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Dietary Supplementation of Isochrysis galbana Biomass in Trachinotus Blochii: Effects on Growth, Feed Utilisation and Physiological Responses

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ABSTRACT: This study examined the use of dried Isochrysis galbana biomass as a functional feed ingredient for Trachinotus blochii. T.blochii with an initial body weight of 42 ± 2 g were acclimatised for 14 days before being fed experimental diets containing control (T0), 0.5% (T1), 1.0% (T2) or 1.5% (T3) dried algal biomass for 60 days. Each treatment was tested in triplicate, with 10 fish per tank. The diets contained 38.87–39.95% crude protein and 6.14–6.51% crude lipid. Growth differences among treatments were not statistically significant. However, the reported feed conversion ratio (FCR) decreased from 1.77 in the control to 1.66 in T2 and T3, while feed conversion efficiency (FCE) reached 0.605 in T3. Whole-body protein and lipid contents varied among treatments; the highest reported protein content was 72.30% in T1 and the highest lipid content was 15.68% in T2. Significant dietary effects were reported for serum cholesterol, albumin, total protein and triglycerides. In T2, cholesterol and total protein reached 75.67 mg dL^{-1} and 5.38 g dL^{-1}, respectively. Liver and intestinal amylase activities were also highest in T2, at 45.89 and 75.88 U mg^{-1} protein, respectively, with significant differences reported among treatments. Superoxide dismutase (SOD) and catalase (CAT) activities showed numerical increases, while the manuscript contains conflicting statements about flesh-colour responses that require confirmation. Overall, the results suggest that dried I. galbana biomass may influence selected aspects of feed utilisation, digestive activity and physiological responses. T2 (1.0% inclusion) showed comparatively high values for several measured parameters, but the inconsistencies identified in the manuscript should be resolved before firm conclusions are drawn.

1. INTRODUCTION

Sustainable and efficient marine finfish aquaculture relies on feeds that meet fish nutritional needs while supporting growth, feed utilisation and normal physiological function. Feed ingredients supply essential nutrients and can also affect digestion, metabolism, antioxidant defences and tissue condition. For this reason, researchers are increasingly investigating natural ingredients that may provide nutritional value alongside other functional benefits.

Marine microalgae are of interest as feed ingredients because their biomass can contain proteins, lipids, polyunsaturated fatty acids, pigments, minerals and other biologically active compounds. These components may contribute to the nutrient supply of cultured fish and could also influence physiological processes. The value of a microalgal ingredient therefore depends on its overall composition, rather than on any single constituent alone.

Isochrysis galbana is a marine microalga with potential for use in aquaculture feeds. Its biomass contains protein and lipid, as well as polyunsaturated fatty acids and carotenoid pigments, including fucoxanthin. This combination makes *I. galbana* worth investigating as both a source of nutrients and a possible functional ingredient in formulated diets.

Using the whole algal biomass is useful because any response in fish may reflect the combined effects of its different constituents. Protein can contribute to tissue development, while lipids and fatty acids support energy metabolism and other cellular functions. Carotenoids and other compounds may also contribute to antioxidant activity. Studying whole biomass therefore provides information about the ingredient as a whole, although it does not allow an observed effect to be attributed to fucoxanthin alone.

Silver pompano (*Trachinotus blochii*) is a commercially important marine fish that is farmed under controlled aquaculture conditions. Appropriate nutrition is essential for supporting its growth, efficient feed use and physiological condition. Evaluating alternative feed ingredients for this species may help identify practical ways to improve diet formulation and production efficiency.

Growth alone does not provide a complete picture of how fish respond to a functional feed ingredient. Feed conversion, body composition, digestive enzyme activity, serum biochemical parameters, antioxidant enzyme activity, flesh colour and tissue structure can provide additional information about nutrient use and physiological status. Considering these measures together allows a more balanced assessment of a dietary ingredient.

In this study, dried *I. galbana* biomass containing naturally occurring fucoxanthin was added to practical diets for juvenile *T. blochii* at inclusion levels of 0.5%, 1.0% and 1.5%. During a 60-day feeding trial, the fish were assessed for growth, feed utilisation, proximate composition, digestive enzymes, serum biochemical parameters, antioxidant enzyme activity and flesh colour. Histological observations of the gills, intestine and liver were also included. The aim was to assess the potential of whole dried *I. galbana* biomass as a feed ingredient and identify any treatment-associated responses, while recognising that whole biomass contains several potentially active components.

2. MATERIALS AND METHODS

2.1 Experimental design and fish

Healthy juvenile silver pompano (*Trachinotus blochii*), with an initial mean body weight of 42 ± 2 g, were used in the feeding trial. Before the experiment, the fish were acclimatised for 14 days in fibreglass-reinforced plastic (FRP) tanks. They were then randomly assigned to four dietary treatments, each with three replicate tanks and 10 fish per tank. In total, the experiment involved 12 tanks and 120 fish.

