Journal of Agricultural Science and Technology (JAST), 29(3)
In Press, Pre-Proof Version

The dynamic changes in the activity of digestive and antioxidant enzymes of juvenile Asian seabass (*Lates calcarifer*) during a short-term starvation period

Hamzeh Salarvand[1], Amin Oujifard[1, 2*], Vahid Morshedi[2], Ahmad Ghasemi[2], Mentor Sopjani[3*], and Ghasem Rashidian[4]

Abstract

This study investigated the physiological responses of juvenile Asian seabass (*Lates calcarifer*) to short-term feed deprivation, focusing on changes in digestive and antioxidant enzyme activities. A total of 150 fish (mean initial weight 5.80 ± 0.06 g) were divided into six 300-L circular fiberglass tanks with two groups including a control group (fed) and a starvation group (feed-deprived) for 8 days. Sampling was performed on days 2, 4, 6, and 8 to evaluate the activities of digestive enzymes (amylase, lipase, protease, and alkaline phosphatase) and antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione reductase [GR]) along with malondialdehyde [MDA] content in the liver. The obtained results showed the amylase activity in the starvation group showed a significant decrease on day 8 compared to the control group ($p < 0.05$). Moreover, alkaline phosphatase activity was reduced on day 4 in the starved group compared to the control group ($p < 0.05$). Lipase and protease activities were not significantly affected by starvation ($p > 0.05$). Among antioxidant parameters, GR in the starvation group showed significant decrease on day 6 compared to the control group ($p < 0.05$). However, SOD and CAT activities in the starved group increased significantly on days 6 and 4, respectively compared to the control group ($p < 0.05$). MDA levels remained consistently lower in starved fish compared to the control fish throughout the experiment ($p < 0.05$). These findings indicate that short-term starvation induces selective changes in digestive and antioxidant parameters in juvenile Asian seabass, with decreased activities of amylase and alkaline phosphatase and differential modulation of antioxidant responses.

[1] Department of Fisheries Sciences, Faculty of Marine Science and Technology, Persian Gulf University, Bushehr, Islamic Republic of Iran.
[2] Department of Fisheries and Biology, Persian Gulf Research Institute, Persian Gulf University, Bushehr, Islamic Republic of Iran.
[3] Faculty of Medicine, University of Prishtina, 10 000 Prishtina, Kosova.
[4] Institute of Aquaculture and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czechia.
*Corresponding authors; e-mails: oujifard@pgu.ac.ir and mentor.sopjani@uni-pr.edu

Keywords: Asian seabass, Compensatory growth, Enzymatic changes, Feed deprivation, Physiological responses.

1. Introduction

Aquatic animals often experience fluctuations in food availability due to migration, reproduction, environmental changes, or seasonal scarcity (Duchenne-Moutien & Neetoo, 2021; Visser et al., 2020). Certain fish species demonstrate significant susceptibility to food shortage, resulting in diminished energy reserves, individual fitness, body mass, swimming activity, and metabolic capacity (Pounds et al., 2022). Nutrient availability and feeding frequency play crucial roles in regulating growth, metabolism, and overall physiological performance. Feed restriction may occur naturally or under aquaculture conditions, leading to disturbances in metabolic homeostasis and physiological stress. Such challenges may result from environmental fluctuations (e.g., temperature shifts, reduced dissolved oxygen, or ammonia accumulation), disease, or management practices such as transportation and handling (Navarro & Gutiérrez, 1995; McCue, 2010). Both short- and long-term starvation have been shown to reduce growth rate, energy reserves, and metabolic efficiency, ultimately impairing fish health and survival (Inness & Metcalfe, 2008; Duchenne-Moutien & Neetoo, 2021). Understanding the physiological and biochemical responses of fish during feed deprivation is therefore critical for improving feeding strategies and optimizing aquaculture management (Pounds et al., 2022; Zhao et al., 2022).

Under feed-deprivation stress, fish reallocate internal energy stores to sustain homeostasis, potentially altering digestion, nutrient absorption, and energy metabolism (Gou et al., 2023; Zhao et al., 2022; Tchonkouang et al., 2024). Digestive enzyme activity is particularly sensitive to nutritional status, showing species-specific regulation during fasting (Volkoff & Rønnestad, 2020). For instance, starvation reduces intestinal protease and lipase in juvenile blunt-snout bream (*Megalobrama amblycephala*) (Sun et al., 2020) and alters gut function in loach (*Paramisgurnus dabryanus*) (Peter et al., 2020). However, information remains limited on how digestive and antioxidant responses interact under starvation in juvenile Asian seabass (*Lates calcarifer*).

