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Effect of dietary tryptophan supplementation on growth, body composition and digestive enzymes activity of juvenile silver pompano *Trachinotus blochii* (Lacepede, 1801)

MEENU DEVASSYKUTTY, BOBY IGNATIUS, P. VIJAYAGOPAL, SANAL EBENEEZAR, PARAMITA BANERJEE SAWANT, A. M. BABITHA RANI, ELDHO VARGHESE, P. SAYOOJ AND K. A. SAJINA

ICAR-Central Institute of Fisheries Education, Versova, Mumbai - 400 061, Maharashtra, India
**ICAR- Central Marine Fisheries Research Institute, Kochi - 682 018, Kerala, India*
e-mail: bobycmfri@gmail.com

ABSTRACT

A 60-day feeding trial was conducted to study the effect of dietary supplementation of tryptophan in juvenile silver pompano *Trachinotus blochii* (Lacepede, 1801) (Average initial weight=6.81±0.05 g). Five isonitrogenous and isolipidic feeds supplemented with tryptophan at different levels, 0 (TRP_0), 0.5 ($TRP_{0.5}$), 1.0 ($TRP_{1.0}$), 1.5 ($TRP_{1.5}$) and 2.0 ($TRP_{2.0}$) g 100 g^{-1} of diets were formulated. The weight gain%, specific growth rate, hepato-somatic index, digestives enzymes of stomach and intestine except protease activity in stomach, red blood cells, white blood cells, crude protein, fat and ash content of the fish were significantly influenced ($p<0.05$) by tryptophan supplementation. The viscero-somatic index, intraperitoneal fat ratio, muscle ratio, protease activity in the stomach and acid insoluble ash were unaffected by the treatment. Best observations in terms of growth, body composition and digestive enzyme activity among the treatments were obtained in the fish group fed with tryptophan at a level of 0.5 g 100 g^{-1} feed. From the current observations it can be concluded that supplementation of tryptophan at the rate of 0.5 g 100 g^{-1} diet can positively influence the growth of *T. blochii*.

Keywords: Essential amino acids, Growth, Silver pompano, Tryptophan

Introduction

The behavioural, structural, pathological, reproductive and physiological aspects of the fish rely mainly on nutrition (Guillame *et al*., 2001). Among all the nutrients, protein is one of the vital macronutrients as it is the building block of muscles and organs. The wholesomeness of protein relies completely on the essential amino acid (EAA) composition (Moraes and de Almeida, 2020). Protein provides amino acids for proteogenesis, generating energy and restoring impaired tissues. In addition to protein synthesis, EAAs have significance in many of the metabolic and nutritional processes of animals including regulation of gene expression, cell signalling, appetite stimulation, food intake, nutrient metabolism, digestion, growth and development, proper energy consumption, immune response, removing ammonia and other physiological processes such as osmoregulation, metamorphosis, pigmentation and reproduction (Wu *et al*., 2014). The deficiency of EAAs impairs growth, feeding efficiency and fish's immune response (Pianesso *et al*., 2015). Incorporation of EAA in the right quantity can develop balanced aquafeeds, which will enhance the quality of feed and thus increase the profitability of aquaculture (Li *et al*., 2009).

Tryptophan is an aromatic amino acid that should be supplied through diet. It has versatile effect on the physiology of teleosts (Hoseini *et al*., 2019). Growth, survival, sexual behaviour, reproduction and immunity of the fish are linked to this essential amino acid, tryptophan. This indoleamine neurotransmitter plays a vital role in the fish's behavioural as well as physiological functioning (Sahu *et al*., 2020). Tryptophan is the precursor of serotonin and kynurenine (Le Floch *et al*., 2011). Serotonin is a crucial neuromodulator that is an active participant in stress suppression. It regulates pituitary growth hormone secretion (Musumeci *et al*., 2013). Kynurenine and its breakdown products have diverse biological functions. Deficiency in tryptophan causes cataracts and spinal deformities in fish (Sahu *et al*., 2020). Effect of tryptophan in improving growth, immune system and stress mitigation roles were reported in various species (Akhtar *et al*., 2013a; Tejpal *et al*., 2014; Ciji *et al*., 2015; Morandini *et al*., 2015). L-tryptophan amino acid as the precursor of serotonin (5-HT) can modulate the role of brain and cortisol during stress response (Cabanillas-Gamez *et al*., 2018; Gonzalez-Silvera *et al*., 2018). The tryptophan requirements of different fish species

lie between 0.3-1.3% of dietary protein level and can vary according to species (Hoseini *et al*., 2019).

