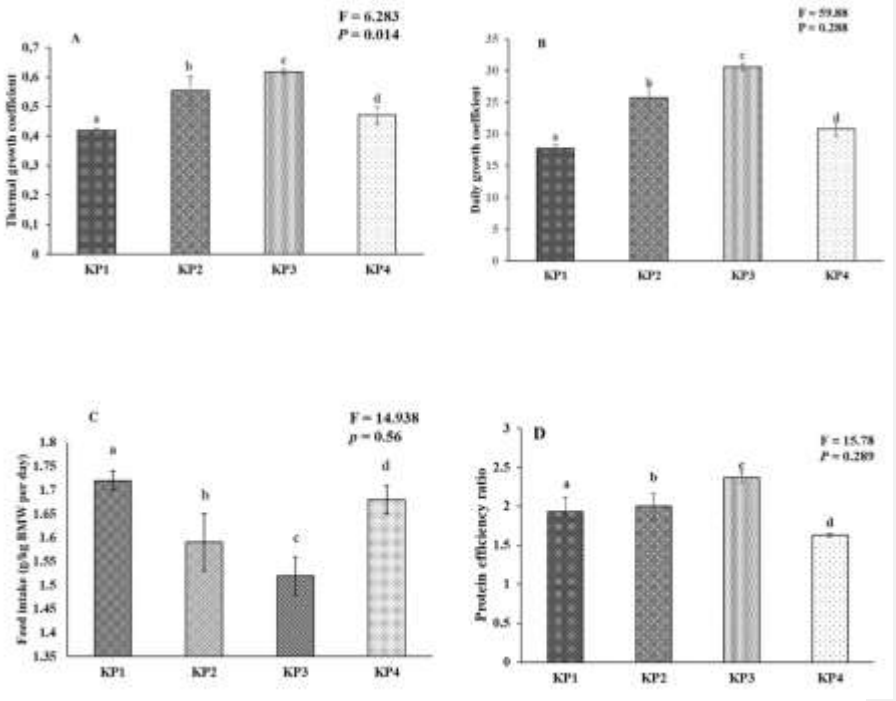

Figure 2. Growth coefficient and feed utilization of the giant gourami juveniles reared under different diets during 90 days of the experiment period. (A) Thermal growth coefficient (TGC), (B) daily growth coefficient (DGC), (C) feed intake (FI), and (D) protein efficiency ratio (PER). The mean value and standard deviation (mean ± SD) are presented for giant gourami (n = 3). Different superscripts in the bar diagram of the giant gourami juvenile TGC, DGC, FI, and PER indicate significant differences among other diets ($P < 0.05$, One-way ANOVA Duncan Post-Hoc)

Furthermore, the thermal growth coefficient (TGC) has often been used to predict growth performance and production performance of aquaculture using water temperature at the fish-rearing location. This study presents the relationship between thermal growth coefficient and condition factor, daily growth coefficient, and protein efficiency ratio (Figure 3). The thermal growth coefficient had strong relationships with the condition factor (r^2 = 0.777, figure 3A), daily growth coefficient (r^2 = 0.999, figure 3B), and protein efficiency ratio (r^2 = 0.749, figure 3D), while the thermal growth coefficient had a moderate relationship with the feed intake (r^2 = 0.699, figure 3C).

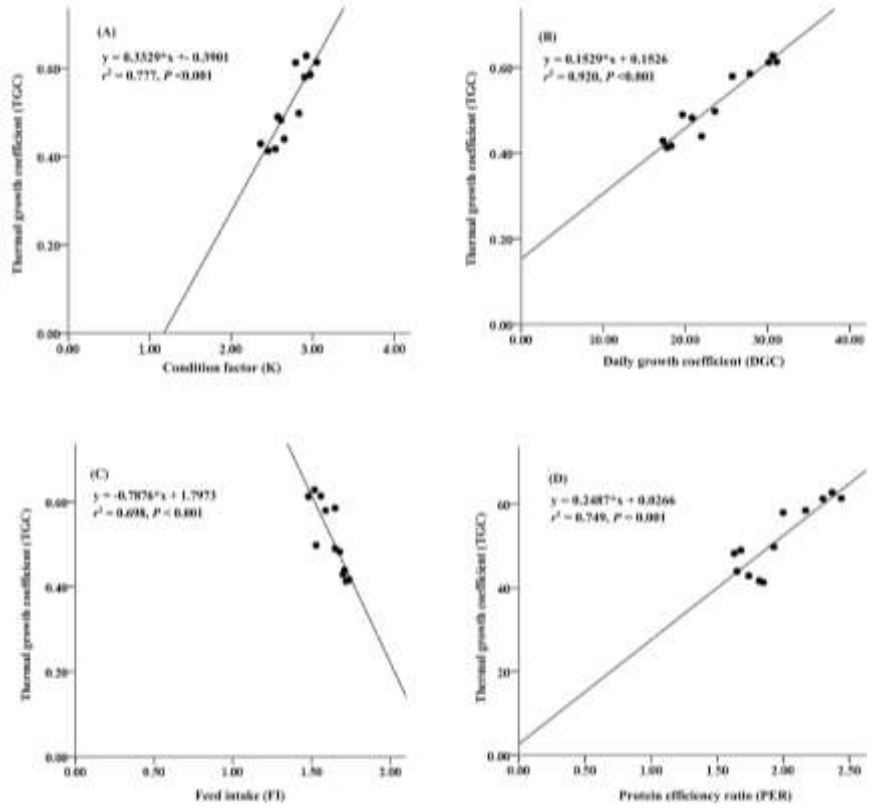

Figure 3. Relationships between thermal growth coefficient and condition factor (A), daily growth coefficient (B), feed intake (C) and protein efficiency ratio (D) for giant gourami (*O. gourami*) over 90 days.

Condition factor and body indices of giant gourami after 90 days of feeding

The condition factor was significantly different between diets ($F_{(3,8)}$ = 19.98, P = 0.566) in the present study; while the GSI, HIS, and VFSI displayed marginally significant differences between diets. The HIS 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). The GSI value of giant gourami was significantly ($F_{(3,8)}$ = 10.492, P = 0.243) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 diets. The VFSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's post-hoc test revealed that the HIS (1.30 ± 0.13%), GSI (4.15 ± 0.36%), and VFSI (2.75 ± 0.34%) were significantly higher (P < 0.05) in the KP3 diet than in the other diets. Meanwhile, BSI showed no significant difference (P > 0.05) among the treatment diets (Table 3).

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Table 3. Mean (± SD) value condition factor and body indices of giant gourami during the 90-day experimental period. Note: Numbers followed by different superscript of letters in the same row indicate a significant difference (P < 0.05). Numbers followed by superscript of the same letter in the same row showed no significant difference (P > 0.05).

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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}
Viscerosomatic index (GSI%)	3.20 ± 0.21^{a}	3.77 ± 0.09^{b}	4.15 ± 0.36^{c}	3.17 ± 0.02^{d}
Hepatosomatic (HIS%)	0.97 ± 0.05^{a}	1.06 ± 0.19^{ab}	1.30 ± 0.13^{c}	1.04 ± 0.12^{ad}
Visceral fat-somatic indexes (VFSI%)	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

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 ± 1.76 µm), fold width (53.23 ± 0.88 µm), enterocyte height (27.42 ± 0.42 µm), and microvillus height (2.79 ± 0.45 µm) 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 ± 1.43 µm), fold width (61.50 ± 1.02 µm), and enterocytes (32.82 ± 0.54 µm) 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 ($F_{(3,8)} = 253.57$, $P = 0.316$).

Pond water quality

The pond water quality values of the giant gourami juvenile rearing freshwater concrete pond 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 in 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 line are significantly different ($P < 0.05$).