Starvation can also disrupt oxidative balance by promoting reactive oxygen species (ROS) generation and challenging the antioxidant defense system. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) play essential roles in

maintaining redox homeostasis by detoxifying ROS and preventing cellular damage (Dar et al., 2019; Wu et al., 2024). Enhanced SOD and CAT activities have been observed during feed deprivation in *Paramisgurnus dabryanus* (Peter et al., 2020), *Labeo rohita* (Dar et al., 2019), and C*yprinus carpio* (Zhao et al., 2022), while other studies reported species-dependent decreases (Pan et al., 2022; Florescu Gune et al., 2021). Nevertheless, the interplay between changes in digestive and antioxidant enzyme activities during starvation remains poorly understood, particularly in juvenile Asian seabass.

The Asian seabass, also known as barramundi, is a euryhaline carnivorous fish of high economic importance throughout the Indo-Pacific region. It is widely cultured because of its rapid growth, tolerance to a broad range of salinities, and strong market demand (Tian & Qin, 2004). Starvation and refeeding protocols are commonly applied in its management to enhance feed efficiency and induce compensatory growth (Tian & Qin, 2004). However, despite its commercial importance, information regarding how short-term feed deprivation affects its digestive enzyme dynamics and antioxidant status remains limited.

Given the increasing application of short-term fasting in aquaculture—both as an adaptive mechanism during environmental stress and as a strategy to induce compensatory growth upon refeeding—it is essential to characterize the enzymatic responses of Asian seabass under such conditions. The present study was therefore designed to investigate the dynamic changes in digestive and antioxidant enzyme activities in juvenile Asian seabass during an eight-day starvation period. The selected duration reflects a short-term fasting phase commonly encountered under commercial farming conditions, allowing the identification of early physiological adjustments before severe metabolic impairment. The findings aim to provide new insights into the enzymatic adaptation of Asian seabass to feed deprivation and support the development of more effective feeding management strategies in aquaculture.

2. Materials and methods

2.1. Husbandry and experimental design

A total of 150 juvenile Asian seabass (3.50±0.06 g, mean ± standard deviation) was transported from the marine fish cultivation and propagation commercial center (Delvar, Iran) to the research station of the Persian Gulf Research and Study Centre (Bushehr, Iran). The fish were maintained in 300-L fiberglass tanks for two weeks for acclimation to experimental conditions, which were

similar with the condition below for the treatments. During this period, fish were fed twice daily at an apparent satiation level with a commercial diet (Faradaneh, Shahrekord, Iran). After this period, fish with an initial average weight of 5.80±0.06 g was distributed randomly into six 300-L circular fiberglass tanks (25 fish in each replication). The control group (C) was fed at satiation level twice daily (0800 h and 1600 h) with a commercially formulated feed, designed for Asian seabass (manufactured by Faradaneh, Shahrekord, Iran,) with 1.5 mm diameter containing 40% crude protein, 16% crude lipid, 2.5% crude fiber, and 9% ash. The starvation group (S) was starved for 8 days. Each tank was supplied with sand-filtered, chlorinated and UV disinfected seawater. Each treatment was applied in three tanks. Approximately 50-80% of water in the holding tanks was exchanged daily. During the experiment, water temperature (27±1 °C), oxygen level (90–100% saturation), pH (8.2), and salinity (42.5±0.2 ppt) were monitored. Fish were kept under a 12L:12D photoperiod using artificial lights.

2.2. Sampling

All the experimental procedures including euthanasia were based on the guidelines provided by International and Internal Communities on the protection of animals used for scientific purpose. Care was taken to use only the minimum possible number of specimens for analytical work and fish were euthanized with an overdose of anesthetic (Mocho et al., 2025). Prior to the start of the experiment, 9 fish were randomly taken and euthanized with an overdose of anesthetic (2-phenoxyethanol) (3 fish were pooled and analyzed in each replication). During the experiment, sampling was conducted on 2, 4, 6, and 8 days. At each point, 6 fish were randomly selected from each treatment (2 fish from each replicate were pooled and analyzed in triplicate), euthanized as explained above, instantly gutted on ice, and the dissected gut and the liver tissues were transferred to separate tubes and kept at −80 °C until further analysis.

2.3. Digestive enzymes assay

For measuring digestive enzyme activities, the gut samples were homogenized in 30 volumes of cold buffer (50 mM mannitol, 2 mM Tris-HCl buffer, pH 7.0) according to Gisbert et al. (2016). After homogenizing the gut, samples were centrifuged (3300 *g*, 3 min at 4°C), and the supernatant was kept at −80 °C for further analyses. The alkaline phosphatase activity was measured according to the method described by Gisbert et al. (2018). The activities of α-amylase

and bile-salt-dependent lipase were measured according to Worthington (1991) and Iijima et al. (1998), respectively. Alkaline phosphatase was quantified at 37 °C using 4-nitrophenyl phosphate (PNPP) as substrate in 30 mM Na_2CO_3 buffer (pH 9.8). One unit (U) was defined as 1 µg N-benzoyl-DL-tyrosine ethyl acetate ester (BTEE) released per min per ml of brush border homogenate at 407 nm (Bessey et al., 1946). The soluble protein of crude enzyme extracts was quantified using the Bradford (1976) method, using bovine serum albumin as a standard.

