Fishery Technology 60 (2023) : 157 - 162

Digestive Enzyme Profiling in Different Life Cycle Stages of Rainbow Trout (*Oncorhynchus mykiss,* Walbaum 1792) from Kashmir Valley

Asifa Wali*, Tasaduq Hussain Shah, Farooz Ahmad Bhat, Adnan Abubakr, Bilal Ahmad Bhat, Tariq Hussain Bhat, Ishrat Mohd

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) Rangil, Ganderbal 190 006, Jammu and Kashmir, India

Abstract

For most fish species, the digestive tract produces enzymes related to metabolism, digestion, absorption and assimilation of nutrients including proteins, lipids and carbohydrates. A total of 200 intestine samples of *O. mykiss* from the national trout fish farm, Kokernag were screened to estimate the enzyme profiles of fish from fry to broodstock stages. The highest trypsin activity was obtained in the fry stage (95 U/mg), followed by the yearling stage (80 U/mg). The lowest value was observed in table size stage (0.0098 U/mg). The ALP activity was highest in table size stage (85.16 U/mg) followed by yearling stage (39.72 U/mg) while the lowest was at fingerling stage (3.44 U/mg). The total lipase activity was maximum in table size stage (273.16 IU/L) and minimum in yearling stage (25. 38 IU/L). The total amylase activity was highest in yearling stage (208.24 IU/L), followed by fry (151.57 IU/L) and lowest in brood stage (1.73 IU/L). Better understanding of the enzymes in the digestive tract can lead to designing feeds for various life cycle stages of *O. mykiss* keeping in view of the prominent intestinal enzyme stage and will lead to better growth by proper utilization of nutrients which in turn, lead to improved production of trout fish.

Keywords: Digestive-tract, enzymes, fry, metamorphosis, trout

Introduction

Adult rainbow trout weigh 2-3 kg and reach maximum size, weight and age of 120 cm total length (TL), 25.4 kg and 11 years of age (Froese & Pauly, 2008). Rainbow trout live as the inhabitant of the upper, cold water sections of rivers and seas. The factors which usually determine their color and shape include the habitat and food of rainbow trout, as in the case of other trouts. Many improved strains of rainbow trout are produced that have superior traits like roughness, disease resistance, higher growth rate and reproductive ability under culture conditions. Freshwater sources of the Pacific coasts of North America and Asia are the original habitat of rainbow trout. Rainbow trout have been introduced to over 82 countries, since the species can tolerate a wider range of environmental conditions than other trout species. Rainbow trout's natural diet depends on the size and age of the fish, on the size of the food item and on the habitat in which it lives. Unlike other fish species, rainbow trout are aggressive and greedy in feeding (Hoitsy, 2002). They feed on the adult beetles (Coleoptera), flies (Diptera), ants (Formicidae) and the larval stages of Lepidoptera (moths and butterflies) (Montgomery & Bernstein, 2008). Rainbow trout are primarily carnivorous but show few anatomical characteristics for acquiring and digesting prey. In addition to the simple and small size of the teeth, they do not have any elaborate structures for capturing, holding, or swallowing prey. The salmonid swallows its food whole into a Y-shaped stomach, which has a wide oesophagus. Pyloric caeca usually branch into the midgut at the pyloric end and their abundance is often of taxonomic importance among salmonid species. The pancreas cannot be easily seen in the pyloric caeca as it is distributed in the fat and connective tissue. Larger specimens will have a bile duct extending from the middle lobe of the liver to the upper midgut. There is no distinct demarcation between the midgut and the hindgut. In addition to a thin, nearly transparent swim bladder, the kidney

Received 26 July 2022; Revised 17 May 2023; Accepted 05 June 2023

**Email:* *oncorhynchusasifa@gmail.com*

occupies most of the visceral cavity, just dorsal to the swim bladder. Overall, rainbow trouts are usually representative of salmonids. In summary, this is a relatively primitive (unspecialized) fish, a carnivore with good swimming ability for capturing prey, an abdominal cavity that can easily extend posteriorly and a short intestine for consuming food. In general, the length of the gut (oesophagus to anus) is 0.6 to 0.8 times the body length.