	Foregut				Midgut				Hindgut			
	hF (µm)[a]	wF (µm)[b]	hE (µm)[c]	hMV (µm)[d]	hF (µm)	wF (µm)	hE (µm)	hMV (µm)	hF (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[a]	25.62±0.79[d]	2.31±0.07[d]	228.45±1.01[a]	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

Table 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 (^{O}C)	45	28.01 ± 1.06	27 - 30	25-33	Prokoso *et al.*[43]
Dissolved oxygen (mg/L)	14	6.01 ± 0.14	5.80 – 6.20	3-5	Syandri *et al.*[44]
Total alkalinity (mg/L as $CaCo_3$)	14	58.09 ± 3.33	52.5 - 62.5	120	Boyd *et al.*[45]
Hardness (mg/L as $CaCo_3$)	14	66.34 ± 1.32	65 - 68.5	168	Boyd *et al.*[45]
pH	14	7.48 ± 0.19	7.2 – 7.8	6.5 – 9.0	Boyd *et al.*[45]
Nitrates (mg/L)	14	0.04 ± 0.01	0.03 – 0.05	0.2 – 219	Boyd and Tucker[46]

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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[47,48,49]. The giant gourami belongs to the trophic level of herbivorous fish[50]. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish[51,49]. 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[2,52]. 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*[53], and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*[48] and for rearing rohu, *Labeo rohita*[54]. 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, the 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*). In the recent past, the dose used was 300 ml/kg of feed. This method is a new approach that has been developed by Azrita *et al*.[9] to improve feed nutrition and whole-body carcasses, covering fatty acids, the atherogenic index and thrombogenic, feed efficiency, and growth performance of giant gourami. Here, we continued the investigation by reducing the feed dose to 150 ml/kg. This study's results found that supplementing feed with newly formulated products can increase feed nutrition, covering amino acids in diet and body meat, and the growth coefficient of giant gourami. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[12], fish oil[19, 11], and soybean oil[20]. In addition, the provision of feed has been supplemented with probiotics[21], glycine, and prebiotics[22]. 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[55] and Vong *et al.*[56] showed that *Rhizopus oligosporus* can produce various extracellular enzymes. *Aspergillus niger*. has a high capacity to degrade antigenic proteins, including carbohydrases, proteases, lipases, and phosphatases, when used for fermenting plant-sourced fish feed ingredients[12,57]. *Saccharomyces cerevisiae* is one of the most 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[7,58].

The amino acid composition can be used to assess feed quality. Leucine, arginine, and glutamic acid were the most abundant free amino acids in the KP1, KP2, KP3, and KP4 diets. 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 diet[22]. Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid[59], and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein[60]. 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 feed[18]. 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. It was higher in the KP3 diet than the other diets. In contrast, the essential amino acids of fish feed for snubnose pompano were slightly higher than the nonessential amino acids content[60]. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu *et al.*[60] reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet[59] and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet[22]. 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

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cystine in their carcasses than those fed 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[12,57]. The FAA profile differences could be related to different aspects, such as diet composition[61], dietary protein level[62], and methionine levels in the diet[18], including the water quality of the ponds[63]. 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[64,60]. The present study did not examine whether the 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 the insufficient KP1 diet were perhaps due 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 Jannatullah *et al.*[57] and Li *et al.*[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.*[36] 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, the thermal growth coefficient (TGC) strongly correlated with the daily growth coefficient (DGC). Because faster daily fish growth requires a quality diet and

constant water temperature during the rearing period, in this study, water temperature ranged from 27 to 30ºC, and dissolved oxygen was between 5.8 and 6.2 mg /L. According to Besson *et al.*[65], higher daily energy availability in the diet can lead to faster-growing fish, which is supported by constant water temperature and higher daily oxygen levels. The thermal growth coefficient had an essential change in environmental value[66]. Therefore, it was very important to keep the water temperature and dissolved oxygen constant in the aquaculture locations. At the same time, 78% of TGC values were determined by the condition factor connected to whole body weight and the total fish length. TGC of Atlantic cod, *Gadus morhua*, is influenced by body size and condition factors[67].

In this study, a higher value of TGC was detected in fish fed KP3; the effect is that the daily growth coefficient, and the protein efficiency ratio is better. Conversely, decreasing TGC has two effects, *i.e.*, a slow fish growth and lowered daily feed intake. Many scientists state that in aquaculture operations, net yield (kg/m^3) depends upon TGC fluctuation, feed intake, and daily oxygen consumption[65,68,69].

In the present study, feed enrichment with different formulated products did not affect HIS or VFSI except in the KP3 diet. Whereas GSI 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 Arriaga-Hernandez *et al.*[70] for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, Hassan *et al.*[71] 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.*[72] 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.*[64] 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*[44]. 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[18,73]. 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 length[74]. In contrast, substituting as much as 12.5–25% soya protein concentrates with lupin (*Lupinus albus*) meal in carp (*Cyprinus carpio*) diets does not significantly affect the villi length and villi width of the gut[75]. 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 grouper[18], turbot, *Scophthalmus maximus*[12], largemouth bass, *Micropterus salmoides*[22], and common carp, *Cyprinus carpio*[75]. 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.

Conclusions

The present investigation observed that feed enriched with newly formulated products made from mature coconut water and palm sap sugar, and fermented with various mushrooms, given to fish in a dose of 150 ml/kg substantially affected the amino acid composition of the diet and whole-body carcass of giant gourami juveniles. It also affected the growth coefficient, feed utilization, body indices, and gut micromorphology size. The thermal growth coefficient had a strong relationship with the daily growth coefficient (r^2 = 91%) and a moderate relationship with the feed intake (r^2 = 69%). The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass, growth coefficient,

body indices, and the ability of the intestines for feed absorption. Thus, our study also informs fish farmers about culturing good quality giant gourami and fulfilling nutrition requirements for food security.

Data availability

Underlying data

Figshare: Underlying data for 'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles'.
https://doi.org/10.6084/m9.figshare.20407647[76]

This project contains the following underlying data:

- Table 1. Raw data of the experimental diets' proximate composition
- Table 2. Raw data of amino acid of feed experimental
- Table 3. Raw data of whole body carcass proximate composition
- Table 4. Raw data of amino acid of whole-body carcass
- Table 5. Daily growth coefficient, feed utilization and body indices of giant gourami after 90 days of feeding.
- Table 6. Raw data gut micromorphology of giant gourami juveniles fed different diets for 90 days

Competing interests

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

Grant information

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List correction manuscript No. 124706, Des 16, 2022

1. In the method, we revised the 1 litre figure by 1.1 litre and only got 1 litre of solution due to some of it being boiled off to make an 11% palm sap sugar solution (Highlighted in red).
2. In the abstract, we have revised part introduction and method because we have to adjust to1 litre figure by 1.1 litre and the 300 words maximum in the abstract (Highlighted in red)
3. Under the heading, 'Condition factor and body indices of giant gourami after 90 days of feeding', after a number of lines, we have revised the following: The GSI value of giant gourami was significantly ($F_{(3,8)} = 10.492$, $P = 0.243$) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 diets (Highlighted with red).
4. Acknowledgments; we have revised based on your recommendation (Highlighted in red).

Best regards
Azrita

Re: 1 query – and 2 confirmations

Thank you for the resolutions to my text points.

1. Re: an outstanding point. Unfortunately, there is still one small area that does not seem quite right. This concerns the paragraph under the subheading, 'Preparation of formulated product'. This currently runs:

*'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 60⁰ C to make an 11% palm sap sugar solution. 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 the 1.0 litre of palm sap sugar solution. The product was 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.'*

a. Re: sentence 1. The figure of 11% in sentence 1 does not seem correct? (If 100g are cooked in 1 litre, the palm sap sugar makes 1/ 11^{th} of the product, or approximately 9%?)

b. Re: sentence 3. If you created 1.1 litres of palm sap sugar solution in sentence 1 (not 1 litre…) then that is different to 'the 1.0 litre of palm sap sugar solution' you refer to here. Please note that in the Abstract (and possibly elsewhere), you also refer to mixing 2 litres of coconut water with 1.0 litre of palm sap sugar solution.