The four dietary treatments were designated as follows: T0, basal diet without *Isochrysis galbana* biomass (control); T1, basal diet supplemented with 0.5% *I. galbana* biomass; T2, basal diet supplemented with 1.0% *I. galbana* biomass; and T3, basal diet supplemented with 1.5% *I. galbana* biomass. Fish were fed twice daily to apparent satiation for 60 days under continuous aeration and appropriate water-quality conditions.

2.2 Preparation of *Isochrysis galbana* biomass

I. galbana was cultured at ICAR–Central Marine Fisheries Research Institute (CMFRI), Tuticorin, India, using sterilised seawater enriched with suitable nutrients. Cultures were maintained at 25–28 °C under a 12:12 h light–dark cycle with continuous aeration. The culture volume was increased gradually from laboratory flasks to larger systems, while pH, salinity and dissolved oxygen were monitored. Biomass was harvested during the exponential growth phase by centrifugation, washed with distilled water to remove residual salts, shade-dried and ground into a fine powder. The dried biomass was incorporated directly into the experimental diets at 0.5%, 1.0% and 1.5%. The manuscript also reports ethanol extraction of the dried biomass and spectrophotometric measurement at 450 nm to assess fucoxanthin; the extraction calculation and quantitative result should be

supplied from the original laboratory records if this analysis is retained. Whole biomass, rather than purified fucoxanthin, was used in the feeding trial.



Figure 1. Experimental design and fish used in the feeding trial

Note: Healthy *Trachinotus blochii* with an initial body weight of 42 ± 2 g were acclimatized for 14 days before the feeding trial. Experimental feeding tanks showed the four dietary treatments: T0 (control; 0%), T1 (0.5%), T2 (1.0%) and T3 (1.5%) dried *Isochrysis galbana* biomass. Each treatment consisted of three replicate tanks with 10 fish per tank, giving a total of 12 tanks and 120 fish. Fish were fed twice daily to apparent satiation for 60 days under continuous aeration and appropriate water-quality conditions.

2.3 Experimental diet formulation

The control diet was prepared using fish meal, shrimp head meal, meat and bone meal (MBM), GNOC, cottonseed meal, soybean meal, wheat gluten, de-oiled rice bran (DORB), wheat powder, fish oil, sunflower oil, vitamin and mineral mixtures, an antifungal agent, an antioxidant, methionine, lysine, vitamin C and DCP/MCP. The precise meaning of each abbreviation should be provided at first use.

The four diets were prepared to provide increasing levels of dried *I. galbana* biomass across treatments T0–T3. Soybean meal and DORB were adjusted between formulations to accommodate the biomass addition. The ingredient proportions are shown in Table 1. The formulation totals and the adjustments made to individual ingredients should be checked against the original feed-preparation records before submission.

Table 1. Ingredient composition of experimental diets (%)

Ingredient	**T0**	**T1**	**T2**	**T3**
Fish meal	19.00	19.00	19.00	19.00
Shrimp head meal	8.00	8.00	8.00	8.00
MBM	2.00	2.00	2.00	2.00
GNOC	13.00	13.00	13.00	13.00
Cottonseed meal	6.00	6.00	6.00	6.00
Soybean meal	19.00	18.75	18.50	18.25
Wheat gluten	2.00	2.00	2.00	2.00
DORB	1.50	1.25	1.00	0.75

Wheat powder	20.00	20.00	20.00	20.00
Fish oil	2.00	2.00	2.00	2.00
Sunflower oil	2.00	2.00	2.00	2.00
Vitamin mixture	1.50	1.50	1.50	1.50
Mineral mixture	1.50	1.50	1.50	1.50
Antifungal agent	0.10	0.10	0.10	0.10
Antioxidant	0.10	0.10	0.10	0.10
Methionine	0.50	0.50	0.50	0.50
Lysine	0.30	0.30	0.30	0.30
Vitamin C	0.50	0.50	0.50	0.50
DCP/MCP	1.00	1.00	1.00	1.00
Dried *I. galbana* biomass (%)	0	0.5	1	1.5
Total	**100.00**	**100.00**	**100.00**	**100.00**

2.4 Feed preparation

All feed ingredients were weighed according to the planned formulation and mixed thoroughly. The required amount of dried *I. galbana* biomass was then added to each relevant treatment. The mixtures were formed into pellets suitable for juvenile silver pompano and stored under appropriate conditions until use.

The proximate composition of the prepared diets was subsequently determined. The analysed diets contained approximately 38.87–39.95% crude protein, 6.14–6.51% crude fat, 12.19–12.48% ash, 28.11–28.39% carbohydrate, 9.12–9.36% moisture and 4.17–4.76% fibre.

2.5 Feeding trial and growth assessment

Fish were weighed at the start and at the end of the 60-day feeding trial. Feed was offered twice daily until apparent satiation, and feed provision was recorded throughout the experiment.

