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Trypsin-based diet for the growth indices of Spanish mackerel

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ABSTRACT

To protect the sustainability of the aquaculture industry, fishmeal is being replaced with alternative feed ingredients such as plant-based protein components. However, most plant-based feedstuffs contain a wide array of anti-nutritional factors. These factors can potentially hinder nutrient consumption, which in turn can interfere with fish health and performance. Protease enzyme supplements can reverse the impacts of anti-nutritional factors and enhance fish growth. This study aimed to incorporate the trypsin enzyme into the food of Spanish mackerel and explore its effects on growth factors, body composition, and blood biochemical parameters. This study was a fully-randomized experiment with three treatments and three replications. This experiment was carried out as a completely randomized design with three treatments and three replications, and the trypsin enzyme was added to the fish diet at different levels: 0%, 0.015%, and 0.025%. For a timeframe of 60 days, the fish were given experimental diets. The growth indices considered were: specific growth rate (SGR), protein efficiency ratio (PER), condition factor (CF), feed conversion ratio (FCR), weight gain percentage (WGP), and hepatic steatosis index (HSI). The results showed that FCR, PER, SGR, and WGP at enzyme levels of 0.015% and 0.025% were substantially different from the control group.

Keywords: Protease, enzyme, Trypsin, Fish diet, Spanish mackerel.
Article type: Research Article.

INTRODUCTION

To ensure a steady supply of fish, the aquaculture industry has grown dramatically (Opiyo *et al.* 2018; Hodar *et al.* 2020). Furthermore, fish farming is receiving a lot of attention worldwide as a way to combat rising meat prices and meet consumer demand for animal protein sources (Janbakhsh *et al.* 2018; Shen *et al.* 2018; Wang *et al.* 2019; Fattah *et al.* 2020; Tan & Zheng 2020; Zahmatkesh *et al.* 2020). Protein is the most significant component in aquaculture feed (Gasco *et al.* 2018; Siddik *et al.* 2021). Therefore, when formulating fish diets, efforts are made to offer a specific amount of protein that is capable of providing vital amino acids for the excellent health and growth of the farm-raised fish (Kasozi *et al.* 2019; Li, Zheng, *et al.* 2021; Metwally *et al.* 2021). Due to their high protein content, palatability, well-balanced amino acids, and high digestibility, soybean and fish meal

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are the principal protein sources for aquaculture diets (Gyan *et al.* 2019; Nogales-Mérida *et al.* 2019; Jingting *et al.* 2020; Kirimi *et al.* 2020). These are the most expensive protein sources. The need for soybean and fish meal will continue to rise, owing to the aquaculture sector's predicted continued growth. As a result, a worldwide research priority is the adoption of cheaper feed proteases or protein sources to partially or wholly substitute soybean and fish meal. Replacing soybean and fish meal with other, less expensive plant- or animal-protein sources or using protease enzymes is one feeding strategy for reducing the consumption of fish meal or soybean meal (Kumar *et al.* 2018; Samaddar 2018; Frempong *et al.* 2019; Ghafoor *et al.* 2020). Various enzymes, such as proteases, can boost the efficiency of plant protein components (Goda *et al.* 2020; Bui *et al.* 2021; Siddik *et al.* 2021). It has been reported that dietary protease can improve rainbow trout's nutritional digestibility fed a plant protein-based diet and lower dietary fish meal content without impacting white leg shrimp, Prussian carp, or tilapia performance (Hassaan *et al.* 2021; Li *et al.* 2021; Kaiser *et al.* 2022; Xu *et al.* 2022). Furthermore, proteases could be employed to enhance the number of digestible protein diets. When an enzyme is given to a diet, the nutrient matrix values reflect the number of nutrients that may be released. Due to the presence of anti-nutritional factors in plant foods, their utilization in aqua-diets is severely restricted (Kidd *et al.* 2021). In animals, some digested and unabsorbed food is expelled because all enzymes are not present or their number is insufficient to digest all of the food (Vogt 2021; Jagadeesan *et al.* 2022;). Since feed costs have the highest share in the livestock, poultry, and aquaculture industry, synthetic enzymes can reduce production costs (Edwards *et al.* 2019; Naylor *et al.* 2021). Furthermore, by disintegrating anti-nutrient components in food, optimal feed consumption, good health, and pollution reduction can be achieved. Enzyme supplements are used to improve nutrient utilization, maintain the performance of low-quality diets, reduce formulation costs, increase the spectrum of dietary raw materials, overcome maladaptive and anti-nutritional factors of raw material, reduce nutrient excretion in water and improve economic efficiency (Hassan *et al.* 2020; Maas *et al.* 2021). Peptidases are one of the most important groups of commercial enzymes, accounting for over 90% of the enzyme trade worldwide (Da Silva 2018). One of the significant peptidases in the gastrointestinal tract of fish is trypsin. A serine peptidase breaks the peptide bonds mainly at the carboxyl side of the amino acids (arginine or lysine). This enzyme is responsible for activating trypsinogen and other zymogens and digestion of ingested proteins (Wang & Ji 2019; Kuz'mina *et al.* 2022; Muhlia-Almazán & Fernández-Gimenez 2022). Given the positive effect of trypsin supplement on growth in various studies and the importance of fish in the aquaculture industry, in this study, the effect of adding trypsin supplement to the diet of the Spanish mackerel and its possible side effects are investigated.