Perhaps there is an explanation for point b) that has been left out? For example, perhaps you only got 1 litre of solution as a result of the process in sentence 1 due to some of it being 'boiled off' (?). If that was the case, you could add in such a detail and all would make sense. Alternatively, possibly you did obtain 1.1 litres of palm sap sugar solution, but only used 1 litre of it – again, if the text made this clear, then the details would add up. However, '11%' in sentence 1 does seem to be incorrect.

Please clarify how the paragraph should run.

2. I have applied your other text changes. However, you actually sent versions of the manuscripts with slightly different corrections (perhaps they had not been updated?) in two cases. Therefore, please confirm the following text in the manuscript is correct on these two points:

a. Under the heading, 'Condition factor and body indices of giant gourami after 90 days of feeding', after a number of lines you have got:

The GSI value of gourami was significantly ($F_{(3,8)} = 10.492$, P *= 0.243) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 rations.*

b. Under Acknowledgments, I have added your new sentence and left the existing one also. So it runs:

We are grateful to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for funding this research. We would also like to thank the students and fish farmers who helped with data collection in the field and in the laboratory.

Please confirm both these pieces of text in the manuscript are just as they should be.

I look forward to hearing back and sending the manuscript to be printed…

All the best

Jonathan

F1000 Research

PS By the way, I am the editor working on your manuscript, there is no-one else doing so (you sometimes sound like you are unclear on this). Best, Jonathan

Information Classification: General

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Fri, Jan 6, 2023 at 6:26 AM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

I notice from the pdf of corrections that you sent me that aside from the points I queried, you made adjustments to the Abstract, as in order to incorporate the details about the palm sap sugar solution creation process, you had to cut the word length down a bit.

In the current – almost final – version of the manuscript, I have a) made some further small adjustments to the Abstract, as unfortunately, there were a couple of sense issues due to the alterations made.

b) I have in addition, slightly adjusted the wording in the table titles, where this describes the meaning of the superscript letters. I did this as the wording had been altered but did not flow quite right.

c) There is one final query to resolve. This is that there is no reference to Figure 4 in the text at present. Where should this go please?

As I can't send the manuscript to the typesetter until I know where the reference to Figure 4 goes I am attaching the current version here, so you can see the 'tweaks' (ie small changes) I have made to the first bits of the Abstract and the minor alteration to the wording in the table titles as well.

I look forward to hearing back – and to sending the manuscript to the typesetter.

Kind regards

Jonathan

PS You will see that I have taken out a couple of sections – like 'keywords' and 'Grant Information'. This is because the typesetter will take these details from elsewhere, rather than the manuscript copy. Also, I noticed you had changed a country detail in the author addresses section; I have therefore altered this on the manuscript system here as well.

From: azrita ubh <azrita31@bunghatta.ac.id>
Sent: Saturday, December 17, 2022 11:56 PM
To: Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk>
Subject: Re: Final changes

Dear

Jonathan

F1000 Research Team

Thank you for your email on December 15, 2022; We have read the 1 query – and 2 confirmations about manuscript No. 124706. 1 query and two confirmations have been revised. Part revised Highlighted in red (the list correction is attached). Please help us complete this manuscript if there are small things we have not revised.

With best regards

Azrita

On Thu, Dec 15, 2022 at 8:52 PM Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> wrote:

> Dear Azrita
>
> Re: 1 query – and 2 confirmations
>
> Thank you for the resolutions to my text points.
>
> 1. Re: an outstanding point. Unfortunately, there is still one small area that does not seem quite right. This concerns the paragraph under the subheading, 'Preparation of formulated product'. This currently runs:

*'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 60º C to make an 11% palm sap sugar solution. 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 the 1.0 litre of palm sap sugar solution. The product was 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.'*

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b. Re: sentence 3. If you created 1.1 litres of palm sap sugar solution in sentence 1 (not 1 litre…) then that is different to 'the 1.0 litre of palm sap sugar solution' you refer to here. Please note that in the Abstract (and possibly elsewhere), you also refer to mixing 2 litres of coconut water with 1.0 litre of palm sap sugar solution.

Perhaps there is an explanation for point b) that has been left out? For example, perhaps you only got 1 litre of solution as a result of the process in sentence 1 due to some of it being 'boiled off' (?). If that was the case, you could add in such a detail and all would make sense. Alternatively, possibly you did obtain 1.1 litres of palm sap sugar solution, but only used 1 litre of it – again, if the text made this clear, then the details would add up. However, '11%' in sentence 1 does seem to be incorrect.

Please clarify how the paragraph should run.

2. I have applied your other text changes. However, you actually sent versions of the manuscripts with slightly different corrections (perhaps they had not been updated?) in two cases. Therefore, please confirm the following text in the manuscript is correct on these two points:

a. Under the heading, 'Condition factor and body indices of giant gourami after 90 days of feeding', after a number of lines you have got:

The GSI value of gourami was significantly ($F_{(3,8)}$ = 10.492, P *= 0.243) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 rations.*

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Please confirm both these pieces of text in the manuscript are just as they should be.

I look forward to hearing back and sending the manuscript to be printed…

All the best

> Jonathan
>
> F1000 Research
>
> PS By the way, I am the editor working on your manuscript, there is no-one else doing so (you sometimes sound like you are unclear on this). Best, Jonathan
>
> Information Classification: General

Information Classification: General

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azrita ubh <azrita31@bunghatta.ac.id> Sat, Jan 7, 2023 at 1:51 PM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear
Jonathan
F1000 Research

Thank you for your email

I have read and studied your comments to point C regarding Figure 4. You stated that there is currently no reference to Figure 4. I want to clarify that in manuscript No. 124706 no Figure 4 (The manuscript only consists of Figures 1, 2, and 3). Please help us to complete this manuscript.

With best regards

Azrita

[Quoted text hidden]

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

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[b]Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, 28293, Indonesia.
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ABSTRACT

Background: Giant gourami, *Osphronemus goramy* (Lacepede, 1801) is the most important freshwater fish species produced by aquaculture in Indonesia. This study seeks to determine the effect of various newly formulated products on the amino acid composition of the diet and whole-body carcass, and to analyse the growth coefficient, body indices, and gut micromorphology.
Methods: 100 g of palm sap sugar was cooked in 1.1 litre of fresh water for fifteen minutes, to create 1 litre of 11% palm sap sugar solution (after some of it had been boiled off). 2 litres of coconut water were then mixed with the litre of palm sugar solution. 1 litre of this product was added in turn to 2 g of *Aspergillus niger* (CP2), 2 g of *Rhizopus oligosporus* (CP3), and 2 g of *Saccharomyces cerevisiae* (CP4), while freshwater was used as a control (labeled CP1). Aquafeed was added to CP1, CP2, CP3, and CP4, to make diets labeled KP1, KP2, KP3, and KP4. The 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 (2×1×1 m; water volume 1.5 m^3) in a freshwater concrete pond with a stocking density of 30 juveniles/net.
Results: The results supported our hypothesis that different product formulations have a significant effect ($P < 0.05$) on aquafeed nutrition and the whole-body carcass, growth coefficient, feed utilization, body indices, and gut micromorphology of giant gourami juveniles. The thermal growth coefficient strongly correlated with the daily growth coefficient (r^2 = 91%). The KP3 diet contains a higher concentration of amino acids, which increased the growth coefficient, feed utilization, and carcass quality more than the other diets we tested.
Conclusions: Diet KP3 contains higher total amino acids in diets and carcasses and leads to better growth for giant gourami.

INTRODUCTION

In this decade, the production of capture fisheries has decreased; meanwhile, the demand for fish products for human consumption is increasing. Therefore, according to the Food and Agriculture Organisation, 60% of fisheries production in the future will come from aquaculture activities and this figure will continue to rise[1]. The utilization of a variety of fish for aquaculture has now increased the need for commercial feed[2,3,4,5]. At the same time, for aquaculture operations, the cost of aquafeed is still a significant challenge[2,6,7,8]. On the other hand, commercial feed produced by factories still does not contain complete nutrition for fish growth, while being 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 in many important nutrients, including protein, fat, vitamins, and minerals that cultured fish can utilize to increase their growth rate and survival and that is beneficial for human health[4,14,15,16]. Therefore, novel approaches have been developed by scientists to improve the nutrition of fish feeds, such as feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[18], fish oil[19,11], and soybean oil[20]. In addition, supplementing probiotics into the diet[21] and supplemental glycine, prebiotics, and nucleotides in a soybean meal-based diet have been studied[22].