The following growth and feed-utilisation parameters were determined:

Weight gain (WG):	$WG = W_f - W_i$
Weight gain percentage (WGP):	$WGP = (W_f - W_i) / W_i \times 100$
Average daily gain (ADG):	$ADG = (W_f - W_i) / t$
Specific growth rate (SGR):	$SGR = (\ln W_f - \ln W_i) / t \times 100$
Feed conversion ratio (FCR):	FCR = Feed consumed / Weight gain
Feed conversion efficiency (FCE):	FCE = Weight gain / Feed consumed × 100

W_f = final body weight; W_i = initial body weight; t = feeding period (days).

2.6 Proximate composition

Whole-body fish samples were analysed for crude protein, crude fat and ash. Feed samples were analysed for crude protein, crude fat, ash, carbohydrate, moisture and fibre.

The whole-body results were expressed as percentages and used to examine possible differences in nutrient composition among fish receiving the four diets. The complete values and statistical comparisons should be presented alongside the figure where available.

2.7 Collection and preparation of blood samples

At the end of the 60-day trial, blood was collected from representative fish for biochemical analysis. The samples were processed to obtain the serum or plasma fraction required by the assays. The exact sampling and processing procedure should match the method used in the laboratory.

The biochemical parameters evaluated were cholesterol, calcium, glucose, albumin, total protein, triglycerides, SGOT, SGPT and alkaline phosphatase.

2.8 Biochemical assays

Commercial diagnostic kits from Coral Clinical Systems were used to measure glucose, total protein, albumin, triglycerides, cholesterol, SGOT, SGPT, calcium and alkaline phosphatase. Assays were performed according to the instructions supplied with the kits.

2.8.1 Glucose

Serum glucose was determined using the glucose oxidase/peroxidase (GOD/POD) method. According to the kit protocol, glucose is oxidized by glucose oxidase to generate hydrogen peroxide, which reacts in the presence of peroxidase to produce a coloured quinoneimine compound. Absorbance was measured spectrophotometrically at 505 nm. The supplied kit specifies a reaction temperature of 37°C and a 10-min incubation period.

2.8.2 Total protein

Total protein was determined using the Biuret method. Proteins react with cupric ions under alkaline conditions to form a coloured complex, and absorbance was measured at 546 nm. The supplied procedure specifies incubation at 37°C for 10 min.

2.8.3 Albumin

Serum albumin was measured by the bromocresol green method. Albumin forms a coloured complex with bromocresol green under the specified reaction conditions, and absorbance was measured at 630 nm.

2.8.4 Triglycerides

Triglycerides were determined using the glycerol phosphate oxidase/peroxidase (GPO/PAP) method. The reaction produces a coloured quinoneimine product, with absorbance measured at 505 nm according to the supplied kit procedure.

2.8.5 Cholesterol

Serum cholesterol was determined using the cholesterol oxidase/peroxidase (CHOD/PAP) method. The reaction produces a coloured product measured at 505 nm.

2.8.6 SGOT and SGPT

SGOT (AST) and SGPT (ALT) activities were determined using modified IFCC procedures. The supplied protocols use kinetic spectrophotometric measurement at 340 nm, with enzyme activity calculated from the change in absorbance per minute.

2.8.7 Calcium

Serum calcium was determined by the Arsenazo III method. Calcium forms a coloured complex with Arsenazo III, which was measured at 630 nm according to the supplied procedure.

2.8.8 Alkaline phosphatase activity (ALP)

Alkaline phosphatase activity was determined by the IFCC method using p-nitrophenyl phosphate as substrate. The reaction was monitored at 405 nm, and enzyme activity was expressed in units L^{-1}.

2.9 Digestive enzyme assays

At the end of the 60-day feeding trial, representative fish from each treatment were sampled for digestive enzyme analysis. Liver and intestinal tissues were collected separately from fish in T0 (control), T1 (0.5% dried *I. galbana*), T2 (1.0%) and T3 (1.5%). The tissues were processed separately, and the resulting preparations were used to measure amylase, protease and lipase activity.

Amylase activity was determined separately in liver and intestinal preparations and expressed as units per milligram of protein (U mg^{-1} protein). Proteolytic activity was similarly evaluated in the tissue preparations and expressed as U mg^{-1} protein. Lipase activity was determined in liver and intestinal preparations and expressed using the same protein-normalized unit. Enzyme activities were compared among the four dietary treatments to determine whether dietary incorporation of *I. galbana* biomass influenced the digestive capacity of *T. blochii*. The results were expressed as replicate observations for each dietary treatment. The mean enzyme activity and variation among replicates were calculated for each treatment. The digestive enzyme responses were subsequently compared statistically among the T0 (control), T1, T2 and T3 dried *I. galbana* diets.

2.10 Antioxidant enzyme assays

Antioxidant status was assessed by measuring superoxide dismutase (SOD) and catalase (CAT) activity in liver samples collected at the end of the 60-day trial. Samples from the control and supplemented groups were processed for enzyme analysis. SOD activity was used as an indicator of the response to superoxide radicals, while CAT activity was measured as an indicator of hydrogen peroxide removal. Activities were normalised to tissue protein and expressed as U mg^{-1} protein. The results were compared across T0–T3.