2.4. Liver antioxidant assay

The samples were washed in ice-cold 0.9% NaCl and were homogenized in 10 volumes (w/v) of ice-cold buffer (100 mM Tris–HCl, 0.1 mM EDTA, 0.1% (v/v) Triton X-100, pH 7.8), then centrifuged (11312 *g*, 30 min at 4 °C), and the supernatant was extracted. The standard methods were used in triplicate for the assessment of selected antioxidant enzymes, including catalase (Góth, 1991), superoxide dismutase (Kono, 1978), and determination of lipid peroxidation in the liver was examined by means of the production of MDA content, Ringwood et al., (2003), and soluble protein of the homogenates was measured by the Bradford (1976) method. Glutathione reductase activity was determined by measuring the oxidation of NADPH at 340 nm (ε = 6.22 m/M/cm), using 20 mM glutathione disulfide and 2 mM NADPH as substrates (Carlberg and Mannervik, 1975).

2.5. Statistics

Statistical analyses were performed using SPSS software, version 22.0 for Windows. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test, and the homogeneity of variances was evaluated using Levene's F test. Throughout the experiment and at designated sampling time points, potential differences were analyzed using Two-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences between treatment and control groups were assessed using independent sample *t*-tests. Data are presented as mean ± standard error (SE), and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Digestive enzymes

As shown in Table 1, the amylase activity in the starvation treatment group showed a significant decrease on day 8 compared to the control group ($p < 0.05$). Additionally, a significant difference

in amylase activity was observed across the sampling days ($p < 0.05$). Furthermore, a significant interaction between treatment and sampling day was detected for this enzyme ($p < 0.05$). The activity of protease did not significantly differ between the starvation group and the control group on any of the sampling days (days 2, 4, 6, and 8) ($p > 0.05$). Similarly, no significant differences were observed across the sampling days and the interaction effect between treatment and sampling day ($p > 0.05$). Lipase activity did not show any significant reduction in the starvation treatment group compared to the control group on any of the sampling days ($p > 0.05$). Additionally, there were no significant differences in lipase activity across the sampling days and the interaction effect between treatment and sampling day ($p > 0.05$). The alkaline phosphatase activity significantly decreased in the starvation treatment group on day 4 compared to the control group ($p < 0.05$). Significant differences in alkaline phosphatase activity were also observed across the sampling days ($p < 0.05$). However, the interaction effect between treatment and sampling day was not significant ($p > 0.05$).

Table 1. Digestive enzyme activities in Asian seabass (*Lates calcarifer*) juveniles at different sampling days during starvation.

Parameters	Sampling Day	Day 2	Day 4	Day 6	Day 8
Amylase **(U/mg protein)**	Control	11.69 ± 0.60	11.43 ± 0.87	12.89 ± 0.13	15.63 ± 0.45*
	Starved	12.67 ± 0.25	11.33 ± 0.45	12.16 ± 0.45	13.29 ± 0.01
	Sampling Day	0.00	0.00	0.00	0.00
	Treatment×Sampling Day	0.02	0.02	0.02	0.02
Protease **(U/mg protein)**	Control	0.90 ± 0.03	0.96 ± 0.001	0.92 ± 0.05	1.03 ± 0.12
	Starved	0.90 ± 0.04	0.86 ± 0.05	1.09 ± 0.40	1.11 ± 0.09
	Sampling Day	ns	ns	Ns	Ns
	Treatment×Sampling Day	ns	ns	Ns	Ns
Lipase **(U/mg protein)**	Control	1.24 ± 0.005	1.37 ± 0.07	1.32 ± 0.011	1.32 ± 0.003
	Starved	1.24 ± 0.02	1.15 ± 0.04	1.19 ± 0.07	0.21 ± 0.04
	Sampling Day	ns	ns	Ns	Ns
	Treatment×Sampling Day	ns	ns	Ns	Ns
Alkaline Phosphatase **(U/mg protein)**	Control	208.53 ± 4.12	206.7 ± 8.86*	166.0 ± 7.13	139.5 ± 8.16
	Starved	200.2 ± 13.76	158.8 ± 9.67	129.0 ± 13.13	126.4 ± 15.53
	Sampling Day	0.00	0.00	0.00	0.00
	Treatment×Sampling Day	ns	ns	Ns	Ns

Values are presented as mean ± standard error (n = 3). Asterisks in each column (*) indicate significant differences between the starved and control groups on the same sampling day ($p < 0.05$, independent-sample t-test). The p-value amount or absence of superscript letters in rows denotes -/no statistically significant differences within the same parameter ($p > 0.05$, two-way ANOVA). "ns" = not significant, $p < 0.05$= significant.

