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Original Research

Starvation as oxidative stress biomarker in two Indian snakeheads, *Channa striatus* and *Channa marulius*

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ABSTRACT:

Background:

Snakehead species viz: *Channa striatus* and *C. marulius* are unique group of freshwater air breathing fishes well known for their medicinal and recuperative properties. Levels of antioxidant enzymes was used as an indicator and biomarker for the oxidative stress experienced by both the species when subjected to four weeks of starvation and re-feeding.

Materials and methods:

C. striatus and *C. marulius* reared at CARE Aquafarm were starved for a period of 4 weeks followed by a re-feeding for 4 weeks. Four fish of each species were sampled on 0, 2nd and 4th week of starvation and also on 2nd and 4th week of re-feeding. Tissue homogenates were subjected to antioxidant enzymes analyses viz: catalase, super oxide dismutase, glutathione peroxidase, glutathione-S tranferase, glutathione reductase, total reduced glutathione and lipid peroxidation.

Results:

Enzymatic antioxidants like CAT, SOD, GPx, GST and GR were found to be induced in all the three tissues like muscle, liver and gills tested. Lipid peroxidation was also augmented to a greater extent and reduced below normal when the starvation was extended.

Conclusion:

The activities of certain antioxidant enzymes slightly increased and gradually decreased during later period of starvation or initial re-feeding period, which shows that the immune functions were triggered to protect themselves from the unfavourable condition of food deprivation. Few antioxidant activities did not return to normal even after re-feeding for four weeks, which shows that the study needs further extension until recovery.

Keywords:
Channa striatus, C. marulius, antioxidants, starvation, lipid peroxidation

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INTRODUCTION

Fish can withstand an assortment of sustenance hardship periods. Wild fish experience time of nourishment hardship in their lifetime because of movement and occasional change of sustenance accessibility. In aquaculture systems too, even when food is unavailable, fish starve during stress like fluctuation of water quality and disease outbreak. In addition to it, fish also undergo starvation during capture, transportation, and acclimation to farm condition. Hence, investigations on biological responses to starvation in fish are required to provide valid information for comparative physiology of food (Sridee and Boonanuntanasarn, 2012). Limited information is available about how the level and nature of dietary energy affects the oxidative status of a fish.

Antioxidant enzymes go about as scroungers of the exceedingly receptive intermediates created during hydrocarbon metabolism to sustain the cell homeostasis, by reacting distinctively to synthetic compounds and different stress conditions Antioxidant enzyme level can be used as an indicator of the antioxidant status of the organism and can serve as biomarkers of oxidative stress (Livingstone, 2001). To reduce the harmful effects of Reactive Oxygen Intermediates (ROI), fish possess an antioxidant defense system, utilizing enzymatic and non-enzymatic mechanisms. The reduction potential of an antioxidant determines the ability of one antioxidant to reduce another antioxidant or a lipid derived radical (Buettner and Jurkiewicz, 1997).

Snakeheads (Channidae) are carnivorous, air breathing freshwater food fishes which have a medium level of anti-oxidant activities (Lokman, 2006) contributed by some of the major amino acids and fatty acids present in it (Dahlan-Daud *et al*., 2010, Haniffa *et al*., 2014a). Two closely related murrels viz., *C. striatus* and *C. marulius* (Haniffa *et al*., 2014b) are highly valued as food fishes, particularly in India, southeastern Asia, China, and to a lesser extent in Africa (Haniffa *et al*., 2006). Studies on food starvation influencing the antioxidant defenses in fish especially in snakeheads are very scarce. Hence, the present study was attempted to assess the antioxidant potential of two snakehead species viz: *C. striatus* and *C. marulius* after starvation and re-feeding for a period of four weeks each.