The progress of aquaculture biotechnology has stimulated the interest of scientists in improving aquatic animal production, for example, to increase giant gourami production. One of the experimental techniques is to increase feed nutrition used for this purpose, such as, the use of fish meal and Azolla flour as a feed ingredient for giant gourami[23], and the utilization of new products formulated from water coconut, palm sap sugar, and fungus for the enrichment of commercial feed[9]. Additional research has involved a diet supplemented using glutamine[24], feed supplemented with a growth hormone[25], and substitute fish meal incorporating chicken feather[26]. Whether using coconut water and palm sap sugar fermented with mushrooms affects the amino acid composition of the diet, body carcass, growth coefficient, and body indices is still not understood.

Coconut water has extraordinary nutritional value and contains supplements for health like minerals, amino acids, fatty acids, vitamins, enzymes, organic acids, and several phenolic compositions[27,28,29,30]. Palm sap sugar also has health benefits due to its essential nutrient content, such as a low glycaemic index, and it contains antioxidants, vitamins, and minerals[31,32,33,34]. Meanwhile, mushrooms have been widely used in fermentation due to their

ability to degrade antigenic proteins in fish feed ingredients[7,35,36]. Additionally, coconut water is a functional food that can protect the lens from diabetic cataract development in rats[37]. Coconut water is also a treatment for burning pain during urination, dysuria, gastritis, increasing semen, and indigestion[38].

On the other hand, Azrita *et al.*[9] have reported using new formulations of products containing coconut water and palm sap sugar that are fermented with various mushrooms involving a dosage of 300 ml/kg feed. Their newly formulated products can increase fatty acid levels in the diet and whole body carcasses. Besides that, they also improve giant gourami's growth performance and feed efficiency.

However, the effect of these new formulation products at a dosage of 150 ml/kg feed on the diet amino acid composition, and body meat's amino acid composition has not yet been analyzed. In line with that, the relationships between thermal growth coefficient and condition factor, daily growth coefficient, and feed utilization coefficient, including body indices parameters, as well as the gut micromorphology of giant gourami, have not yet been analyzed.

We hypothesized that commercial aquafeed combined with different newly formulated products at the dosage of 150 ml/kg feed could improve the amino acids compositions of the aquafeed and whole body carcass, body indices, and gut micromorphology. Hence, this investigation's first purpose was to analyze the effect of various newly formulated products on the diet's proximate compositions, amino acid composition, and whole-body carcass. The second aim was to analyze the impact of newly formulated products on the growth coefficient and relation to thermal growth coefficient, body indices, and gut micromorphology in giant gourami juveniles.

METHODS

Ethical approval

The Research and Community Service Ethics Committee at Universitas Bung Hatta, West Sumatera, Indonesia approved this research (89/LPPM/Hatta/III-2022) which followed the ARRIVE guidelines. 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. Approval was given by the ethics committee to collect and rear juvenile gurami sago in the aquaculture laboratory, Faculty of Fisheries and Marine Science at Universitas Bung Hatta. All efforts were made to relieve the suffering of experimental animals. Therefore, the animal did not suffer for this study, and they were still in good

condition when returned to the pond after research was completed. Where some fish were euthanized, this was carried out by piercing part of the fish's brain. Gurami sago fish are not classified as a protected animal according to Indonesian legislation.

Preparation of formulated product

We prepared 100 g of palm sap sugar by traditional production and cooked it in 1.1 litre of fresh water for fifteen minutes at 60º C, and only got 1 litre of palm sap sugar solution due to some of it being boiled off. Then, this 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 the 1.0 litre of palm sap sugar solution. The product was 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 48hrs 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) and labeled as the KP2, KP3, and KP4 diets. The aquafeed was supplemented with freshwater (labeled as the KP1 diet; placebo).

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), which 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. The minerals in the 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 freshwater to create the KP1 diet as observed, and the formulated CP2, CP3, and CP4 products were added to the aquafeed at a dosage of 150 ml/kg of feed to create the enriched fish diets. The formulated product added to the aquafeed was mixed manually with it for three minutes to obtain maximum homogenization and then the blend was dried in the open air for thirty minutes. Thereafter, it was given to the trial animal.

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, China). At the same time, a meter 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 nets framed with 2 m^3 (2×1×1 m) PVC pipe (water volume of 1.5 m^3) were placed inside two freshwater concrete ponds with a size of 18 m^3 (6×2×1.5 m). This experiment consisted of four treatments and three replications, 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 17:00hrs) 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 24hrs to empty their intestinal contents.

Proximate and amino acid composition

The diet samples and proximate carcass composition were analyzed using standard AOAC methods[39]. The matter was dried to a constant weight at 105°C. We used the standard Kjeldahl method to analyse crude protein (N × 6.25). We used the Soxhlet method with ether extraction to analyse crude lipids; the ash was incinerated at 550°C for 16 hrs, whereas gross energy was measured in a bomb calorimeter. 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 24hrs at 11°C[40].

Nutrient utilization and body indices

The growth coefficients in the fish experiments were measured by using the thermal growth coefficient (TGC), daily growth coefficient (DGC), total feed intake (FI), and protein efficiency ratio (PER) of giant gourami, assessed using the following formulae:

TGC = [(final weight (g))⅓ – (initial weight (g))⅓] / (mean water temperature (°C)) × duration of rearing period (day)] × 1000

DGC = (Wf⅓ – Wi⅓) / duration of rearing period (day) × 100

FI as feed (FI as feed in g/fish/day) = Total feed fed / (n × t)

PER = wet weight gain / total protein intake

Three fish from each net frame were sacrificed and dissected immediately to determine the Condition factor (CF), Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) as given below:

CF = 100 × [weight of the juvenile (g) / Length of juvenile (cm^3)]

GSI = 100 × [viscera weight (g) / whole body weight (g)]

HSI = 100 × [liver weight (g) / whole body weight (g)]

VFSI = 100 × [visceral fat weight (g) / whole body weight (g)]

BSI = 100 × [Bile weight (g) / weight of liver (g)]

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 24hrs. After sequential dehydration steps in alcohol, the gut samples were embedded in paraffin. The implanted tissue blocks were sectioned at 5 µm, 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 Figure 1.

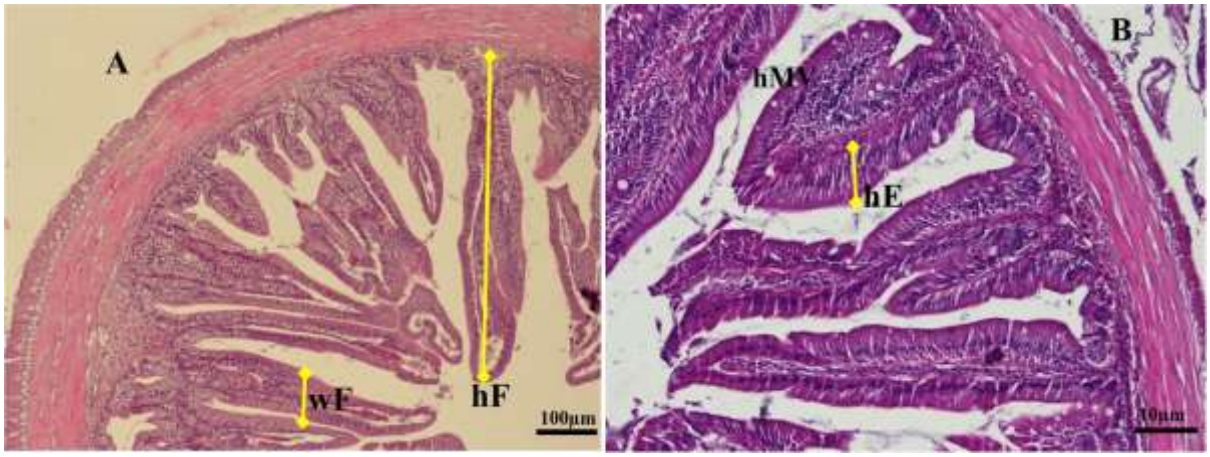

Figure 1. Transversal section photomicrographs of giant gourami juvenile foregut. (A) Fold height and fold width were analyzed in a lower magnification of objective lens of microscope (magnification × 100), (B) Enterocytes height and microvilli height were analyzed using a higher magnification of an objective lens microscope (magnification × 200). hF = fold height, wF = fold width, hE = enterocyte height, hMV = microvillus height (hematoxylin and eosin).