Trachinotus blochii (Lacepede, 1801) (silver pompano or snub nose pompano) has high market demand because of its flesh quality, fast growth rate and wide range of salinity tolerance. Asia-Pacific countries, including Taiwan, Indonesia, China and India, are widely producing this species in ponds and cages (Jayakumar *et al.,* 2014, Kalidas *et al*., 2020). ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), India, developed the spawning stocks and pioneered successful breeding and seed production of the fish (Gopakumar *et al*., 2012). Developing a low-cost, nutritionally balanced feed for the species is now underway (Ebeneezar *et al*., 2019). The present study evaluated the effect of dietary supplementation of tryptophan on the growth. body composition, blood cell count and digestive enzymes activity in juvenile *T. blochiii*.

Materials and methods

The animal experimentation in this study was carried out in accordance with the ARRIVE recommendations (Percie du Sert *et al*., 2020). The live fish were treated in accordance with the UK Animals (Scientific Procedures) Act (1986) and EU Directive 2010/63/EU (2019). The experimental protocols used to execute this study were approved by the ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi, India (MBT/NTM/24).

The fish and experimental design

The experiment was conducted at the Fish Nutrition wet laboratory of ICAR-CMFRI, Kochi. Seventy days old juvenile fish were procured from the finfish hatchery of Rajiv Gandhi Centre for Aquaculture (RGCA), Trivandrum, Kerala, India. The average weight of fish used for the experiment was 6.81±0.05 g. The experiments were conducted in 15 glass tanks of 80 l capacity. A completely randomised design was used in which eight fishes were stocked in each tank of five treatments in triplicates. During the 60-day feeding trial, the fishes were fed twice a day at 10.00 and 16.00 hrs until they appeared satiated. Tanks were fitted with a recirculation system that ensured an uninterrupted flow of water to keep dissolved oxygen (DO) above 5 ppm and to maintain optimal water quality. Salinity was maintained at 15‰. Periodic monitoring of ammonia, nitrite (using test kits, Nice Chemicals) and pH (using an electronic pH meter, Hanna electronics) in the experimental tanks were performed.

Experimental diet

Five isonitrogenous test diets containing varying amounts of tryptophan with a gradation of 0.5 g 100 g^{-1} and a control diet without tryptophan supplementation were formulated (Table 1). The tryptophan in the control diet was calculated as 0.24 g 100 g^{-1} of feed based on the tryptophan content of the ingredients used. Oil and water were mixed with the finely powdered ingredients. The mix

Table 1 Feed formulation (g 100 g $feed^{-1}$) and proximate composition of feed

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5
Fish meal[1]	20	20	20	20	20
Clam meal[2]	12	12	12	12	12
Meat and bone meal[3]	11	11	11	11	11
Wheat flour[4]	21	20.50	20	19.50	19
Soya flour[5]	20.05	20.05	20.05	20.05	20.05
Groundnut oil cake[6]	5	5	5	5	5
Wheat gluten[7]	2	2	2	2	2
L-Tryptophan[8]	0	0.5	1.0	1.5	2
Fish oil[9]	3	3	3	3	3
Vitamin[10]	2	2	2	2	2
Mineral[11]	2	2	2	2	2
BHT+SMB[12]	0.2	0.2	0.2	0.2	0.2
Vitamin C[13]	0.25	0.25	0.25	0.25	0.25
Lecithin[14]	1	1	1	1	1
MCP[15]	0.5	0.5	0.5	0.5	0.5
Crude protein (g 100 g^{-1}, DM basis)	43.43	43.93	43.96	44.3	44.4
Crude fat (g 100 g^{-1}, DM basis)	7.79	7.39	7.46	7.73	7.85
Total ash (g 100 g^{-1}, DM basis)	14.36	14.97	15.11	14.88	14.85