MATERIALS AND METHODS

Adults but sexually immature *C. striatus* (32.7± 1.83 cm and 224 ±16 g) and *C. marulius* (35.8±2.8 cm and 275±21 g) reared at CARE Aquafarm were randomly selected for the study and were maintained at 27.2±2ºC in cement tanks (1m x 1m x 1m) with 12:12 dark/light cycle. Fishes were fed on cleaned and sliced chicken intestine twice a day during the acclimatization and re-feeding period. The fishes were starved for a period of 4 weeks followed by a re-feeding for 4 weeks. Four fish of each species were sampled on 0, 2nd and 4th week of starvation and also on 2nd and 4th week of re-feeding. Water quality parameters and mortality rate were monitored throughout the study. Fishes were randomly sampled

Table 1. SOD activity in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

S. No.	Sample	Starvation period (Weeks)			Re-feeding period (Weeks)	
		0	2	4	2	4
1.	CS Liver	0.315 ±0.075	0.99±0.06	0.66±0.06	0.62±0.55	0.34±0.01
2.	CM Liver	0.59±0.025	0.78±0.02	0.76±0.05	0.68±0.02	0.48±0.04
3.	CS Muscle	0.27±0.04	0.63±0.10	0.30±0.02	0.38±0.04	0.58±0.07
4.	CM Muscle	0.84±0.09	0.66±0.07	0.38±0.04	0.44±0.02	0.47±0.07
5.	CS Gills	1.44±0.10	0.84±0.11	1.24±0.07	0.77±0.10	0.90±0.08
6.	CM Gills	2.32±0.07	1.33±0.07	1.91±0.07	1.78±0.08	1.3±0.05

Values expressed as Units/min/mg protein (mean±SD)

Table 2. CAT activity in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

S. No.	Sample	Starvation period (Weeks)			Re-feeding period (Weeks)	
		0	2	4	2	4
1.	CS Liver	89.27±2.26	69.94±1.45	55.40±3.62	52.19±1.72	58.25±1.50
2.	CM Liver	41.30±1.62	36.58±1.17	30.53±0.93	32.50±1.15	36.13±0.61
3.	CS Muscle	13.67±1.16	24.85±1.60	8.38±0.95	9.90±0.90	12.97±0.67
4.	CM Muscle	4.31±0.04	8.69±1.27	1.18±0.05	1.86±0.18	2.81±0.34
5.	CS Gills	5.21±0.35	3.52±0.12	1.95±0.23	2.74±0.43	2.89±0.68
6.	CM Gills	7.87±0.39	5.29±0.84	3.55±0.25	3.51±0.44	2.93±0.07

Values expressed as µ moles of H_2O_2 consumed/min/mg protein (mean±SD)

from each day and were sacrificed by a blow on the head, and liver, muscle and gills were dissected immediately and made free from blood and immediately frozen in liquid nitrogen and then stored at -80 ºC until use.

One gram of tissue was homogenized in 0.1 M phosphate buffer of pH 7.0 to give a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min at 4˚C to obtain a clear supernatant which was used for further analyses. Superoxide Dismutase (SOD) was estimated following Das *et al*. (2000). One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition. Catalase (CAT) activity was determined following Sinha (1972). The activity of catalase was expressed as µmole of H_2O_2 decomposed/min/mg protein. Glutathione Peroxidase (GPx) was assayed as per Ellman (1959). The activity was expressed in term of µg of glutathione consumed/min/mg protein. Glutathione -S-Transferase (GST) was estimated following Habig and Jakoby (1974). The enzyme activity was calculated in terms of µmoles of CDNB conjugate formed/min/mg protein.

Glutathione Reductase activity (GR) was assayed following the method Beutler and Matsumoto (1975) and was expressed as µ moles of NADPH oxidized/min/mg protein. Total Reduced Glutathione (TRG) was determined following Moron *et al*. (1979) and expressed as µg/g tissue. Lipid Peroxidation (LPO) was estimated by following Niehius and Samuelsson (1968) and reported as µ moles/mg protein. All the values were expressed as Mean± Standard Deviation (Mean±SD) after analyzing the data using Microsoft excel.