A fish's nutritional physiology can be determined by testing the enzymes in its gut. Food quality is directly correlated with its ability to support growth and its nutritional value is determined by how easily it is digested and absorbed by the animal (Akintunde, 1985). As a result of feeding habits, the distribution of digestive enzymes and their specific activity change along the gut (Tengjaroenkul et al., 2000). Different feeding habits at various developmental stages are explained by the digestive processes associated with fish size and food type (Tramati et al., 2005). There exists a relationship between the anatomical and physiological development of the digestive organs. Due to the smaller surface area exposed to enzymatic action, adults tend to capture larger prey in natural conditions. This demands a greater digestive effort. A knowledge of digestive enzymes may be useful in developing more efficient diets and rearing techniques (Uys & Hecht, 1987). Climate and seasons play the most significant roles in influencing aquatic organisms' metabolic rate. Ectothermic animals make up the majority of aquatic organisms. Due to this, their physiological processes such as growth, reproduction and survival, are regulated by the temperature of the environment. Ectothermic animals have the peculiar ability to adapt to different temperatures in their environment, with minimum and maximum climatic tolerance limits. Various studies have determined the effect of water temperature on fish digestion and enzyme activity over a range of seasons. A feed's ability to boost animal growth in a culture operation is governed not only by its nutrient profile but also by an animal's inherent ability to consume, digest, absorb and metabolize those nutrients. Aquatic animals utilize nutrients based on the activity of their digestive enzymes (Areekijseree et al., 2004; Day et al., 2011). This study was conducted to determine the digestive enzyme profile of different stages of Rainbow trout.

Materials and Methods

The digestive enzymes at different stages (fry, fingerling, yearling, tablesize and broodsize) were determined by homogenizing whole gut from each category individually in ice-cold tris-HCL/ distilled water buffer, pH 7.4 with a homogenizer (Polytron) and the homogenates were centrifuged at 13,000 rpm for 2 mins at 4 ºC. Supernatant was collected and stored in aliquots in freezer until use. The activity of alkaline phosphatase, lipase, amylase and trypsin were determined using kits as described by the manufacturer (Coral Clinical Systems).

Results and Discussion

This study was conducted to determine the enzyme levels in the intestine of different stages of *O. mykiss*. The morphometric parameters of Rainbow trout used in the study is given in Table 1. The trypsin activity among different life cycle stages of *O. mykiss* were compared (Fig. 1). The highest value was in fry stage with a value of 95 U/mg followed by yearling stage (80 U/mg). The lowest value was observed in tablesize (TS) stage (0.0098 U/mg). The total ALP activity in all life cycle stages of *O. mykiss*

Table 1. Morphmetric parameters of rainbow trout during various developmental stages

Stage	Total Weight (g)	Total Length (mm)	Whole intestinal length (mm)	Intestinal Coefficient	HSI (%)
Fry	3.60 ± 0.19	63.31 ± 1.32	41.1 ± 1.58	0.77 ± 0.24	1.19 ± 0.08
Fingerling	10.56 ± 0.78	91.51 ± 2.33	69.02 ± 2.18	0.90 ± 0.01	1.11 ± 0.09
Yearling	45.04 ± 1.52	149.37 ± 1.75	109.97 ± 1.59	0.88 ± 0.01	1.38 ± 0.06
Tablesize	235.19 ± 8.85	258.00 ± 3.64	196.05 ± 4.25	0.89 ± 0.01	1.24 ± 0.05
Broodsize	530.69 ± 11.43	341.2 ± 2.96	266.1 ± 5.06	0.91 ± 0.01	1.62 ± 0.06

N=40, *Values expressed as Mean ± SE

are given in Fig. 2. The highest value was observed in table size stage (85.16 U/mg) followed by yearling stage (YL) (39.72 U/mg). The lowest value was in fingerling stage (FL) (3.44 U/mg). The maximum value for lipase activity was observed in table size (273.16 U/L) and minimum was recorded in yearling stage (25. 38 U/L).

The amylase activity in all life cycle stages of *O. mykiss* is presented in Fig. 4. The highest amylase activity was recorded in yearling stage (208.24 U/L) followed by fry (151.57 U/L) and lowest was observed in brood stage (1.73 U/L). The brooder utilizes the pre-accumulated fat to sustain spawning phase when it takes only limited feed and hence there is low amylase activity at this stage. The amylase and ALP activity differ significantly for the fry, fingerling, yearling and broods ($p < 0.01$), while the activity of these digestive enzymes varied non-significantly for the tablesize ($p > 0.05$). The lipase activity was found to be significantly different in fry, yearling, tablesize and broodstock stages ($p < 0.01$). The activity was non-significant in the case of fingerling stage ($p > 0.05$). The trypsin activity varied non-significantly in all the stages ($p > 0.05$). While comparing the enzyme activity of different life cycle stages, the study showed that ALP activity differs significantly in fry, fingerling, yearling, tablesize and broodstock stages. The lipase activity significantly differs in fry, yearling, tablesize and broodstock stages, while the amylase and trypsin activity on comparison within stages showed non-significance (Table 2).