[1]Arbee Aquatic Proteins Pvt Ltd, Kochi, India; [2, 4, 6]Procured from local market, Ernakulam, India; [3]Kerala Veterinary and Animal Science University, Meat Technology Unit, Thrissur, India; [5]Sakthi Soyas, Coimbatore, India; [7]Viveka Essence Mart, Wall Tax Road, Chennai, India; [8]Hi-Media, Mumbai, India; [9]Kiriyanthan Trading Co., Kochi, India; [10]Supplevite-M, Sarabhai Zydus Animal Health Pvt. Ltd., Vadodara, India; [11]Agrimin Forte, Virbac Healthcare India, Pvt. Ltd., Mumbai, India; [12] Butylated hydroxyl toluene and Sodium meta bisulphate, Hi-Media, Mumbai, India;[13]Stay-C, DSM Nutritional Technologies, Mumbai, India; [14]Ocean Nutrition, Belgium; [15] Monocalcium phosphate Loba Chemie Pvt Ltd.

was completely blended in a homogeniser (Foss, Italy) and then passed through a twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India). The pellets were dried in the hot air oven to keep the feed's moisture level under 10% (Labline, India). The feed was crumbled and sieved to appropriate size (2 mm) before being placed in sealed containers for storage.

Sampling

In the final sampling, three fishes from each tank, *i.e.,* nine fishes from each treatment, were taken for various analyses. Fishes were starved for 24 h before sampling. Blood samples were collected by anesthetising the fish with clove oil and puncturing the caudal vein. After taking the body measurements, liver and visceral weights were noted to measure the changes in body condition index. Stomach and intestinal samples were preserved in 0.25 M sucrose solution and then homogenised, centrifuged and the supernatant was collected and stored at -80°C for analysis of digestive enzymes.

Growth, survival and nutritional parameters

The growth of the fish was determined by calculating weight gain (WG%) and specific growth rate (SGR). Feed conversion ratio (FCR), feed efficiency ratio (FE) and protein efficiency ratio (PER) were calculted. Body condition indices such as hepato-somatic index (HSI), viscero-somatic index (VSI), intra peritoneal fat ratio (IPFR) and muscle ratio (MR) were also estimated.

WG (%) = 100 × (Final body weight [g] - Initial body weight [g]) /Iinitial body weight (g)

SGR (%) = 100 × (ln final weight - ln initial weight)/ Days of the experiment

FE = Body weight gain(g)/ Dry feed intake (g)

FCR = Feed fed [dry weight (g)] / Body weight gain (g)

PER = Body weight gain (g)/Total protein ingested (g)

HSI (%) = 100 × [Liver weight (g)] / [Whole body weight (g)]

VSI (%) = 100 × [Viscera weight (g) / [Whole body weight (g)]

IPFR (%) = 100 × [Intra peritoneal fat weight (g) / [Whole body weight (g)]

MR (%) = 100 × (Skeletal muscle weight) / (Whole body weight)

Survival rate = 100 × (No. of fish stocked / No. of fish survived)

Digestive enzymes

Analysis of protease enzyme was performed by the casein digestion method (Drapeau, 1976) for which 2.5 ml casein in 0.01 N NaOH, 0.05 M trisphosphate buffer (pH 7.8) and 0.1 ml tissue samples were mixed, kept for 10 min and then 10% trichloroacetic acid was added to stop the reaction. The reaction mixture was filtered and OD was taken at 280 nm and the protease activity is expressed as Units mg protein^{-1} min^{-1}. Amylase was analysed using the Dinitrosalicylic acid (DNS) method (Rick and Stegbauer, 1974), in which 1% (w/v) starch solution, phosphate buffer (pH 6.9) and 5% tissue homogenate were mixed and incubated at 37°C for 30 min. DNS was added and mixture was kept in a water bath for 5 min. Distilled water was added to dilute the mixture after cooling. The optical density (OD) was measured at 540 nm and the amylase activity is expressed in terms of moles of maltose released from starch per min per mg protein (Units mg protein^{-1} min^{-1}). Lipase estimation was done by the procedure given by Cherry and Crandall (1932). Tissue homogenate was mixed with distilled water, phosphate buffer and olive oil (substrate). After shaking well, the mixture was incubated at 37°C for 24 h and 3 ml of 95% alcohol and two drops of phenolphthalein indicator were added and the mixture was titrated against 0.05N NaOH until the appearance of a permanent pink colour. The amount in terms of milli equivalent of alkali consumed gave the lipase activity and expressed as Units mg protein^{-1} min^{-1}.