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 10:00am at a depth of 20 cm from each concrete pond to determine the water temperature, dissolved oxygen, and pH value. In addition, we also measured the total alkalinity, hardness, and nitrates of the water in the pond experiments. A thermometer (Celsius scale) was used to measure water temperature. To measure water dissolved oxygen (O_2; mg L^{-1}), we used 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 experiments. The level of nitrate-nitrogen (NO_3-N; mg L^{-1}), alkalinity (mg L^{-1}), and hardness (mg L^{-1}) were measured according to standard procedures[41].

Calculations and statistical method

The data from this study were reported in the form of the mean ± standard deviation for each treatment. Data were analysed using 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[42]. To create the figures, Microsoft Office Professional Plus 2019 was used.

RESULTS

Proximate and amino acid profiles of the diets

Commercial feed supplemented with different formulated products with the dosage of 150 ml/kg of feed significantly affects 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±0.17%) was significantly higher ($P < 0.05$) in the KP3 diet than in the other treatments, while the carbohydrate (31.19 ± 0.38%), crude fibre (2.82±0.06%), and ash (6.67±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 with a dosage of 150 ml/kg of feed 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 significant 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 ± 0.011%), nonessential amino acids (12.91 ± 0.004%), 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 ± SD*. Note: Numbers followed by different superscript letters in the same row are significantly different ($P < 0.05$). Numbers with the same superscript letter in the same row show no significant difference ($P > 0.05$).

	KP1	KP2	KP3	KP4
Proximate composition	%, dry weight basis			
Dry matter	38.42 ± 0.25^{a}	38.27 ± 0.01^{a}	37.59 ± 0.16^{a}	38.41± 0.10^{a}
Crude protein	19.68 ± 0.41^{a}	20.27 ± 0.13^{b}	21.70 ± 0.18^{c}	20.44 ± 0.10^{d}
Crude lipid	3.41 ± 0.02^{a}	3.67 ± 0.13^{b}	3.50 ± 0.02^{ac}	3.48 ± 0.04^{ad}
Carbohydrate	26.37 ± 0.17^{a}	29.50 ± 0.54^{b}	31.19 ± 0.38^{c}	30.57 ± 0.06^{d}
Crude fibre	2.23 ± 0.05^{a}	2.36 ± 0.01^{b}	2.82 ± 0.06^{c}	2.45 ± 0.06^{d}
Ash	2.75 ± 0.03^{a}	6.66 ± 0.05^{b}	6.57 ± 0.04^{c}	6.67 ± 0.06^{d}
Energy total (kg calorie/100 g)	240.87 ± 0.38^{a}	234.41 ± 0.30^{b}	240.88 ± 0.74^{ac}	237.11 ± 0.43^{d}
Amino acid composition				
EAA				
Leucine	1.36 ± 0.01^{a}	1.42 ± 0.01^{b}	1.46 ± 0.01^{c}	1.36 ±0 .01^{d}
Isoleucine	0.76 ± 0.01^{a}	0.79 ± 0.01^{b}	0.81 ± 0.01^{c}	0.76 ± 0.01^{d}
Lysine	0.95 ± 0.01^{a}	1.10 ± 0.01^{b}	0.98 ± 0.01^{c}	1.20 ± 0.01^{d}
Valine	0.86 ± 0.01^{a}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}	0.89 ± 0.01^{d}
Threonine	0.79 ± 0.02^{a}	0.92 ± 0.01^{b}	1.04 ± 0.01^{c}	0.83 ± 0.01^{d}
Arginine	1.02 ± 0.01^{a}	1.19 ± 0.01^{b}	1.30 ± 0.01^{c}	1.03 ± 0.01^{d}
Phenylalanine	0.67 ± 0.01^{a}	0.93 ± 0.01^{b}	1.05 ± 0.01^{c}	0.77 ± 0.01^{d}
Tyrosine	0.43 ± 0.01^{a}	0.53 ± 0.00^{b}	0.57 ± 0.06^{c}	0.45 ± 0.01^{d}
Methionine	0.18 ± 0.01^{a}	0.26 ± 0.01^{b}	0.30 ± 0.01^{c}	0.21 ± 0.01^{d}
Histidine	0.40 ± 0.01^{a}	0.50 ± 0.01^{b}	0.57 ± 0.01^{c}	0.43 ± 0.01^{d}
Tryptophan	0.06 ± 0.01^{a}	0.11 ± 0.01^{b}	0.07 ± 0.00^{bc}	0.09 ± 0.01^{bd}
NEAA				

Alanine	0.85 ± 0.01[a]	0.94 ± 0.01[b]	0.87 ± 0.06[c]	0.97 ± 0.01[bd]
Serine	1.01 ± 0.01[a]	1.12 ± 0.01[b]	1.23 ± 0.01[c]	1.01 ± 0.01[d]
Glycine	1.15 ± 0.01[a]	1.32 ± 0.01[b]	1.29 ± 0.01[c]	1.19 ± 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.

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

Commercial feed combined with a new formulation product significantly affected 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 ± 0.17%), fat (3.47 ± 0.03%), carbohydrates (31.18 ± 0.37%), and crude fibre (2.81 ± 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 whole-body meat. 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 provided the KP1, KP2, and KP4 diets.

Table 2. Whole-body proximate and amino acid composition of giant gourami after a 90-day feeding trial. Note: Numbers followed by different superscript letters in the same row are significantly different ($P < 0.05$). Numbers with the same superscript letter in the same row show no significant difference ($P > 0.05$).

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

Alanine	1.86 ± 0.01[a]	2.08 ± 0.01[b]	2.92± 0.01[c]	1.97 ± 0.01[d]
Serine	1.28 ± 0.01[a]	1.31 ± 0.01[b]	1.26 ± 0.01[c]	1.31 ± 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]
∑EAA	12.68 ± 0.003[a]	15.13 ± 0.005[b]	15.82± 0.001[c]	13.61 ± 0.008[d]
∑NEAA	12.91 ± 0.007[a]	14.32 ± 0.01[b]	15.69 ± 0.002[c]	13.50 ± 0.001[d]
∑AA	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.

Growth coefficient and survival

The growth coefficient and feed utilization of the giant gourami juveniles displayed significant differences among the diets. One-way ANOVA results exhibited a marginally significant difference between experimental diets in the case of the thermal unit growth coefficient ($F_{(3,8)} = 153.99$, $P = 0.458$), and daily growth coefficient ($F_{(3,8)} = 59.88$, $P = 0.288$), while total feed intake (% BW day-1) ($F_{(3,8)} = 14.938$, $P = 0.56$), and protein efficiency ratio ($F_{(3,8)} = 15.78$, $P = 0.29$) also showed significant differences ($P < 0.05$) among the treatment diets (Figure 2).