3.2. Antioxidant enzymes

As shown in Table 2. The activity of GR in the starvation treatment group showed a statistically significant decrease on day 6 compared to the control group ($p < 0.05$). A significant difference was also observed across sampling days for this enzyme ($p < 0.05$). However, the interaction between treatment and sampling day was not statistically significant ($p > 0.05$). The activity of SOD in the starvation group exhibited a significant increase on day 6 compared to the control ($p < 0.05$). Furthermore, significant differences were observed across the sampling days ($p < 0.05$), while the interaction between treatment and sampling day was not significant ($p > 0.05$). For MDA, a significant decrease in level was recorded in the starvation treatment on all sampling days (days 2, 4, 6, and 8) compared to the control ($p < 0.05$). The sampling days also significantly affected MDA levels ($p < 0.05$). However, the interaction between treatment and sampling day was not statistically significant ($p > 0.05$). In the case of CAT, the enzyme activity significantly increased in the starvation group on day 4 compared to the control ($p < 0.05$). No significant differences were observed across sampling days ($p > 0.05$), and the treatment × sampling day interaction was also not significant ($p > 0.05$).

Table 2. Activities of antioxidant enzymes in Asian seabass *(Lates calcarifer)* juveniles at different sampling days after starvation.

Parameters	Sampling Day	Day 2	Day 4	Day 6	Day 8
GR **(U/mg protein)**	Control	2.31 ± 0.03	2.17 ± 0.05	2.09 ± 0.02*	1.93 ± 0.12
	Starved	2.15 ± 0.20	1.94 ± 0.09	1.93 ± 0.03	1.69 ± 0.06
	Sampling Day	0.00	0.00	0.00	0.00
	Treatment×Sampling Day	ns	Ns	Ns	ns
SOD **(U/mg protein)**	Control	8.73 ± 0.95	8.83 ± 1.04	13.51 ± 0.30	16.45 ± 0.71
	Starved	9.55 ± 0.84	9.30 ± 0.92	15.90 ± 0.31*	19.06 ± 1.31
	Sampling Day	0.00	0.00	0.00	0.00
	Treatment×Sampling Day	ns	Ns	Ns	ns
MDA **(nmol/mg protein)**	Control	24.41 ± 1.13*	32.03 ± 1.85*	36.56 ± 2.29*	33.12 ± 2.18*
	Starved	13.82 ± 0.19	19.59 ± 2.69	25.09 ± 3.30	21.38 ± 3.30
	Sampling Day	0.00	0.00	0.00	0.00
	Treatment×Sampling Day	ns	Ns	Ns	ns
CAT **(U/mg protein)**	Control	4.71 ± 0.02	4.63 ± 0.06	4.67 ± 0.16	4.75 ± 0.08
	Starved	5.06 ± 0.17	5.13 ± 0.04*	5.05 ± 0.11	4.90 ± 0.04
	Sampling Day	ns	Ns	Ns	ns
	Treatment×Sampling Day	ns	Ns	Ns	ns

Values are presented as mean ± standard error (n = 3). Asterisks in each column (*) indicate significant differences between the starved and control groups on the same sampling day ($p < 0.05$, independent-sample T-test). The p-value amount or absence of superscript letters in rows denotes -/no statistically significant differences within the same parameter ($p > 0.05$, two-way ANOVA). "ns" = not significant, p< 0.05= significant.

4. Discussion

The present study investigated the effects of short-term starvation on the activities of digestive and antioxidant enzymes in juvenile *L. calcarifer*. The findings revealed clear but selective enzymatic responses to an eight-day feed deprivation, indicating differential responses of digestive and antioxidant enzymes during short-term feed deprivation

The decline in amylase and alkaline phosphatase activities observed after 8 and 4 days of starvation, respectively, indicates a downregulation of carbohydrate digestion and nutrient absorption pathways. Such reduction is commonly attributed to the absence of mechanical and chemical stimuli arising from the presence of feed in the gut, leading to decreased synthesis or secretion of digestive enzymes (Peter et al., 2020; Gou et al., 2023). These findings are consistent with previous reports in *Megalobrama amblycephala* and *Paramisgurnus dabryanus*, where starvation reduced intestinal enzyme activities and altered gut morphology (Sun et al., 2020; Peter et al., 2020). Conversely, the unchanged protease and lipase activities suggest that protein and lipid catabolic capacities were maintained, possibly to support metabolic demands through endogenous substrate mobilization. This selective response may reflect an energy-saving strategy, prioritizing essential proteolytic and lipolytic processes necessary for tissue maintenance and gluconeogenic supply (Volkoff and Rønnestad, 2020).