Proximate composition analysis

The standard methodology of AOAC (2005) was used for proximate composition analysis. The sample was oven-dried at 105^{0}C until a constant weight was achieved for estimating the moisture content. The Kjeldahl method was used for determining crude protein with a semi-automated Kjeldahl system (FOSS Kjeltec 2300) after acid digestion of the sample. The Soxhlet system (FOSS Soxtec2043) determined crude lipid, which employs the ether extraction methodology. The sample was incinerated in a muffle furnace at 550°C for 3 h to determine the ash content. Acid insoluble ash (AIA) was determined by boiling the ash in acid (5N HCl), washed and filtered in ashless filter paper which was incinerated in muffle furnace.

Red blood cells (RBC) and white blood cells (WBC) count

Neubauer's hemocytometer was used to count the number of red blood cells (RBCs) and white blood cells (WBCs) of the sampled fishes and expressed in terms of number cells per ml of blood.

Statistical analysis

Statistical analysis was done using the SAS 9.4 (SAS Institute, Cary, North Carolina, USA) software for Windows. Significant difference among the five levels of tryptophan, each replicated three times, was determined

by one-way ANOVA and Tukey's HSD test ($p<0.05$) was used for the multiple comparisons of treatment effects.

Results

The dietary tryptophan levels significantly influenced the growth performance, blood cell counts (RBC and WBC), digestive enzyme activity and proximate composition of silver pompano. The growth in terms of average final weight, was observed to be poor in fishes fed with the minimum (TRP_0) and maximum (TRP_2) levels of dietary tryptophan (Table 2). Weight gain percentage and specific growth rate were significantly lower in the control as compared to other groups, among which no significant difference was observed. Among the treatment groups, the weight gain percentage of silver pompano ranged from 197.15 to 300.62%. The highest weight gain (300.62%) was observed in the treatment group fed with diet containing tryptophan at 0.5 g 100 g^{-1}. The SGR of silver pompano among the treatment groups ranged from 1.81 to 2.26. FCR of the fish in the five treatments ranged from 1.67 to 2.32. Among the treatment groups, best FCR was found to be 1.67 in the treatment group fed with 0.5% tryptophan. FCR, FE and PER values showed no significant difference among treatments. The survival rate was 100% in all the dietary tryptophan levels except in the treatment group, where minimum tryptophan was available in the feed (TRP_0). HSI was observed to be significantly different ($p<0.005$) among treatments and VSI, MR and IPFR were not affected by tryptophan supplementation.

In this study, tryptophan supplementation significantly influenced the fish's digestive enzyme activity (Table 3). Amylase activity in the intestine of fish was observed to be same in TRP_0 and $TRP_{0.5}$ where as in stomach the amylase activity was found to increase initially and which then decreased and was observed to be high in the treatment group $TRP_{0.5}$. Protease and lipase activity in the intestine was high in $TRP_{1.0}$, whereas the treatment did not affect protease activity of the stomach. Lipase activity in the stomach was found to be the highest in $TRP_{1.5}$ and lowest in $TRP_{2.0}$.

The body composition of silver pompano, fed with graded levels of tryptophan is given in Table 4, in which all the proximate parameters of the fish body were found to be significantly influenced by dietary tryptophan supplementation. Crude protein, crude fat and crude ash content were observed to be highest in the group $TRP_{0.5}$. The RBC and WBC were high in the treatment group fed with the highest tryptophan level *i.e.,* $TRP_{2.0}$ and the treatment group $TRP_{0.5}$ had no significant difference with $TRP_{2.0}$. Accordingly, the present study indicates that supplementing tryptophan at 0.5 g 100 g^{-1} feed can enhance growth in juvenile silver pompano.

Table 2. Growth performance, survival, feed utilisation and body condition indices of *T. blochii* fed with graded levels of tryptophan