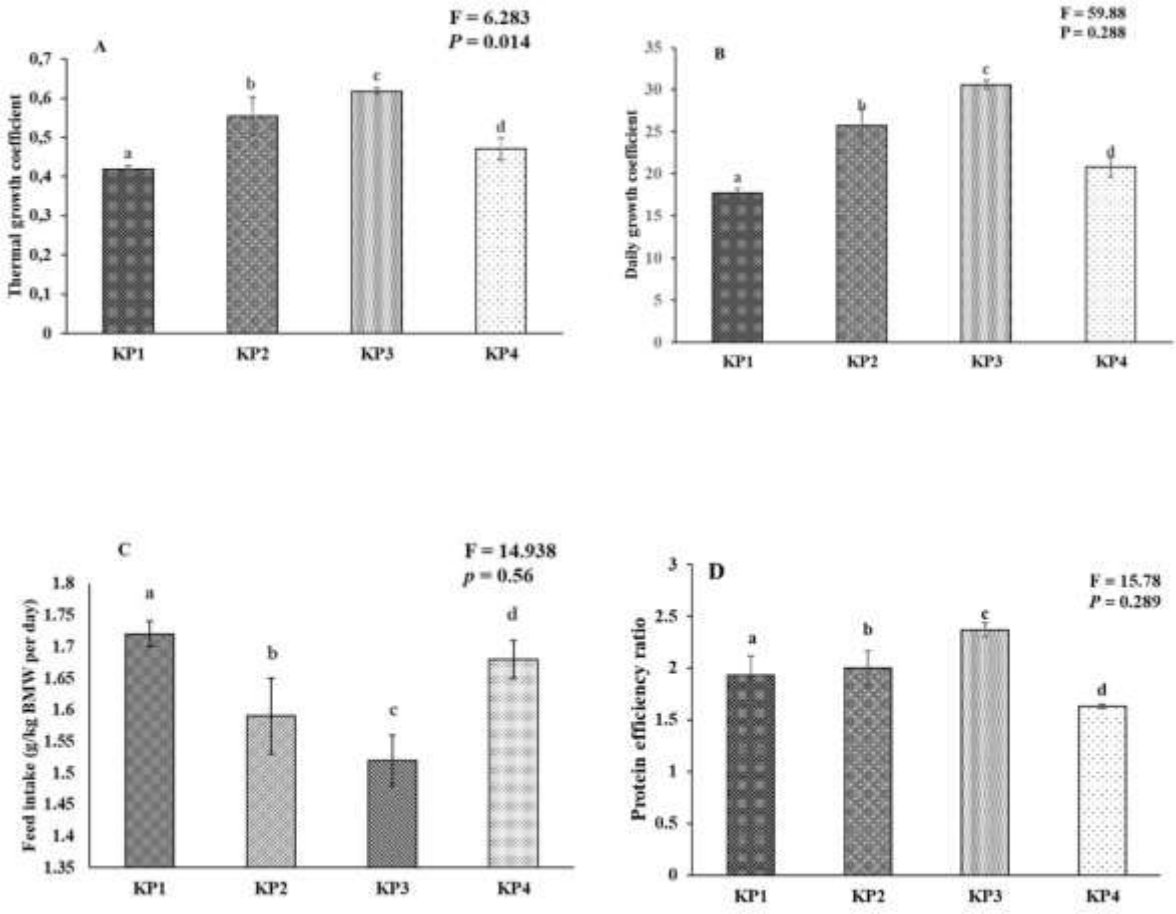

Figure 2. Growth coefficient and feed utilization of the giant gourami juveniles reared under different diets during 90 days of the experiment period. (A) Thermal growth coefficient (TGC), (B) daily growth coefficient (DGC), (C) feed intake (FI), and (D) protein efficiency ratio (PER). The mean value and standard deviation (mean ± SD) are presented for giant gourami (n = 3). Different superscripts in the bar diagram of the giant gourami juvenile TGC, DGC, FI, and PER indicate significant differences among other diets ($P < 0.05$, One-way ANOVA Duncan Post-Hoc).

Furthermore, the thermal growth coefficient (TGC) has often been used to predict growth performance and production performance of aquaculture using water temperature at the fish-rearing location. This study presents the relationship between thermal growth coefficient and condition factor, daily growth coefficient, and protein efficiency ratio (Figure 3). The thermal growth coefficient had strong relationships with the condition factor (r^2 = 0.777, figure 3A), daily growth coefficient (r^2 = 0.999, figure 3B), and protein efficiency ratio (r^2 = 0.749, figure 3D), while the thermal growth coefficient had a moderate relationship with the feed intake (r^2 = 0.699, figure 3C).

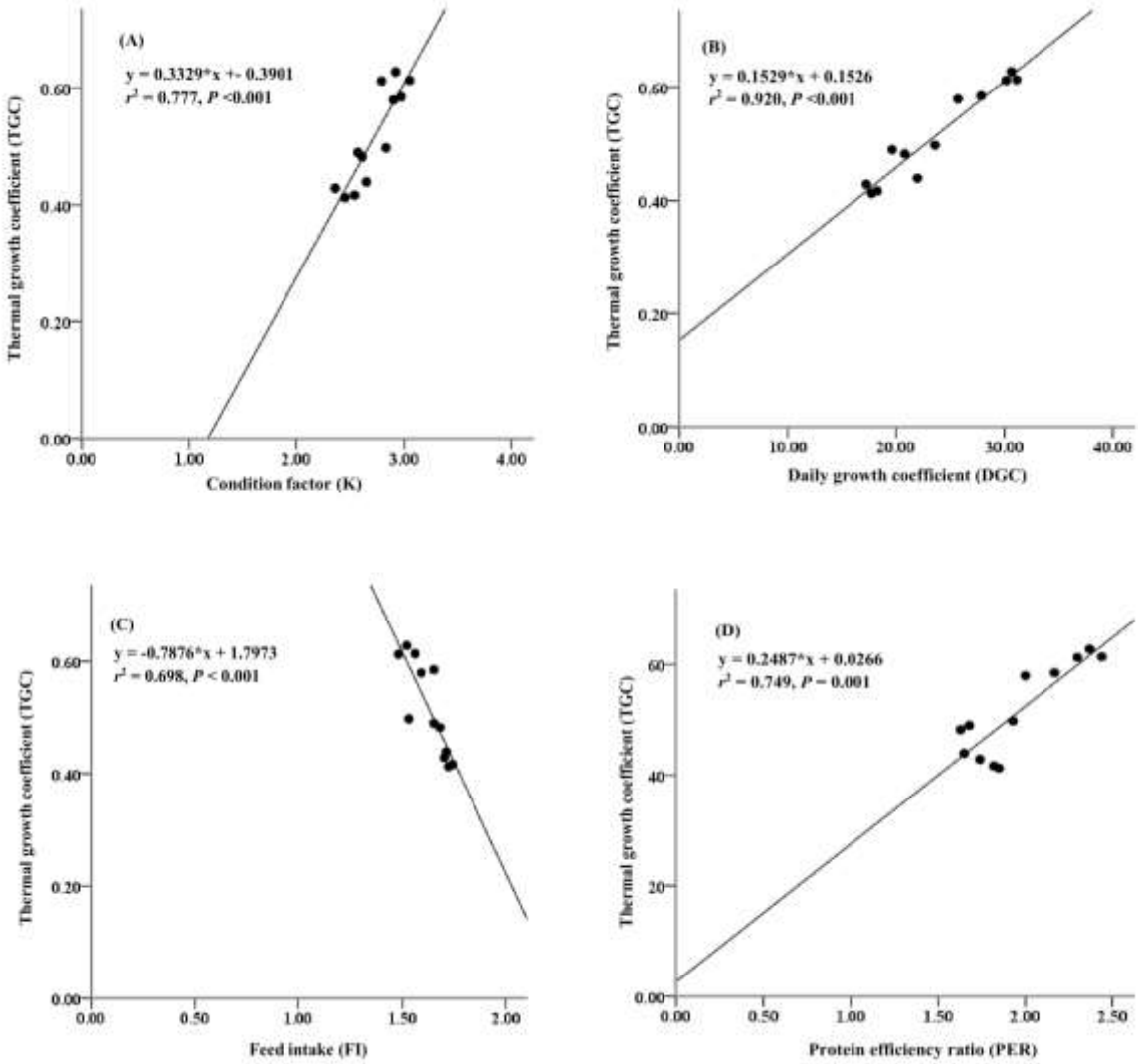

Figure 3. Relationships between thermal growth coefficient and condition factor (A), daily growth coefficient (B), feed intake (C) and protein efficiency ratio (D) for giant gourami (*O. gourami*) over 90 days.