Research has proven that the age and growth of fish exhibit a strong correlation. A strong correlation exists between total length, standard length and weight in rainbow trout at every stage, from fry to brood. Research involving the evaluation of differ-

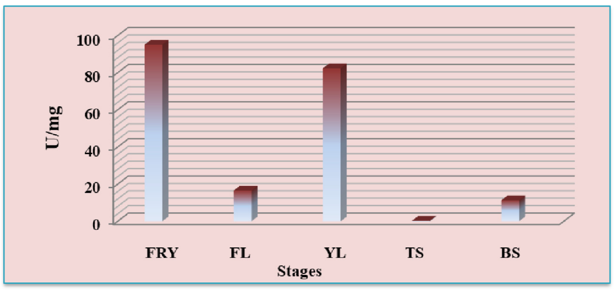

Fig. 1. Total trypsin activity in all life cycle stages of *O. mykiss*. FL-fingerling, YL-yearling, TS-tablesize and BS-broodstock

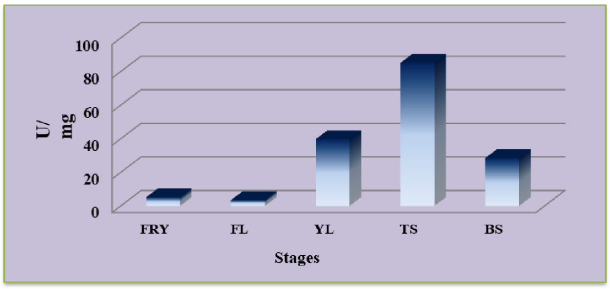

Fig. 2. Total ALP activity in all life cycle stages of *O. mykiss*. FL-fingerling, YL-yearling, TS-table size and BS-broodstock

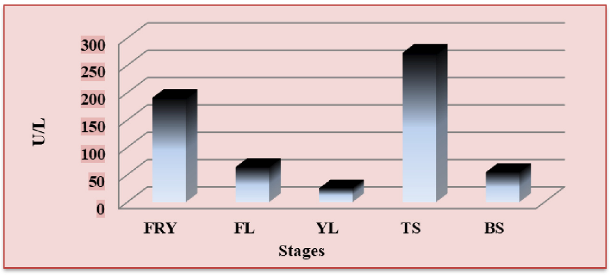

Fig. 3. Total Lipase activity in all life cycle stages of *O. mykiss*. FL-fingerling, YL-yearling, TS-tablesize and BS-broodstock

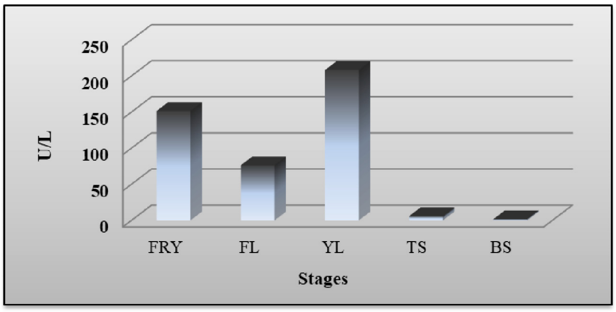

Fig. 4. Total Amylase activity in all life cycle stages of *O. mykiss*. FL-fingerling, YL-yearling, TS-tablesize and BS-broodstock

ent life cycle stages of fishes has shown that the formation and differentiation of organs can influence the production of enzymes from the alimentary canal (Kuz'mina & Gelman, 1998; Cara et al., 2003). Trypsin activity in the gut of trout fry (95.01 ± 42.20 U/mg) and yearling (82.36 ± 37.44 U/mg) was highest, as observed in some species of Caspian brown trout (Zamani et al., 2009). This might be due to the development and appearance of the digestive and reproductive organs at early stages. The tablesize stage being the adult phase of fish, represents the active participation in spawning and