Parameters	Dietary tryptophan levels (g 100 g $feed^{-1}$)					SEM	p>F *
	TR TRP_0	$TRP_{0.5}$	$TRP_{1.0}$	$TRP_{1.5}$	$TRP_{2.0}$		
Average final weight (g)	20.11^{c}	27.74^{a}	26.42^{ab}	24.90^{ab}	24.03^{b}	0.75	<0.001
Weight gain (%)	197.15^{b}	300.62^{a}	288.72^{a}	264.43^{a}	257.28^{a}	12.73	0.002
Specific growth rate (%)	1.81^{b}	2.31^{a}	2.26^{a}	2.15^{a}	2.12^{a}	0.06	<0.001
Feed conversion ratio	1.93^{a}	1.67^{a}	1.91^{a}	1.94^{a}	2.32^{a}	0.15	0.11
Feed efficiency	0.53^{a}	0.60^{a}	0.54^{a}	0.52^{a}	0.43^{a}	0.04	0.15
Protein efficiency ratio	1.32^{a}	1.51^{a}	1.34^{a}	1.30^{a}	1.08^{a}	0.11	0.15
Hepato-somatic index	1.06^{ab}	0.90^{ab}	1.62^{a}	0.78^{b}	1.29^{ab}	0.16	0.02
Viscero-somatic index	7.69^{a}	7.02^{a}	9.52^{a}	7.13^{a}	8.37^{a}	0.56	0.06
Intra peritoneal fat ratio	1.31^{a}	0.96^{a}	1.78^{a}	0.97^{a}	0.98^{a}	0.49	0.15
Muscle ratio	54.20^{a}	52.36^{a}	52.36^{a}	52.34^{a}	55.66^{a}	1.31	0.28
Survival (%)	87.50^{a}	100^{a}	100^{a}	100^{a}	100^{a}	3.73	0.15

Data represent the averages of triplicate observations and expressed as Mean±SE; *Level of significance from ANOVA table; SEM: Standard Error of Mean

Table 3. Digestive enzyme activity of *T. blochii* fed with graded levels of tryptophan

Enzyme level (Units mg $protein^{-1}$ min^{-1})	TRP_0	$TRP_{0.5}$	$TRP_{1.0}$	$TRP_{1.5}$	$TRP_{2.0}$	SEM	p>F*
Amylase intestine	3.69^{b}	3.32^{b}	9.20^{a}	8.54^{a}	7.53^{a}	0.58	<0.001
Amylase stomach	6.55^{ab}	9.48^{a}	7.44^{ab}	6.29^{b}	7.22^{ab}	0.65	0.04
Protease intestine	1.27^{bc}	1.39^{b}	1.78^{a}	1.27^{bc}	0.96^{c}	0.07	<0.001
Protease stomach	2.88^{a}	1.45^{a}	0.87^{a}	0.40^{a}	3.85^{a}	0.92	0.12
Lipase intestine	4.83^{bc}	5.34^{ab}	5.65^{a}	4.12^{c}	5.23^{ab}	0.18	0.001
Lipase stomach	12.49^{ab}	13.35^{ab}	10.95^{ab}	15.25^{a}	8.69^{b}	1.15	0.02

Data represent the averages of triplicate observations and expressed as Mean±SE; *Level of significance from ANOVA table; SEM: Standard Error of Mean

Table 4. Proximate composition (on dry matter basis), RBC and WBC count of *T. blochii* fed with graded levels of tryptophan

Parameters	TRP_0	$TRP_{0.5}$	$TRP_{1.0}$	$TRP_{1.5}$	$TRP_{2.0}$	SEM	p>F*
Crude protein (%)	52.95^{e}	56.87^{a}	55.95^{b}	54.19^{c}	53.52^{d}	0.10	<0.001
Crude fat (%)	11.18^{d}	15.26^{a}	14.53^{b}	13.52^{c}	11.24^{d}	0.11	<0.001
Total ash (%)	10.49^{c}	13.04^{a}	12.56^{ab}	12.30^{ab}	11.71^{b}	0.2	<0.001
AIA (%)	0.36^{a}	0.53^{a}	0.42^{a}	0.46^{a}	0.49^{a}	0.05	0.296
RBC ($\times 10^9$ ml^{-1})	2.83^{b}	3.33^{ab}	2.83^{b}	2.78^{b}	3.62^{a}	0.13	0.003
WBC ($\times 10^6$ ml^{-1})	5.5^{b}	7.0^{ab}	7.2^{ab}	6.0^{b}	8.0^{a}	0.40	0.01

Data represent the averages of triplicate observations and are expressed as Mean±SE; *Level of significance from ANOVA table; AIA: Acid insoluble ash; SEM: Standard Error of Mean

Discussion

Proper protein utilisation and growth in fishes require all the essential amino acids in an adequate composition. Dietary deficiency of one or more essential amino acids will remove the amino group of other amino acids and convert it into ammonia in the liver, which will further affect the protein synthesis and growth of the organism (Von der Decken and Lied, 1993; Wilson, 2002). Tryptophan is a secondary limiting amino acid after lysine and methionine and inadequate level of tryptophan in feed can inhibit the proper utilisation of other indispensable amino acids in fish (Nguyen *et al*., 2018).