2.11 Flesh colour

At the end of the feeding trial, representative muscle samples were collected to assess whether dried *I. galbana* biomass was associated with changes in flesh colour. Colour was measured using the CIE L*a*b* system. L* indicates lightness, a* represents the red–green axis and b* represents the yellow–blue axis. Measurements were compared among T0, T1, T2 and T3.

Histological examination was used to describe the structure of the gills, intestine and liver after 60 days of feeding. Representative fish from the control and supplemented treatments were sampled, and the tissues were processed using routine histological procedures. Samples were fixed, dehydrated through graded alcohol, cleared, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (H&E). Sections were examined under a light microscope. Gill observations focused on primary filaments and secondary lamellae; intestinal observations focused on mucosal folds, epithelial integrity and general tissue organisation; and liver observations focused on hepatic architecture and cellular arrangement. Fixation time, section thickness, magnification and the number of fish examined should be added from the laboratory records to make the method reproducible.

2.13 Statistical analysis

Results were summarised as mean ± standard deviation (SD) for the three replicate observations reported for each treatment. A one-way analysis of variance (ANOVA) was used to compare T0, T1, T2 and T3, with statistical significance set at $P < 0.05$. The experimental unit and any post-hoc test used should be stated clearly and should reflect the actual statistical analysis.

3. RESULTS

3.1 Nutrient composition of experimental diets

The analysed diets had broadly similar proximate compositions, with some small differences among treatments. T2 had the highest crude protein content (39.95 ± 0.03%), while T1 had the lowest (38.87 ± 0.01%). Crude fat was highest in T1 (6.51 ± 0.11%), and ash was highest in T3 (12.45 ± 0.03%). Carbohydrate values were similar across the diets, ranging from 28.14% to 28.35%. Moisture was slightly higher in T2 (9.34 ± 0.03%), while fibre was highest in T1 (4.76 ± 0.10%). Overall, the measured diets were compositionally similar, although the individual values should be checked against Table 2 before publication.

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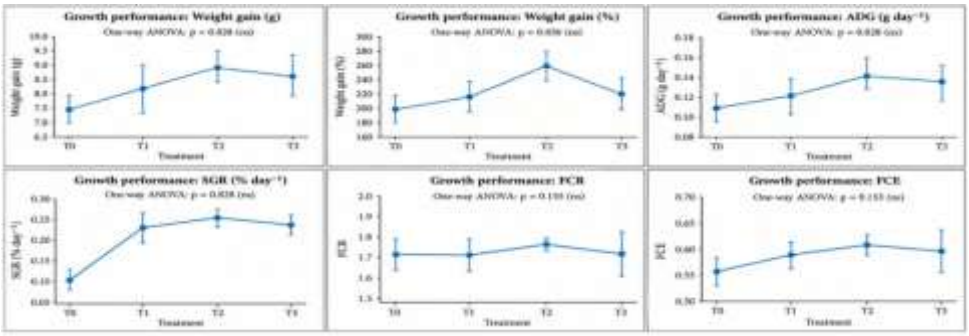
Table 2. Analysed proximate composition of experimental feeds

Parameter (%)	T0	T1	T2	T3
Crude protein	39.56 ± 0.04	38.87 ± 0.01	39.95 ± 0.03	39.51 ± 0.02
Crude fat	6.16 ± 0.04	6.51 ± 0.11	6.14 ± 0.04	6.24 ± 0.03
Ash	12.23 ± 0.05	12.29 ± 0.03	12.25 ± 0.04	12.45 ± 0.03
Carbohydrate	28.17 ± 0.05	28.35 ± 0.04	28.16 ± 0.04	28.14 ± 0.02
Moisture	9.15 ± 0.03	9.26 ± 0.03	9.34 ± 0.03	9.33 ± 0.02
Fibre	4.72 ± 0.10	4.76 ± 0.10	4.17 ± 0.06	4.33 ± 0.03

3.2 Growth performance and feed utilisation

Growth and feed-utilisation measures varied across the four treatments during the 60-day trial. The manuscript reports the highest weight gain (8.92 ± 0.44 g), weight-gain percentage (259.56 ± 14.45%), average daily gain (0.145 ± 0.007 g day^{-1}) and specific growth rate (0.252 ± 0.012% day^{-1}) in T2. FCE was also reported as highest in T2 (0.603 ± 0.002), while the Results section identifies T1 as having the lowest FCR (1.718 ± 0.066). However, no significant differences in growth performance were detected ($P > 0.05$).