Condition factor and body indices of giant gourami after 90 days of feeding

The condition factor was significantly different between diets ($F_{(3,8)} = 19.98$, $P = 0.566$) in the present study; while the GSI, HIS, and VFSI displayed marginally significant differences between diets. The HIS 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). The GSI value of giant gourami was significantly ($F_{(3,8)} = 10.492$, $P = 0.243$) different between diets, and the GSI of giant gourami fed KP3 rations was higher than if fed KP1, KP2, or KP4 diets. The VFSI was not considerably different among the KP1, KP2, and KP4 diets. The Duncan's post-hoc test revealed that the HIS (1.30 ± 0.13%), GSI (4.15 ± 0.36%), and VFSI (2.75 ± 0.34%) were significantly higher ($P < 0.05$) in the KP3 diet than in the other diets. Meanwhile, BSI showed no significant difference ($P > 0.05$) among the treatment diets (Table 3).

Table 3. Mean (± SD) value condition factor and body indices of giant gourami during the 90-day experimental period. Note: Numbers followed by different superscript letters in the same row are significantly different ($P < 0.05$). Numbers with the same superscript letter in the same row show no significant difference ($P > 0.05$).

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}
Viscerosomatic index (GSI%)	3.20 ± 0.21^{a}	3.77 ± 0.09^{b}	4.15 ± 0.36^{c}	3.17 ± 0.02^{d}
Hepatosomatic (HIS%)	0.97 ± 0.05^{a}	1.06 ± 0.19^{ab}	1.30 ± 0.13^{c}	1.04 ± 0.12^{ad}
Visceral fat-somatic indexes (VFSI%)	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

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 ± 1.76 µm), fold width (53.23 ± 0.88 µm), enterocyte height (27.42 ± 0.42 µm), and microvillus height (2.79 ± 0.45 µm) 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 ± 1.43 µm), fold width (61.50 ± 1.02 µm), and enterocytes (32.82 ± 0.54 µm) 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 ($F_{(3,8)} = 253.57$, $P = 0.316$).

Pond water quality

The pond water quality values of the giant gourami juvenile rearing freshwater concrete pond 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 in 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 line are significantly different ($P < 0.05$).

	Foregut				Midgut				Hindgut			
	hF (µm)[a]	wF (µm)[b]	hE (µm)[c]	hMV (µm)[d]	hF (µm)	wF (µm)	hE (µm)	hMV (µm)	hF (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[d]	25.62±0.79[d]	2.31±0.07[d]	228.45±1.01[d]	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

Table 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 (^{O}C)	45	28.01 ± 1.06	27 - 30	25-33	Prokoso *et al.*[43]
Dissolved oxygen (mg/L)	14	6.01 ± 0.14	5.80 – 6.20	3-5	Syandri *et al.*[44]
Total alkalinity (mg/L as $CaCo_3$)	14	58.09 ± 3.33	52.5 - 62.5	120	Boyd *et al.*[45]
Hardness (mg/L as $CaCo_3$)	14	66.34 ± 1.32	65 - 68.5	168	Boyd *et al.*[45]
pH	14	7.48 ± 0.19	7.2 – 7.8	6.5 – 9.0	Boyd *et al.*[45]
Nitrates (mg/L)	14	0.04 ± 0.01	0.03 – 0.05	0.2 – 219	Boyd and Tucker[46]

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[47,48,49]. The giant gourami belongs to the trophic level of herbivorous fish[50]. Generally, herbivorous fish require a lower dietary protein level than carnivorous fish[51,49]. 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[2,52]. 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*[53], and lower than the feed fat content for the herbivorous fish *Ancistrus cirrhosis*[48] and for rearing rohu, *Labeo rohita*[54]. 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, the 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*). In the recent past, the dose used was 300 ml/kg of feed. This method is a new approach that has been developed by Azrita *et al.*[9] to improve feed nutrition and whole-body carcasses, covering fatty acids, the atherogenic index and thrombogenic, feed efficiency, and growth performance of giant gourami. Here, we continued the investigation by reducing the feed dose to 150 ml/kg. This study's results found that supplementing feed with newly formulated products can increase feed nutrition, covering amino acids in diet and body meat, and the growth coefficient of giant gourami. Several authors have reported increasing feed nutrition and maximizing the digestive enzyme activity of aquacultured fish by providing feed supplemented with EPA and DHA[17], iodine and selenium[10], methionine[12], fish oil[19, 11], and soybean oil[20]. In addition, the provision of feed has been supplemented with probiotics[21], glycine, and prebiotics[22]. 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[55] and Vong *et al.*[56] showed that *Rhizopus oligosporus* can produce various extracellular enzymes. *Aspergillus niger*. has a high capacity to degrade antigenic proteins, including carbohydrases, proteases, lipases, and phosphatases, when used for fermenting plant-sourced fish feed ingredients[12,57]. *Saccharomyces cerevisiae* is one of the most 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[7,58].

The amino acid composition can be used to assess feed quality. Leucine, arginine, and glutamic acid were the most abundant free amino acids in the KP1, KP2, KP3, and KP4 diets. 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 diet[22]. Feed for pacu, *Piaractus mesopotamicus*, was supplemented with an essential amino acid[59], and feed for snubnose pompano, *Trachinotus blochii*, was supplemented with different levels of protein[60]. 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 feed[18]. 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. It was higher in the KP3 diet than the other diets. In contrast, the essential amino acids of fish feed for snubnose pompano were slightly higher than the nonessential amino acids content[60]. This difference may be caused by differences between freshwater fish and marine fish. As in the present study, Prabu *et al.*[60] reported that different dietary protein levels also caused different pools of FAAs, including limiting essential amino acid types in the diet[59] and supplemental glycine, prebiotic, and nucleotide levels in the soybean meal-based diet[22]. 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 those fed 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[12,57]. The FAA profile differences could be related to different aspects, such as diet composition[61], dietary protein level[62], and methionine levels in the diet[18], including the water quality of the ponds[63]. 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[64,60]. The present study did not examine whether the 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 the insufficient KP1 diet were perhaps due 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 Jannatullah *et al.*[57] and Li *et al.*[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.*[36] 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, the thermal growth coefficient (TGC) strongly correlated with the daily growth coefficient (DGC). Because faster daily fish growth requires a quality diet and

constant water temperature during the rearing period, in this study, water temperature ranged from 27 to 30ºC, and dissolved oxygen was between 5.8 and 6.2 mg /L. According to Besson *et al.*[65], higher daily energy availability in the diet can lead to faster-growing fish, which is supported by constant water temperature and higher daily oxygen levels. The thermal growth coefficient had an essential change in environmental value[66]. Therefore, it was very important to keep the water temperature and dissolved oxygen constant in the aquaculture locations. At the same time, 78% of TGC values were determined by the condition factor connected to whole body weight and the total fish length. TGC of Atlantic cod, *Gadus morhua*, is influenced by body size and condition factors[67].

In this study, a higher value of TGC was detected in fish fed KP3; the effect is that the daily growth coefficient, and the protein efficiency ratio is better. Conversely, decreasing TGC has two effects, *i.e.*, a slow fish growth and lowered daily feed intake. Many scientists state that in aquaculture operations, net yield (kg/m^3) depends upon TGC fluctuation, feed intake, and daily oxygen consumption[65,68,69].

In the present study, feed enrichment with different formulated products did not affect HIS or VFSI except in the KP3 diet. Whereas GSI 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 Arriaga-Hernandez *et al.*[70] for white snook (*Centropomus viridis*) juveniles fed a 15% replacement of fish meal with soybean meal. Moreover, Hassan *et al.*[71] 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.*[72] 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.*[64] 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*[44]. 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[18,73]. 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 length[74]. In contrast, substituting as much as 12.5–25% soya protein concentrates with lupin (*Lupinus albus*) meal in carp (*Cyprinus carpio*) diets does not significantly affect the villi length and villi width of the gut[75]. 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 grouper[18], turbot, *Scophthalmus maximus*[12], largemouth bass, *Micropterus salmoides*[22], and common carp, *Cyprinus carpio*[75]. 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.