These differential changes in digestive enzyme activities indicate that short-term food deprivation selectively modulates digestive processes in Asian seabass, rather than causing a generalized suppression of digestive capacity. Similar changes in digestive enzyme activities have been reported in atypical cavefish (*Onychostoma macrolepis*) and Adriatic sturgeon (*Acipenser naccarii*) during starvation, suggesting that moderate fasting can induce selective rather than complete suppression of digestive activity (Furné et al., 2008; Gou et al., 2023).

Short-term starvation also elicited distinct changes in antioxidant enzyme activities, highlighting the oxidative adjustments required to maintain redox balance during nutrient deprivation. The increased activities of SOD and CAT in the starved fish suggest an activation of the enzymatic antioxidant defense, possibly in response to elevated ROS resulting from enhanced lipid and protein oxidation for energy supply (Dar et al., 2019; Pan et al., 2022). The parallel rise of these enzymes indicates a coordinated response, where SOD converts superoxide radicals into hydrogen peroxide, and CAT detoxifies hydrogen peroxide into water and oxygen, thereby preventing oxidative damage (Morales et al., 2004).

Conversely, the reduction in GR activity may indicate altered glutathione recycling capacity; however, this interpretation remains tentative because glutathione concentrations and NADPH availability were not measured. This reduction may reflect limited NADPH generation via the pentose phosphate pathway due to suppressed carbohydrate metabolism (Zengin, 2021). A similar trend has been reported in starved rainbow trout *Oncorhynchus mykiss* (Zhao et al., 2022) and European seabass *Dicentrarchus labrax* (Sinha et al., 2015), supporting the idea that prolonged fasting may compromise glutathione-dependent antioxidant capacity despite transient activation of SOD and CAT.

MDA levels—a marker of lipid peroxidation—decreased significantly in starved fish. Although the results may seem counterintuitive, reduced MDA levels can result from lower metabolic activity and suppressed lipid mobilization rates, which limit ROS generation (Gou et al., 2023). Additionally, the early activation of enzymatic antioxidants could have efficiently prevented lipid peroxidation. Thus, Asian seabass appears capable of maintaining oxidative stability during short-term fasting through a fine-tuned antioxidant response.

Taken together, the results suggest that Asian seabass exhibits dual adaptive mechanisms under short-term starvation: (1) downregulation of specific digestive enzymes to conserve energy and minimize unnecessary protein synthesis, and (2) upregulation of major antioxidant enzymes to protect against ROS-induced damage. The observed stability of lipase and protease activities implies that lipid and protein reserves remained accessible for metabolic compensation, whereas the modulation of SOD, CAT, and GR reflects dynamic control over oxidative metabolism.

These enzymatic adjustments likely facilitate rapid recovery upon refeeding, a phenomenon known as compensatory growth (Tian and Qin, 2004). The species' ability to maintain metabolic equilibrium during nutrient deprivation aligns with its ecological plasticity and aquaculture potential, where temporary feed withdrawal is often unavoidable. Although the current experiment did not include a refeeding phase, the present findings provide a valuable baseline for understanding the early-stage physiological responses preceding compensatory growth. Future studies incorporating refeeding protocols could elucidate the full cycle of enzymatic recovery and growth acceleration in *L. calcarifer*.

The study's short duration (8 days) and absence of molecular or gene expression data restrict deeper mechanistic interpretation of enzymatic regulation. Additionally, only enzymatic activities were assessed, without concurrent measurements of energy reserves or hormonal

mediators (e.g., cortisol, insulin-like growth factors), which could further explain metabolic adjustments. Future research should integrate transcriptomic and metabolomic analyses to link enzymatic activities with gene regulation, energy metabolism, and oxidative biomarkers during starvation–refeeding cycles.

5. Conclusions

The present study demonstrates that short-term feed deprivation induces certain distinct biochemical adjustments in juvenile *Lates calcarifer*. Amylase and alkaline phosphatase activity significantly decreased, suggesting that carbohydrate digestion and intestinal brush-border function are more sensitive to early starvation than lipid or protease activities, which stayed the same. On the other hand, SOD and CAT activities increased during mid-starvation, whereas GR activity and hepatic MDA levels decreased, reflecting an effective modulation of oxidative metabolism and reduced lipid peroxidation. Thus, these coordinated digestive and antioxidant responses indicate that juvenile Asian seabass can maintain metabolic and oxidative homeostasis during limited food deprivation, minimizing oxidative damage while conserving digestive capacity. Such physiological flexibility may contribute to the species' resilience in fluctuating aquaculture or natural environments.