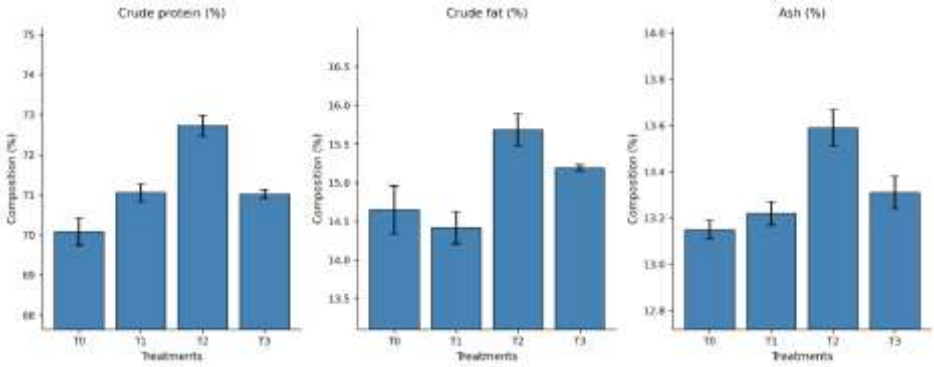
Fig 3. Growth performance and feed utilisation of *T. blochii* after 60 days



3.3 Whole-body proximate composition

Whole-body proximate composition was examined after the 60-day trial to assess whether the diets were associated with differences in nutrient composition. The parameters included crude protein, crude fat, ash and moisture. The values should be reported with means, standard deviations and the relevant statistical results; the figure alone does not provide enough detail for readers to assess the differences fully.

Fig 4. Whole-body proximate composition of *T. blochii*



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3.4 Serum biochemical parameters

The manuscript reports significant treatment-related differences in serum cholesterol, albumin, total protein and triglycerides. Cholesterol increased from T0 to T2 and then fell slightly in T3. Calcium increased up to T2 before declining in T3, while glucose rose from the control and remained relatively stable among the supplemented groups. Albumin, total protein and triglycerides also increased towards T2 and then showed a slight decrease in T3. Exact means, variation and statistical comparisons should be provided in a table so these statements can be evaluated.

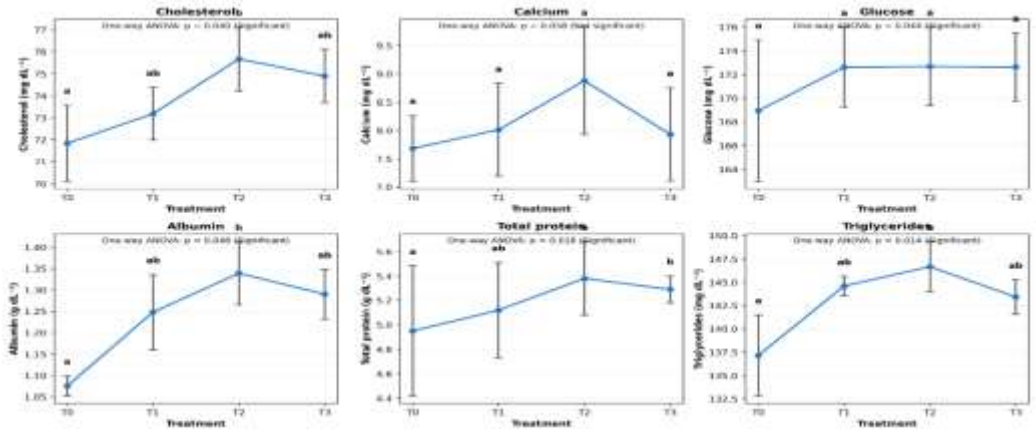


Fig 5. Serum biochemical characteristics of *T. blochii*

3.5 Serum enzyme activities

Serum enzyme activities differed numerically among treatments. ALP increased from 19.89 ± 1.91 U L^{-1} in T0 to 24.48 ± 0.53 U L^{-1} in T3. SGPT was highest in T2 (14.80 ± 0.58 U L^{-1}), followed by T3 (14.18 ± 0.92 U L^{-1}), and lowest in T0 (12.39 ± 1.01 U L^{-1}). SGOT was also highest in T2 (14.55 ± 1.16 U L^{-1}) and lowest in T0 (12.93 ± 1.21 U L^{-1}). These values describe the observed pattern, but significance should not be claimed unless supported by the corresponding statistical tests.