MATERIALS AND METHODS

In a fish breeding complex, 120 Spanish mackerel with an average weight of 15.6 ± 0.5g were prepared and adapted in 2500-L tanks with 1500 L of water and 10 L per minute water flow for 25 days. The experiment was carried out for 60 days. Following the bioassay, the fish were randomly allocated to one of three experimental groups, each with three replications. Every week, standard methods were used to test and record daily water temperature, acidity, and dissolved oxygen. For this study, Alltech Coppens commercial diet with 49% protein, 12% fat, 6.0% fibre, 1.5% calcium, 8% moisture, 1.5% phosphorus, and 8.0% ash along with 0%, 0.015%, and 0.025% trypsin enzyme were used as a source of food for Spanish mackerel (Table 1).

Table 1. Prepared different diets for various treatments.

Group	Diet
T_1 (control group)	Basic commercial diet with 0.000% trypsin supplement
T_2	Basic commercial diet with 0.015% trypsin supplement
T_3	Basic commercial diet with 0.025% trypsin supplement

Sodium phosphate buffer with pH 7.4 was used to dissolve the enzyme. The solution containing the enzyme was sprayed on the diets. Finally, a constant amount of water-soluble jelly powder was sprayed on all diets to reduce leaching. Feeding was carried out every day at 10 AM and 7 PM at a rate of 3% body weight. The fish were weighed using a 0.01 g precision scale once every ten days to track their growth. The amount of food was adjusted according to weight gain. Using data obtained from bioassays, as well as the protein content in both food and carcasses, growth factors including specific growth rate (SGR), condition factor (CF), feed conversion ratio (FCR), the protein efficiency ratio (PER), weight gain percentage (WGP), and hepatic steatosis index (HSI) were calculated (Ye *et al.* 2011; Li *et al.* 2021; Smiley & Abedian 2021). According to AOAC recommendations, approximate fish carcass chemicals were analysed to assess moisture, fat, protein, and ash. To measure the moisture content of the diet, an oven of 115 °C was used for 20 hours, and to measure the crude protein; the

Kjeldahl method was used (Barlow *et al.* 1981). The Kjeldahl method for protein measurement consists of three steps: titration, distillation, and digestion. Soxhlet and ether solvent were used to measure the amount of crude fat. The amount of ash was measured by burning the samples in an electric oven at 600 °C. At the end of the rearing period, blood samples were taken from 3 fish in each experimental unit after anaesthesia. Blood was obtained by a sterile syringe, then transferred into a 3-mL tube, centrifuged at 3000 rpm for 15 min, and then transferred to a new sterile tube. Serum samples were stored at -95 °C until use. Blood biochemical indices were measured using commercial clinical kits, including glucose, blood urea nitrogen (BUN), total protein, albumin, globulin, and albumin/globulin (A/G) ratio. Three fish were euthanized from each replication and quickly dissected in a cold environment to minimize enzymatic activities. The Bradford protein assay determined the total protein concentration in a sample. Data were analysed using SPSS software by the one-way analysis of variance (ANOVA). Duncan's multiple domain test at a 95% confidence level was used to compare the means between treatments.

RESULTS AND DISCUSSION

Physicochemical parameters of water were normal throughout the rearing period, and no mortality was recorded between treatments. At the end of 60 days of rearing, the indices such as final weight, WGP, SDR, and PER in fish-fed diets containing trypsin supplement (T_2 and T_3 = 0.015 and 0.025%) were significantly higher than the control group ($p < 0.05$). There was no significant difference between T_2 and T_3 ($p > 0.05$). FCR was higher in the control (T_1) than in the other groups ($p > 0.05$). CF in different treatments did not significantly differ ($p > 0.05$). HSI in T_3 was significantly higher than in the other groups ($p < 0.05$; Fig. 1).