In the current experiment, the growth and feed utilisation were observed to be high in the treatment group fed with tryptophan level of 0.5 100 g feed^{-1}. Excess tryptophan intake causes adverse effects such as reduced feed intake and impaired growth in *Labeo rohita* (Abidi and Khan, 2010). Similarly in the current experiment, tryptophan levels beyond and below the adequate level in the feed depressed growth of silver pompano. The negative effect of excess tryptophan can be due to the amino acid toxicity and ingestion of excessive tryptophan (Abidi and Khan, 2010; Moehn *et al*., 2012). Consumption of disproportionate or excess amino acids causes amino acid toxicity (Harper *et al*., 1970). It is caused either by the accumulation of amino acid or its products from degradation, which may further lead to stress in the fish's enzymatic system, generating possible toxicity and accumulation (Alam *et al*., 2003). Reduction in growth with supplementation of tryptophan beyond 0.5% can be due to excess consumption of tryptophan. The current study did not find nutritional deficiency disorders in fish fed with a lower level of tryptophan. FCR was observed to be 1.67 in the feed containing 0.5% of tryptophan. FCR value of 1.67 points out the fact that the feed was of higher quality. Similarly best value for FE and PER were obtained in $TRP_{0.5}$.

The influence of tryptophan on body condition indices was reported in juvenile *Sciaenops ocellatus* (Pewitt *et al*., 2017) and juvenile *Oreochromis niloticus* (Nguyen *et al*., 2018). In the current experiment, HSI was observed to be significantly affected by tryptophan supplementation whereas, other biometric indices studied were unaffected. An intermediate value of 0.90 for HSI was obtained in the treatment group fed with an adequate level of tryptophan *i.e.,* 0.5 g 100 g feed^{-1}. The lowest and highest HSI values observed can be attributed to liver abnormalities due to over and under supplementation of tryptophan (Ahmed, 2012; Hansson *et al*., 2017). Similar to the current observation, IPFR was not affected by tryptophan levels in the feed in juvenile *O. niloticus* (Nguyen *et al*., 2018).

The influence of tryptophan levels in the diet on the digestive process of fish is yet to be explored (Diogenes *et al*., 2019). The increase in intestinal amylase activity in $TRP_{1,}$ $TRP_{1.5}$ and TRP_2 groups could be attributed to the capability of the amino acid to activate the amylase enzymes as its protein structure contains tryptophan units (Kushak *et al*., 2002). The lowest lipase activity in the stomach and protease activity in the intestine of fish recorded in $TRP_{2.0}$ could be due to the inhibitory action of dietary tryptophan on the digestive enzymes, similar to the reports in salmonid species (Mardones *et al*., 2018).

Even though weight gain is considered the most common measure of growth rate in fishes, the body composition is also equally important in determining the requirement of amino acids in the fish (Cowey, 1992). Similar to the reports in *Plotosus canius* (Ahmed, 2012), *Cirrhinus mrigala* (Abidi and Khan, 2010) and *Labeo rohita* (Tejpal *et al*., 2009), the crude protein and crude lipid composition were observed to be increasing with supplementation of tryptophan. Ahmed (2012), reported that the supplementation of tryptophan in the diet could improve the protein utilisation of the fish. Increase in the whole-body protein content of the fish with tryptophan supplementation can be due to the proper utilisation of the dietary protein. Similarly, in the case of whole-body crude fat content, lowest value was obtained in the fish fed with the lowest amount of tryptophan which is in agreement with the findings of Poston and Rumsey (1983) in *Oncorhynchus mykiss*.

According to Hoseini *et al*. (2020), the increase in RBC count indicates fish welfare or improved health. In the current experiment, the adequate tryptophan level of 0.5 g per 100 g feed was found to improve hematopoiesis in fish. These observations are in line with the studies done in *O. mykiss* (Hoseini *et al*., 2020) and *Heteropneustes fossilis* (Ahmed, 2012).

From the present study, it can be concluded that the supplementation of tryptophan in diets at 0.5 g 100 g^{-1} can increase the growth rate, well-being as well as nutritional quality of juvenile silver pompano. The present study will act as a base for optimising the dietary tryptophan requirement in silver pompano.

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