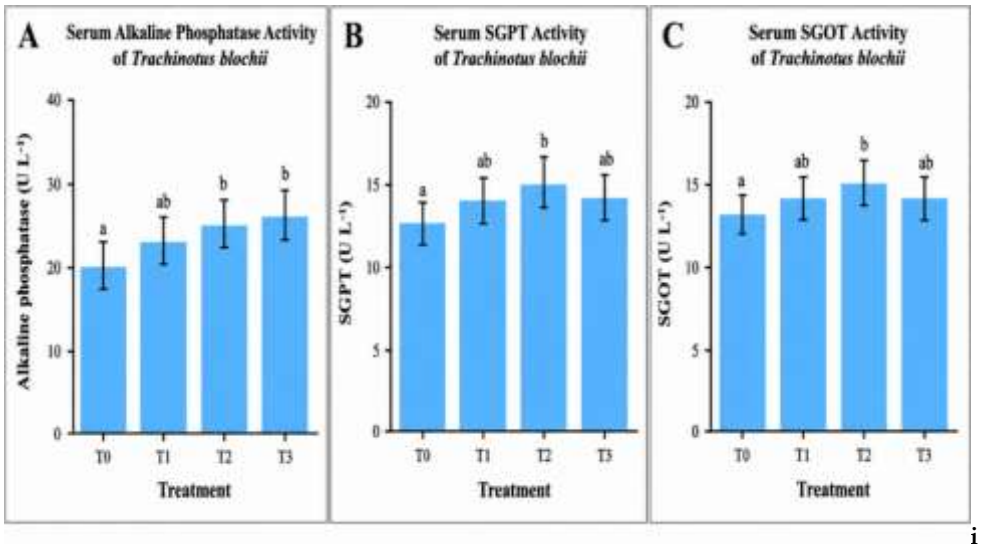


Fig 6. Serum enzyme activities of *T. blochii*

3.6 Digestive enzyme activities

Digestive enzyme activities in the liver and intestine varied among the treatments. Liver amylase was highest in T2 (45.89 ± 0.34 U mg^{-1} protein), and intestinal amylase was also highest in T2 (75.88 ± 1.19 U mg^{-1} protein); the manuscript reports significant differences for both measures ($P < 0.05$). Liver protease and intestinal protease were highest in T2 at 0.00079 ±

0.00012 and 0.00119 ± 0.00014 U mg⁻¹ protein, respectively, but these differences were not significant (P > 0.05). Liver and intestinal lipase were likewise highest in T2, at 7.35 ± 1.45 and 9.89 ± 1.97 U mg⁻¹ protein, respectively, without significant treatment effects (P > 0.05).

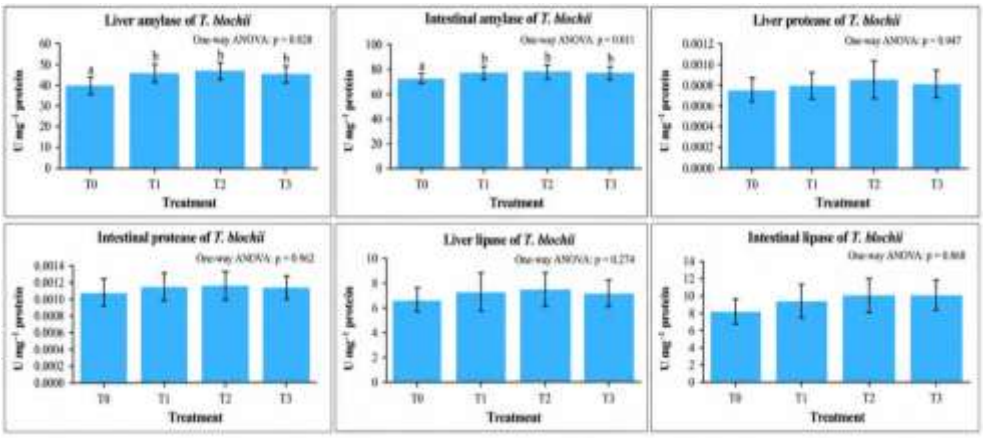


Fig 7. Digestive enzyme activities in *T. blochii*

3.7 Antioxidant enzyme activities

SOD and CAT activities showed numerical variation among the treatments. SOD was highest in T2 (79.31 ± 4.88 U mg⁻¹ protein), followed by T1 (77.66 ± 4.89 U mg⁻¹ protein) and T0 (76.50 ± 4.39 U mg⁻¹ protein); the lowest value was reported in T3 (75.36 ± 4.25 U mg⁻¹ protein). CAT was also highest in T2 (22.82 ± 3.17 U mg⁻¹ protein) and lowest in T0 (20.49 ± 1.24 U mg⁻¹ protein). These are numerical trends only unless statistical testing confirms differences among treatments.