Table 2. Stage wise comparison of enzyme activities of *O. mykiss*

Stages	ALP	Lipase	Amylase	Trypsin
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Fry	5.148 ± 2.319	190.866 ± 27.843	151.570 ± 133.782	95.016 ± 42.213
FL	3.440 ± 2.569	64.264 ± 15.197	77.323 ± 34.615	16.519 ± 7.277
YL	39.720 ± 26.250	25.390 ± 18.800	208.244 ± 44.155	82.362 ± 37.443
TS	85.167 ± 29.580	273.167 ± 67.210	6.089 ± 3.480	0.334 ± 0.110
BS	28.453 ± 25.927	54.868 ± 20.268	1.740 ± 0.435	11.290 ± 5.432
C.D.	N/S	113.186	N/S	N/S
SE	21.205	35.462	64.896	25.559

FL-fingerling, YL-yearling, TS-tablesize and BS-broodstock

fish requires more energy for the development of gonads which is provided by fats and is seen by high levels of lipases in tablesize stage. On the contrary, for growth and development fry stage requires more proteinaceous food and hence showed high levels of trypsin. Profiling of ALP during the investigation revealed the highest values in the tablesize stage (85.16 ± 29.58 U/mg). Lojda et al. (1979), also reported the presence of alkaline phosphatase enzyme in the active transport that occurs in the membranes of the fish alimentary canal and that it characterizes the development of enterocytes. When the fish reaches reproductive phase, since all the cellular effort is aimed at spawning, broodfish tend to have low enzyme activity levels. Additionally, brood fishes have been reported to have low feeding intensity levels. Higher amylase activity was recorded for the yearling stage (208.24 ± 44.15 U/L). A higher amylase concentration is more closely associated with a programmed gene expression (Infante & Cahu, 2001). Benthophage fish (Bream, Carp and Roach) that eat chironomid larvae have been found to have higher amylase activity than other fish species (Pike, Burbot, Perch) by Kuz'mina et al. (2003), and omnivorous fish have higher amylase activity levels than carnivorous fish (Hidaalgo et al., 1999). Omnivore fish feed on a wide range of plant and animal matter compared to other fish species. Certain studies have found a link between fish growth and trypsin, chymotrypsin and alkaline phosphatase activity levels (Lemieux et al., 1999: Rungruangsak-Torrissen et al., 2006). Trypsin activity did not vary in sticklebacks exposed to 21 °C, it changed when the temperature was decreased (Hani et al., 2018) and the difference in mean weights between sticklebacks in the 21 °C group and the other groups could be explained by low trypsin activity levels. In our experiment, trypsin activity was observed highest for the fry stage and lowest for tablesize. The elevation of trypsin activity for fry can be due to greater food intake or higher metabolic rates coinciding with the ambient temperatures. In reality, one of the strategies for balancing increasing energy demand is increased feed consumption. The activity of digestive enzymes is usually more due to this increased feed consumption (Karasov & Douglas, 2013). High temperatures have been shown to boost the metabolic rate of living organisms (Wootton, 1984). The optimal energy allocation is hampered in this condition and the energy balance is shifted to maintenance rather than expansion (Klepsatel et al., 2016). Reduced growth could result from an active metabolism after eating a certain amount of food (Imsland & Jonsdottir, 2002). A water temperature of 21 °C corresponds to the IPCC's (2013) forecast summer temperature in a few decades and this can have a significant impact on digestive enzyme profile of fishes.

Clarification on some aspects of nutritive physiology can help to solve nutritional problems in fish feeding. Assessing the development of the digestive system helps in the precise understanding of feed and dietary requirements at different developmental stages. The study confirms the stages at which suitable food could be given depending on the enzyme activities that lead to better survival and precisely what food the fish is able to digest and absorb exogenous nutrients. It provides better feeding and rearing practices for trout in raceways. Further, the knowledge gained under the experiment is useful for making up a baseline for the healthy trout gut, which helps to understand the changes to the digestive system at different life cycle stages of trout, leading to the development of an informed aquaculture program and the management of trout stocks.

Acknowledgement

Authors are highly thankful to the division of FRM, Faculty of Fisheries, SKUAST-K for providing support while conducting this experiment.