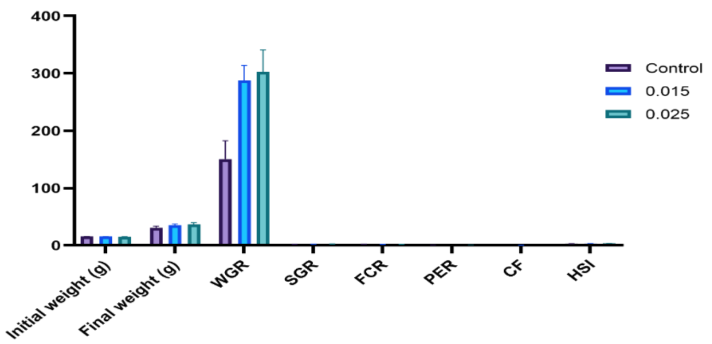

Fig. 1. Growth indices of farmed Spanish mackerel in different treatments.

Fig. 2 depicts the results related to adding different amounts of trypsin in the diet on the farmed Spanish mackerel's body composition. The highest amount of protein belonged to T_3, which was significantly higher than the control treatment (T_1; $p < 0.05$). Moisture and fat values did not show significant differences between different treatments. The ash in T_3 was significantly higher than in the other treatments ($p < 0.05$).

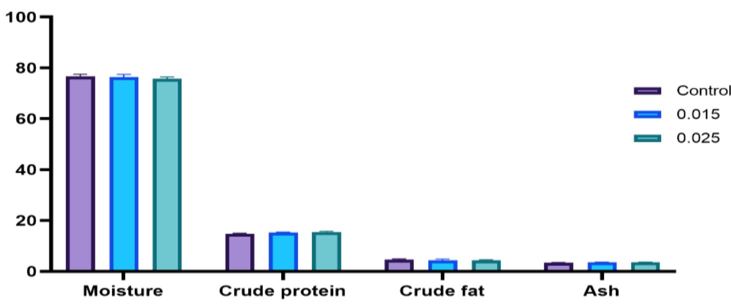

Fig. 2. Biochemical composition of fish carcasses in different treatments (based on percentage).

Fig. 3 shows the biochemical factors of blood, including glucose, blood urea nitrogen (BUN), total protein, albumin, globulin, and A/G ratio in different treatments. Based on the results, no significant difference was observed between treatments fed with diets containing different levels of trypsin supplementation enzyme ($p > 0.05$).

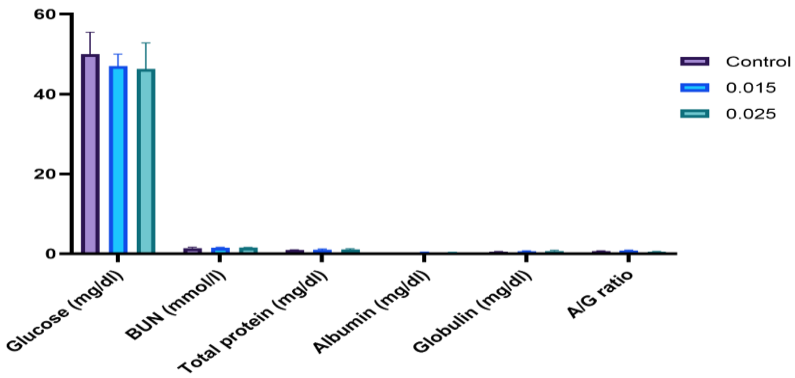

Fig. 3. Biochemical variables in the blood of fish given various diets.

The results of this study showed that using trypsin supplementation in the Spanish mackerel diet, along with reducing the conversion ratio, improves growth and increases the amount of protein and carcass ash, however, exhibits no significant effect on biochemical blood parameters. In the present study, by elevating the amount of trypsin in the diet, growth raised, so that the best growth rate was observed in T_3. In addition, this group exhibited the best feed conversion ratio. In this treatment, HSI was significantly higher than in the other groups. Similar results were obtained in a study on rohu (*Labeo rohita*). So that, the fish-fed porridge containing 0.02% trypsin showed more growth rate than in the control group (Kumari *et al.* 2013). Also, using Avizyme (containing protease) in the diet of rainbow trout improved growth and survival indices (Kanani *et al.* 2014; Elbesthi *et al.* 2020). The results of the present study showed that using trypsin supplements in the Spanish mackerel diet did not affect the moisture and fat content of fish carcasses. Still, in T_3, the amount of ash and protein in fish carcasses was higher than in the control group. Also, it was revealed that the trypsin supplement in the Spanish mackerel diet does not affect biochemical blood factors.

CONCLUSION

In general, the present study showed that trypsin supplementation in the Spanish mackerel diet increases the efficiency of the diet and improves growth indices. Using higher amounts of this enzyme or other application methods may exhibit better results, however, its confirmation will require additional studies. On the other hand, using trypsin did not harm the survival rate of the fish and exhibited no side effects. Further studies on the safety of this enzyme for Spanish mackerel may be performed in the future.

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