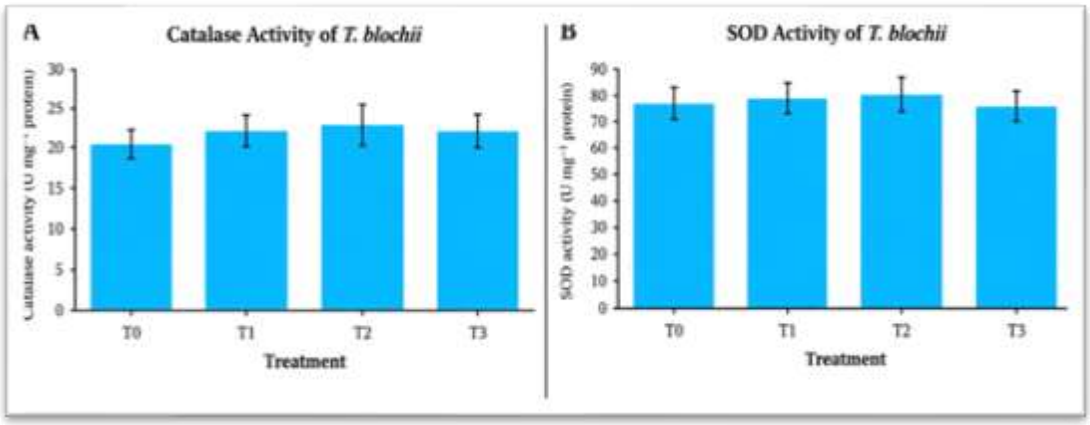


Fig 8. Liver antioxidant enzyme activities

3.8 Flesh colour

The reported CIE L*a*b* values varied among treatments. Lightness (L*) was highest in T2 (78.62 ± 1.00) and lowest in T1 (77.73 ± 0.43). Redness (a*) was highest in T2 (1.43 ± 0.11) and lowest in T0 (1.01 ± 0.12). Yellowness (b*) was highest in T3 (1.26 ± 0.06), closely followed by T2 (1.25 ± 0.07), while T0 had the lowest value (1.10 ± 0.13). The Results section reports significant differences for L* (P = 0.012), a* (P = 0.021) and b* (P = 0.033). This conflicts with the abstract's statement that flesh colour was unaffected, so the statistical output must be checked and the manuscript made consistent.

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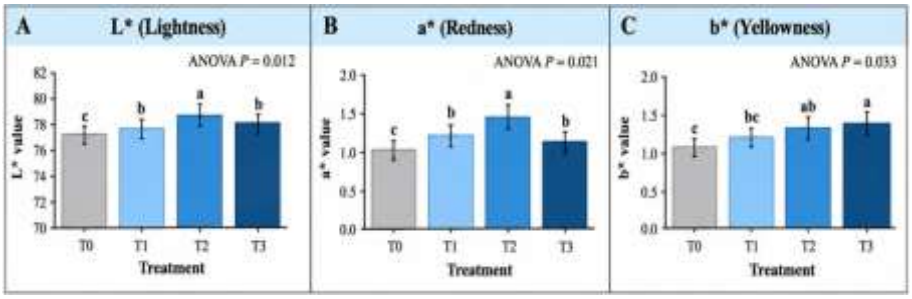


Fig 9. Flesh colour characteristics of *T. blochii*

3.9 Histological observations

3.9.1 Gill histology

Gill sections from the control group (T0) showed intact primary filaments and recognisable secondary lamellae. Similar overall structures were described in T1, T2 and T3. The T2 sections were noted to have a comparatively regular arrangement of secondary lamellae, while the other supplemented groups retained their general gill architecture. No major structural abnormalities were reported in the examined sections. These observations suggest that obvious gill damage was not seen in the samples examined; they do not, by themselves, establish long-term safety.

3.9.2 Intestinal histology

The intestinal sections from the control fish showed distinct mucosal folds and a recognisable epithelial lining. Similar features were reported in the supplemented groups, suggesting that the general intestinal structure was maintained during the feeding trial. T2 was described as having comparatively well-organised mucosal folds, and T1 also showed good mucosal organisation. T3 retained its overall structure, with no obvious severe epithelial damage or extensive degeneration reported in the examined sections.

3.9.3 Liver histology

Liver sections from the control and supplemented groups retained their general tissue architecture. The control samples showed no obvious signs of extensive damage, and similar overall features were described in T1–T3. T2 was noted to have comparatively uniform hepatic organisation. No prominent pathological changes were reported in the sections examined. These are descriptive observations; a stronger assessment would require clearly reported sampling details and, where appropriate, a quantitative histological scoring method.

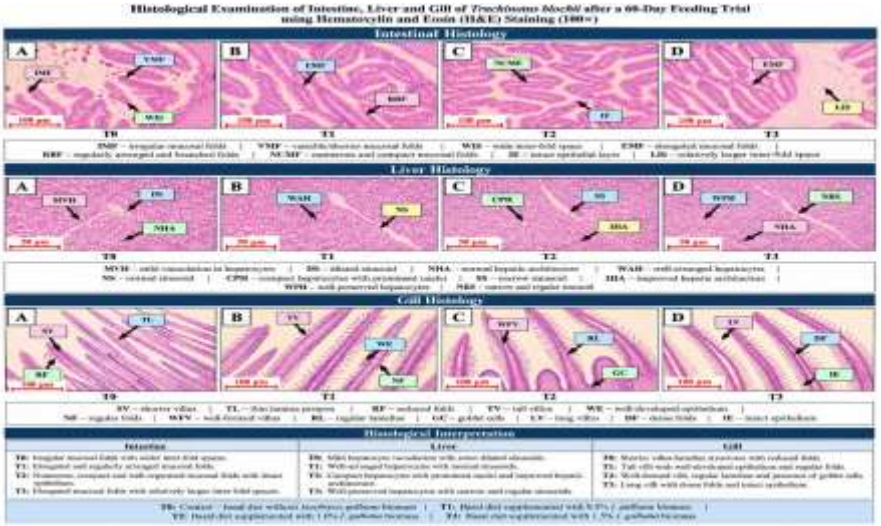


Figure 2. Histological Examination of Intestine, Liver and Gill of *Trachinotus blochii* after a 60-Day Feeding Trial using Hematoxylin and Eosin (H&E) Staining

4. DISCUSSION

This study examined dried *I. galbana* biomass as a dietary ingredient for silver pompano over a 60-day feeding period. The assessment included growth, feed utilisation, body composition, serum biochemistry, digestive and antioxidant enzymes, flesh colour and tissue structure. Growth performance did not differ significantly among treatments, although several biochemical and digestive measures varied. T2 (1.0% biomass) showed comparatively high values for a number of parameters, particularly amylase activity. These findings suggest that the response to a functional ingredient should be assessed using several measures rather than growth alone.