CONCLUSIONS

The present investigation observed that feed enriched with newly formulated products made from mature coconut water and palm sap sugar, and fermented with various mushrooms, given to fish in a dose of 150 ml/kg substantially affected the amino acid composition of the diet and whole-body carcass of giant gourami juveniles. It also affected the growth coefficient, feed utilization, body indices, and gut micromorphology size. The thermal growth coefficient had a strong relationship with the daily growth coefficient (r^2 = 91%) and a moderate relationship with the feed intake (r^2 = 69%). The CP3 formulation was optimal for feed quality, and the KP3 diet was optimal for body carcass, growth coefficient, body indices,

and the ability of the intestines for feed absorption. Thus, our study also informs fish farmers about culturing good quality giant gourami and fulfilling nutrition requirements for food security.

DATA AVAILABILITY

Underlying data

Figshare: Underlying data for 'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles'.
https://doi.org/10.6084/m9.figshare.20407647[76]

This project contains the following underlying data:

- Table 1. Raw data of the experimental diets' proximate composition
- Table 2. Raw data of amino acid of feed experimental
- Table 3. Raw data of whole body carcass proximate composition
- Table 4. Raw data of amino acid of whole-body carcass
- Table 5. Daily growth coefficient, feed utilization and body indices of giant gourami after 90 days of feeding.
- Table 6. Raw data gut micromorphology of giant gourami juveniles fed different diets for 90 days

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Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Quick query (urgent)

2 messages

Haynes, Jonathan <Jonathan.Haynes@tandf.co.uk> Fri, Jan 20, 2023 at 4:24 AM
To: azrita ubh <azrita31@bunghatta.ac.id>

Hi Azrita

Just before I send the m/s to production, it seems worth double-checking a point I have already asked about in the text.

In the Abstract, the first sentence of Methods reads: *100 g of palm sap sugar was cooked in 1.1 litre of fresh water for fifteen minutes, to create 1 litre of 11% palm sap sugar solution (after some of it had been boiled off).*

You'll remember that I queried if '11%' here, was the correct figure before. (Although the amounts are not comparable, since you were mixing 'grammes' of a solution in 'litres' of water – ie the substances are not equivalent - and although I do not know the position because some of the resulting palm sugar solution was boiled off…I'd wondered if it should be '9%' or less, as for example, 100ml of solution X dissolved in 1litre of solution Y would create a 9% solution of X, and 100ml of solution X dissolved in 1.1 litres of solution Y would create an 8.3% solution of X.)

So please can you confirm that '11% palm sap sugar solution' here, is correct? (You did not explicitly say this before, you see…)

This is all that needs doing, and the m/s can go to the typesetter straightaway :)

I look forward to hearing back.

Best wishes

Jonathan

F1000 Research editorial team

azrita ubh <azrita31@bunghatta.ac.id> Fri, Jan 20, 2023 at 8:58 AM
To: "Haynes, Jonathan" <Jonathan.Haynes@tandf.co.uk>

Dear
Haynes, Jonathan
F1000 Research

We have made every effort to revise manuscript No. 124706 according to your suggestion, and we state that the 11% palm sugar sap solution is correct. We'd love to hear from you if the m/s can go to the typesetter straightaway :)

With best regards

Azrita

[Quoted text hidden]

Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Your article 124706 is now accepted

2 messages

editorial@f1000research.com <editorial@f1000research.com> Wed, Jan 25, 2023 at 4:32 PM
To: azrita31@bunghatta.ac.id

Dear Azrita

'Effect of feed enriched by products formulated from coconut water, palm sap sugar, and mushroom on the chemical composition of feed and carcass, growth performance, body indices, and gut micromorphology of giant gourami, *Osphronemus goramy* (Lacepède, 1801), juveniles' -
Undefined A, Syandri H, Aryani N and Mardiah A

We have now accepted your article for publication in F1000Research. It will be sent to the typesetters and a member of the Production team will send you a proof in due course.

If you are yet to suggest reviewers for your article please note that your article will only be published once you have suggested 5 suitable reviewers who meet our reviewer criteria. Please do not contact your suggested reviewers, as this has the potential to influence and invalidate their review. Our editorial team will contact any suitable reviewers on your behalf and will be your main contact once your article has been published.

Best wishes,

Jonathan
The Editorial Team, F1000Research

azrita ubh <azrita31@bunghatta.ac.id> Thu, Jan 26, 2023 at 5:58 AM
To: editorial@f1000research.com

Dear
Jonathan
The Editorial Team, F1000Research

Thank you for your email; we are pleased to receive information from you that manuscript No. 124706 has now been accepted for publication on F1000 Research. We will soon be suggesting five reviewers for this article. Thank you for our discussions so far.

Best regards

Azrita

[Quoted text hidden]

Webmail Univ. Bung Hatta

azrita ubh <azrita31@bunghatta.ac.id>

Article 124706 - Query

5 messages

production@f1000research.com <production@f1000research.com> Mon, Jan 30, 2023 at 10:32 PM
To: azrita31@bunghatta.ac.id

Dear Azrita

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

Please click here to download the PDF proof of your F1000Research article.

Note: Please mention "*" in table 2 body.

Corrections at this stage may require further typesetting and therefore cause some delays. If any corrections are necessary, please mark them directly on the PDF file using the commenting and markup tools in software such as Adobe Reader.

Please return your proof corrections to us via email - please note that after the article has been published, any requests for minor corrections will only be considered on a case-by-case basis. Therefore, we encourage you to check your proofs carefully at this stage.

If there are any outstanding queries on your reviewer suggestions, then we will be in touch with you shortly.

Best regards,

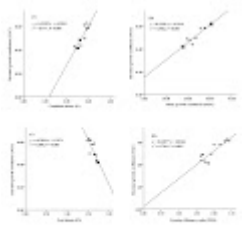
Manahil
The Editorial Team, F1000Research

azrita ubh <azrita31@bunghatta.ac.id> Tue, Jan 31, 2023 at 6:25 PM
To: production@f1000research.com

Dear
Manahil
The Editorial Team, F1000 Research
Thank you for your email on January 30, 2023; we have revised article No. 124706. Figure 3, we replaced with Figure 3 in the final revised manuscript on January 7, 2023 (attached). Revised yellow highlight.

With best regards
Azrita
[Quoted text hidden]

3 attachments



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F1000.Production.Research <production.research@f1000.com> Wed, Feb 1, 2023 at 5:25 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita,

Thank you for your email.

Apologies for the confusion – can you please mark on the PDF which of the cells in table 2 should be marked with a '*' unless this can be removed from the table legend?

Kind Regards,

Manahil

Manahil Aslam

on behalf of F1000 Research

Production Editor

E manahil.aslam@f1000.com

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London

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[Quoted text hidden]
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> [Quoted text hidden]
> Information Classification: General

azrita ubh <azrita31@bunghatta.ac.id> Thu, Feb 2, 2023 at 6:27 AM
To: "F1000.Production.Research" <production.research@f1000.com>

Dear
Manhil Aslam
on behalf of F1000 Research
Production Editor

Thank you for your email on February 1, 2023. We want to explain that the symbol '*' in Mean ± SD*, written in the title of Table 2, explains *Values represent the means of triplicate samples. They were written in the notes below Table 2.

The same symbol for Mean ± SD* is also written in Table 1 (please see Table 1). With pleasure, we hope you can agree.

With best regards

Azrita

[Quoted text hidden]

F1000.Production.Research <production.research@f1000.com> Thu, Feb 2, 2023 at 10:47 PM
To: azrita ubh <azrita31@bunghatta.ac.id>

Dear Azrita,

Thank you for the clarification.

We will proceed with the next steps and return the corrections to the typesetters.

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