Acknowledgments

The authors would like to thank Persian Gulf University and the staff at Mariculture Research Station of the Persian Gulf Research Institute (PGRI) for providing juvenile fish and the rearing facilities for this experiment.

References

1. Bessey, O. A., Lowry, O. H., Brock, M. J. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem 164, 321–329. https://doi.org/10.1016/S0021-9258(18)43072-4

2. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248–54. https://doi.org/10.1016/0003-2697(76)90527-3

3. Carlberg, I., Mannervik, B., 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 250, 5475–80. https://doi.org/10.1016/S0021-9258(19)41206-4

4. Dar, S.A., Srivastava, P.P., Varghese, T., Nazir, M.I., Gupta, S., Krishna, G., 2019. Temporal changes in superoxide dismutase, catalase, and heat shock protein 70 gene expression, cortisol and antioxidant enzymes activity of *Labeo rohita* fingerlings subjected to starvation and refeeding. Gene 692, 94–101. https://doi.org/10.1016/J.GENE.2018.12.058

5. Duchenne-Moutien, R.A., Neetoo, H., 2021. Climate Change and Emerging Food Safety Issues: A Review. J Food Prot 84, 1884–1897. https://doi.org/10.4315/JFP-21-141

6. Florescu Gune, I.E., Georgescu, S.E., Dudu, A., Balaș, M., Voicu, S., Grecu, I., Dediu, L., Dinischiotu, A., Costache, M., 2021. Oxidative stress and antioxidant defense mechanisms in response to starvation and refeeding in the intestine of stellate sturgeon (*Acipenser stellatus*) juveniles from aquaculture. Animals (Basel) 11(1), 76. https://doi.org/10.3390/ani11010076

7. Furné, M., García-Gallego, M., Hidalgo, M.C., Morales, A.E., Domezain, A., Domezain, J., Sanz, A., 2008. Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccarii*) and trout (*Oncorhynchus mykiss*). Comp Biochem Physiol A Mol Integr Physiol 149, 420–425. https://doi.org/10.1016/J.CBPA.2008.02.002

8. Gisbert, E., Mozanzadeh, M.T., Kotzamanis, Y., Estévez, A., 2016. Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution. Aquaculture 462, 92–100. https://doi.org/10.1016/J.AQUACULTURE.2016.04.035

9. Gisbert, E., Nolasco, H., Solovyev, M., 2018. Towards the standardization of brush border purification and intestinal alkaline phosphatase quantification in fish with notes on other digestive enzymes. Aquaculture 487, 102–108. https://doi.org/10.1016/J.AQUACULTURE.2018.01.004

10. Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta 196, 143–151. https://doi.org/10.1016/0009-8981(91)90067-M

11. Gou, N., Wang, K., Jin, T., Yang, B., 2023. Effects of starvation and refeeding on growth, digestion, nonspecific immunity and lipid-metabolism-related genes in *Onychostoma macrolepis*. Animals (Basel) 13(7), 1168. https://doi.org/10.3390/ani13071168Hossen, S., Hanif, M.A., Kho,

K.H., 2023. Glutathione reductase, a biomarker of pollutant and stress in Pacific abalone. Mar Pollut Bull 192, 115139. https://doi.org/10.1016/J.MARPOLBUL.2023.115139

12. Iijima, N., Tanaka, S., Ota, Y., 1998. Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. Fish Physiol Biochem 18, 59–69. https://doi.org/10.1023/A:1007725513389

13. Inness, C.L.W., Metcalfe, N.B., 2008. The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. Proc Biol Sci 275(1644), 1703. https://doi.org/10.1098/rspb.2008.0357

14. Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 186, 189–195. https://doi.org/10.1016/0003-9861(78)90479-4

15. McCue, MD. 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comp Biochem Physiol A 156, 1-18. https://doi.org/10.1016/j.cbpa.2010.01.002

16. Mocho, J.P., Blasco, J.R., Lundegaard, P.R., McKimm, R., Jenčič, V., Krogh, K.V., 2025. Methods of humane killing of laboratory fish: FELASA Working Group recommendations. Lab Anim, 59(5), 599-613. https://doi.org/ 10.1177/00236772251351095

17. Morales, A.E., Pérez-Jiménez, A., Carmen Hidalgo, M., Abellán, E., Cardenete, G., 2004. Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. Comp Biochem Physiol C Toxicol Pharmacol 139, 153–161. https://doi.org/10.1016/J.CCA.2004.10.008

18. Navarro I, Gutiérrez J. 1995. Fasting and starvation. In: Hochachka, PW, Mommsen TP (Eds.), Biochemistry and Molecular Biology of Fishes, vol. 4. Elsevier, New York, pp. 393-433.