References

Akintunde, E.A. (1985) Digestive enzymes in the gut of *Sarotherodon galilaeus* (Syn. *Tilapia galilea*, Family Cichlidae) of Lake Kainji, Nigeria. Niger. J. Sci. 18: 22-25

Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P. and Rungruangsak-Torrissen, K. (2004) Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus*, Simpson 1900. Aquac. 234(1-4): 575-587

Cara, J.B., Moyano, F.J., Cardenas, S., Fernandez-Diaz, C. and Yuffera, M. (2003) Assessment of digestive enzyme activities during larval development of white bream. J. Fish Biol. 63: 48-58

Day, R.D., German, D.P. and Tibbetts, I.R. (2011) Why can't young fish eat plants? Neither digestive enzymes nor gut development preclude herbivory in the young of a stomach less marine herbivorous fish. Comp. Biochem. Physiol. B. Biochem. 158(1): 23-29

Erlanger, B.F., Kokowsky, N. and Cohen, W. (1961) The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95(2): 271-278

Froese, R. and Pauly, D. (2008) Fishbase (version 07/2008) http://www.fishbase.org (Accessed on 19 June 2023)

Hani, Y.M.I., Marchand, A., Turies, C., Kerambrun, E., Palluel, O., Bado-Nilles, A., Beaudouin, R., Porcher, J.M., Geffard, A. and Dedourge-Geffard, O. (2018) Digestive enzymes and gut morphometric parameters of threespine stickleback (*Gasterosteus aculeatus*): Influence of body size and temperature. PLoS One. 13(4): e0194932

Hidalgo, M.C., Urea, E. and Sanz, A. (1999) Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic andamylase activities. Aquac. 170: 267-283

Hoitsy, G. (2002) A Pisztráng tenyésztése és horgászata. 152 oldal

Imsland, A.K. and Jonsdottir, O.D.B. (2002) Is there a genetic basis to growth in Atlantic cod? Fish Fish. 3(1): 36-52

Infante, J.Z. and Cahu, C.L. (2001) Ontogeny of the gastrointestinal tract of marine fish larvae. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 130(4): 477-487

IPCC. (2013) Climate change. The Physical Science Basis: The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), 1535 p, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Karasov, W.H. and Douglas, A.E. (2013) Comparative Digestive Physiology. Compr. Physiol. 3(2): 741

Klepsatel, P., Galikova, M., Xu, Y. and Kuhnlein, R.P. (2016) Thermal stress depletes energy reserves in Drosophila. Sci. Rep. 6(1): 33667

Kuz'mina, V., Glatman, L., Drabkin, V. and Gelman, A. (2003) Amylolytic activity in fish intestinal mucosa: temperature effects. Comp. Biochem. Physiol. B. Biochem. 134(3): 529-534

Kuz'mina, V.V. and Gelman, A.G. (1998) Traits in the development of the digestive function in fish. J. Ichthyol. 39(1): 106-115

Lemieux, H., Blier, P. and Dutil, J.D. (1999) Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadusmorhua*)? Fish Physiol. Biochem. 20(4): 293-303

Lojda, Z. Gossrau, R. and Schiebler, T.H. (1979) Enzyme Histochemistry: A Laboratory Manual, 339 p, Springer-Verlag, New York

Montgomery, W.L. and Bernstein, Y. (2008) Rainbow trout (*Oncorhynchus mykiss*): a technical conservation assessment. Rocky Mountain Region, Species Conservation Project. USDA Forest Service. 47: 76-92

Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg, A. and Waagbo, R. (2006) Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 32(1): 7-23

Tengjaroenkul, B., Smith, B.J., Caceci, T. and Smith, S.A. (2000) Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. Aquac.182: 317-327

Tramati, C., Savona, B. and Mazzola, A. (2005) A study of the pattern of digestive enzymes in *Diplodus puntazzo* (Cetti 1777) (Osteichthyes, Sparidae): evidence for the definition of nutritional protocols. Aquac. Int. 13: 89-95

Uys, W. and Hecht, T. (1987) Assays on the digestive enzymes of sharp tooth catfish *Clarias gariepinus* (Pisces: Clariidae). Aquac. 63: 301-313

Wootton, R.J. (1994) Energy allocation in the threespine stickleback. In: The Evolutionary Biology of the Threespine Stickleback (Bell, M.A. and Foster, S.A., Eds), pp 114-143, Oxford University Press, New York

Wootton, R.J. (1984) A functional biology of sticklebacks: Croom Helm, London. Mitochondrial DNA B: Resour. 69

Zamani, A., Hajimoradloo, A., Madani, R. and Farhangi, M. (2009) Assessment of digestive enzymes activity during the fry development of the endangered Caspian brown trout *Salmo caspius*. J. Fish Biol. 75(4): 932-937