The experimental diets had similar measured proximate compositions: crude protein ranged from 38.87% to 39.95%, and crude fat ranged from 6.14% to 6.51%. This similarity provides useful context for interpreting the treatment responses. Nevertheless, dried *I. galbana* contains a mixture of nutrients and bioactive compounds, and the present design does not establish which constituent was responsible for any observed response. In particular, the findings should not be attributed to fucoxanthin alone because the fish received whole algal biomass.

Growth performance is an important measure when assessing a feed ingredient. T2 had the highest reported values for weight gain, weight-gain percentage, average daily gain and specific growth rate. However, the differences among treatments were not statistically significant. The numerical trends therefore do not demonstrate that supplementation improved growth. Feed conversion measures also varied, but the FCR values and treatment rankings conflict between sections of the manuscript and need to be verified against the original records before interpretation.

The whole-body proximate composition also varied among treatments. Differences in protein, lipid and ash may reflect variation in nutrient deposition, but the available narrative does not provide all means, standard deviations and statistical comparisons needed to evaluate these patterns. The complete dataset should be presented and checked before drawing conclusions about the effect of the diets on body composition.

SGOT and SGPT were reported to be highest in T2, while ALP was highest in T3. These results indicate differences in the numerical patterns of the measured serum enzymes. However, enzyme activity alone cannot establish either improved organ function or tissue damage. Interpretation should take account of the statistical results and the histological observations, and should avoid claiming significance where it has not been demonstrated.

The digestive enzyme results provide information about how the fish responded to the experimental diets. Liver and intestinal amylase activities were reported to be significantly higher in the supplemented groups, with the highest values in T2. By contrast, protease and lipase did not differ significantly among treatments, despite also having their highest numerical values in T2. The amylase pattern may indicate an effect on carbohydrate-related digestion, but higher enzyme activity does not necessarily mean that nutrient absorption or growth improved. Digestibility measurements would be needed to test that possibility directly.

SOD and CAT activities were numerically highest in T2. Both enzymes are involved in cellular antioxidant defence, but the reported trends do not establish an improvement in antioxidant protection unless the differences are statistically supported. Because the fish received whole *I. galbana* biomass, further work would be needed to identify which components, if any, contributed to the measured responses.

The reported flesh-colour measurements showed the highest L* and a* values in T2 and the highest b* value in T3. The Results section gives significant P-values for all three coordinates, but the abstract states that colour was unaffected. This inconsistency must be resolved using the original data and statistical output. Any interpretation of the colour changes should also avoid attributing them specifically to algal pigments without direct supporting evidence.

The examined gill, intestinal and liver sections were described as retaining their general structure across the four treatments. The gills had identifiable filaments and secondary lamellae, the intestine retained organised mucosal folds, and the liver showed generally preserved architecture. No major abnormalities were reported in the sections examined. These findings are reassuring at a descriptive level, but they do not rule out subtle changes or establish long-term safety, particularly without quantitative scoring and full sampling details.

Taken together, the results suggest that dried *I. galbana* biomass was associated with variation in selected biochemical and digestive responses, while growth performance remained statistically similar among treatments. T2 (1.0% biomass) had comparatively high numerical values for several parameters, including amylase activity and some serum measurements.

However, the available results do not establish 1.0% as the optimal inclusion level. Since the ingredient was whole dried biomass, the observed patterns may reflect its combined nutritional and bioactive components rather than fucoxanthin alone.

This study adds information on the possible use of dried *I. galbana* biomass in silver pompano diets. Further studies should report the algal biomass composition in detail, measure nutrient digestibility, define the tank as the experimental unit where appropriate, and use clearly described sampling and quantitative histological assessment. Longer trials would help determine whether the observed trends are repeatable and translate into practical benefits for aquaculture. The present findings support further investigation, but the efficacy and suitable inclusion level require confirmation.

5. CONCLUSION

Dried *I. galbana* biomass may have potential as a low-inclusion feed ingredient for *T. blochii*. In this 60-day trial, supplementation did not significantly improve growth performance, although the manuscript reports differences in digestive amylase activity and selected serum biochemical parameters. The 1.0% treatment showed comparatively high values for several measures and may warrant further investigation, but the current results do not establish it as the optimum level. Because whole biomass was used, the responses cannot be attributed to fucoxanthin alone. Further studies should include digestibility measurements, immune and oxidative-stress indicators, and more detailed, preferably quantitative, histological assessment.

6. Ethics statement

Insert the actual institutional animal ethics committee name, approval number and approval date, or provide the target journal's applicable exemption statement. Do not submit this placeholder wording as the final ethics statement.

7. Conflict of interest

The authors declare that they have no conflict of interest associated with this study.

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