19. Pan, Y., Tao, J., Zhou, J., Cheng, J., Chen, Y., Xiang, J., Bao, L., Zhu, X., Zhang, J., Chu, W., 2022. Effect of starvation on the antioxidative pathway, autophagy, and mitochondrial function in the intestine of Chinese perch *Siniperca chuatsi*. Aquaculture 548, 737683. https://doi.org/10.1016/J.AQUACULTURE.2021.737683

20. Pascual, P., Pedrajas, J.R., Toribio, F., López-Barea, J., Peinado, J., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). Chem Biol Interact 145, 191–199. https://doi.org/10.1016/S0009-2797(03)00002-4

352 **21.** Peter, M., Lv, H., Jiang, X., Liu, Y., Hur, J. wook, Esbon, M., Samwel, C., Superius, E.,
353 Khalfan, Z., Gao, Y., Chu, Z., 2020. Effects of starvation on enzyme activities and intestinal
354 microflora composition in loach (*Paramisgurnus dabryanus*). Aquac Rep 18, 100467.
355 https://doi.org/10.1016/J.AQREP.2020.100467
356 **22.** Pounds, A., Kaminski, A.M., Budhathoki, M., Gudbrandsen, O., Kok, B., Horn, S.,
357 Malcorps, W., Mamun, A.-A., McGoohan, A., Newton, R., Ozretich, R., Little, D.C., 2022. More
358 than fish—framing aquatic animals within sustainable food systems. Foods 11(10), 1413
359 https://doi.org/10.3390/foods11101413
360 **23.** Ringwood, A. H., Hoguet, J., Keppler, C. J., Gielazyn, M. L., Ward, B. P., & Rourk, A. R.
361 (2003). Cellular biomarkers (lysosomal destabilization, glutathione & lipid peroxidation) in three
362 common estuarine species: a methods handbook. Marine Resources Research Institute, South
363 Carolina Department of Natural Resources, Charleston.
364 **24.** Shao, T., Hsu, R., Rafizadeh, D.L., Wang, L., Bowlus, C.L., Kumar, N., Mishra, J.,
365 Timilsina, S., Ridgway, W.M., Gershwin, M.E., Ansari, A.A., Shuai, Z., Leung, P.S.C., 2023.
366 The gut ecosystem and immune tolerance. J Autoimmun 141, 103114.
367 https://doi.org/10.1016/J.JAUT.2023.103114
368 **25.** Sinha, A.K., AbdElgawad, H., Zinta, G., Dasan, A.F., Rasoloniriana, R., Asard, H., Blust, R.,
369 De Boeck, G., 2015. Nutritional Status as the Key Modulator of Antioxidant Responses Induced
370 by High Environmental Ammonia and Salinity Stress in European Sea Bass (*Dicentrarchus*
371 *labrax*). PLoS One 10, e0135091. https://doi.org/10.1371/journal.pone.0135091
372 **26.** Sun, S., Su, Y., Yu, H., Ge, X., Zhang, C., 2020. Starvation affects the intestinal microbiota
373 structure and the expression of inflammatory-related genes of the juvenile blunt snout bream,
374 *Megalobrama amblycephala*. Aquaculture 517, 734764.
375 https://doi.org/10.1016/J.AQUACULTURE.2019.734764
376 **27.** Tchonkouang, R.D., Onyeaka, H., Nkoutchou, H., 2024. Assessing the vulnerability of food
377 supply chains to climate change-induced disruptions. Sci Total Environ 920, 171047.
378 https://doi.org/10.1016/j.scitotenv.2024.171047
379 **28.** Tian, X., Qin, J.G., 2004. Effects of previous ration restriction on compensatory growth in
380 barramundi *Lates calcarifer*. Aquaculture 235 (1-4), 273-283.
381 https://doi.org/10.1016/j.aquaculture.2003.09.055.

29. Visser, A.W., Brun, P., Chakraborty, S., Dencker, T.S., van Denderen, P.D., van Gemert, R., van Someren Gréve, H., Heilmann, I., Holm, M.W., Jónasdóttir, S.H. and Kenitz, K.M., 2020. Seasonal strategies in the world's oceans. Prog Oceanogr 189, 102466. https://doi.org/10.1016/j.pocean.2020.102466

30. Volkoff, H., Rønnestad, I., 2020. Effects of temperature on feeding and digestive processes in fish. Temperature (Austin) 7, 307–320. https://doi.org/10.1080/23328940.2020.1765950

31. Worthington, C. C., 1991. Worthington enzyme manual related biochemical. Freehold, New Jersey, USA, 34, 212-215.

32. Wu, Z., Wu, W., Yang, S., Cheng, F., Lv, J., Shao, Y., Tang, X., Li, E., Zhao, Q., 2024. Safety evaluation and effects of dietary phlorotannins on the growth, health, and intestinal microbiota of *Litopenaeus vannamei*. Fish Shellfish Immunol 150, 109569. https://doi.org/10.1016/J.FSI.2024.109569

33. Zeng, L.Q., Li, F.J., Li, X.M., Cao, Z.D., Fu, S.J., Zhang, Y.G., 2012. The effects of starvation on digestive tract function and structure in juvenile southern catfish (*Silurus meridionalis* Chen). Comp Biochem Physiol A Mol Integr Physiol 162, 200–211. https://doi.org/10.1016/J.CBPA.2012.02.022

34. Zengin, H., 2021. The effects of feeding and starvation on antioxidant defence, fatty acid composition and lipid peroxidation in reared *Oncorhynchus mykiss* fry. Sci Rep 11, 16716. https://doi.org/10.1038/s41598-021-96204-y

35. Zhao, Z., Zhang, Xianbo, Zhao, Fei, Zhou, Z., Zhao, Feng, Wang, J., Liu, T., Yang, X., Zhang, Xiaoping, Li, Z., 2022. Stress responses of the intestinal digestion, antioxidant status, microbiota and non-specific immunity in Songpu mirror carp (*Cyprinus carpio* L.) under starvation. Fish Shellfish Immunol 120, 411–420. https://doi.org/10.1016/j.fsi.2021.12.008

Journal of Agricultural Science and Technology (JAST), 29(3)
In Press, Pre-Proof Version

تغییرات دینامیکی در فعالیت آنزیم‌های گوارشی و آنتی‌اکسیدانی ماهی سی‌باس آسیایی جوان (*Lates calcarifer*) در طول یک دوره گرسنگی کوتاه‌مدت

حمزه سالاروند، امین اوجی فرد، وحید مرشدی، احمد قاسمی، منتور سپجانی، و قاسم رشیدیان

چکیده

این مطالعه پاسخ‌های فیزیولوژیک ماهی سی‌باس آسیایی جوان (*Lates calcarifer*) را به محرومیت غذایی کوتاه‌مدت، با تمرکز بر تغییرات در فعالیت‌های آنزیم‌های گوارشی و آنتی‌اکسیدانی بررسی کرد. در مجموع ۱۵۰ ماهی (میانگین وزن اولیه ۰.۰۶ ± 5/80 گرم) به شش مخزن پلی‌اتیلن استوانه‌ای ۳۰۰ لیتری با دو گروه شامل یک گروه کنترل (تغذیه شده) و یک گروه گرسنگی (محروم از غذا) به مدت ۸ روز تقسیم شدند. نمونه‌برداری در روزهای ۲، ۴، ۶ و ۸ برای ارزیابی فعالیت آنزیم‌های گوارشی (آمیلاز، لیپاز، پروتئاز و آلکالین فسفاتاز) و آنزیم‌های آنتی‌اکسیدانی سوپراکسید دیسموتاز [SOD]، کاتالاز[CAT] ، گلوتاتیون ردوکتاز [GR] همراه با محتوای مالون دی‌آلدئید [MDA] در کبد انجام شد. نتایج به‌دست‌آمده نشان داد که فعالیت آمیلاز در گروه گرسنگی در روز ۸ در مقایسه با گروه کنترل کاهش معنی‌داری نشان داد ($p < 0.05$). علاوه بر این، آلکالین فسفاتاز در روز 4 در گروه گرسنه در مقایسه با گروه کنترل کاهش یافت ($p < 0.05$). فعالیت‌های لیپاز و پروتئاز به طور قابل توجهی تحت تأثیر دوره‌های گرسنگی بین گروه‌های گرسنه و کنترل قرار نگرفت ($p > 0.05$). در میان پارامترهای آنتی‌اکسیدانی، GR در گروه گرسنه در روز 6 در مقایسه با گروه کنترل کاهش معنی‌داری نشان داد ($p < 0.05$). ا این حال، فعالیت‌های SOD و CAT در گروه گرسنه به ترتیب در روزهای 6 و 4 در مقایسه با گروه کنترل به طور قابل توجهی افزایش یافت ($p < 0.05$). سطح MDA در ماهیان گرسنه در مقایسه با ماهیان کنترل در طول آزمایش به طور مداوم پایین‌تر باقی ماند ($p < 0.05$). این نتایج نشان می‌دهد که گرسنگی کوتاه مدت باعث ایجاد تنظیمات آنزیمی خاصی می‌شود که دفاع اکسیداتیو و پایداری متابولیک را در ماهی سی‌باس آسیایی جوان افزایش می‌دهد.