RESULTS AND DISCUSSION

Water quality parameters were measured and recorded as pH – 7.0, chloride – 250 ppm, total hardness

Table 3. GPx activity in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

S. No	Sample	Starvation period (Weeks)			Re-feeding period (Weeks)	
		0	2	4	2	4
1.	CS Liver	37.89±2.06	26.67±4.54	19.43±0.14	19.13±1.30	22.42±1.03
2.	CM Liver	28.22±1.22	20.79±0.16	14.14±0.62	16.02±0.65	18.19±0.15
3.	CS Muscle	48.49±1.30	53.82±0.60	59.19±0.82	50.64±0.95	45.63±0.80
4.	CM Muscle	57.21±0.56	66.08±1.09	64.23±1.02	54.03±0.92	52.10±0.94
5.	CS Gills	38.63±0.88	54.29±0.97	74.89±1.38	71.05±0.88	67.72±0.94
6.	CM Gills	83.57±1.01	119.87±1.15	104.79±2.73	108.09±1.53	117.23±0.52

Values expressed as µ moles of GSH oxidized/min/mg protein (mean±SD)

Table 4. GST activity in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

S. No	Sample	Starvation period (Weeks)			Re-feeding period (Weeks)	
		0	2	4	2	4
1.	CS Liver	222.06±1.28	194.05±2.89	174.48±4.31	185.48±0.83	193.34±1.80
2.	CM Liver	286.79±2.66	210.59±0.82	173.29±3.76	169.59±2.36	181.32±2.86
3.	CS Muscle	13.90±0.52	16.73±0.59	18.16±0.66	18.50±0.87	16.86±0.52
4.	CM Muscle	11.28±0.63	15.21±0.80	18.62±0.56	17.56±0.58	14.53±0.36
5.	CS Gills	18.46±1.60	48.43±1.20	40.71±1.15	45.2±1.24	42.89±1.28
6.	CM Gills	14.68±0.64	34.94±1.47	27.43±0.60	24.61±1.04	18.21±1.57

Values expressed as µ moles of CDNB conjugation formed/min/mg protein (mean±SD)

– 500 ppm, fluoride – 0.5 mg/l, iron - 0.5 mg/l, residual chlorine - nil and nitrate – 0.55 mg/l. No mortality was observed throughout the study period. The weight of the fishes reduced to a greater extent by the end of starvation and the compensatory growth during re-feeding was also noticed.

Liver is the main organ responsible to allocate the nutrient storage for vital process during starvation period. Gingerich (2010) reported that the effect of food deprivation was found in the relative liver size. In the present study, liver SOD increased by second week and it gradually decreased when starvation was extended, whereas, even after re-feeding, SOD values decreased gradually by 2nd and 4th week in both *C. striatus* and *C. marulius* (Table 1). CAT, GPx and GST values decreased with increase in starvation period and their activities gradually increased during re-feeding in both the species as in Tables 2, 3 and 4. In *C. marulius*, GR activity decreased by 2nd week and increased during 4th week of starvation, whereas, it again decreased and increased during re-feeding period. In *C. striatus*, GR values increased during starvation and decreased during re-feeding period (Figure 1a, 1c and 1e). TRG level decreased during starvation and slightly increased after 2 weeks of re-feeding in *C. striatus* whereas no such changes were recorded for *C. marulius* (Figure 1b, 1d and 1f).

In muscle of *C. marulius,* SOD decreased during starvation and slightly increased after re-feeding, as in Table 1. A rapid increase in SOD of *C. striatus* was noticed during 2nd week and decreased to the initial level by 4th week of starvation, with a gradual increase in re-feeding period. Similarly, CAT also showed maximum increase during 2nd week and decreased by 4th of starvation, which gradually increased during re-feeding period in both the species (Table 2). GPx and GST values increased during starvation and gradually decreased during re-feeding in both *C. striatus* and *C. marulius* (Table 3 and 4). GR and TRG level decreased during starvation and increased during re-feeding in both species. In *C. marulius,* GR level dropped after 2 weeks of re-feeding (Figure 1).

The gills are extremely important for respiration, osmoregulation, acid base balance and excretion of nitrogenous wastes in fish, representing greatest surface area of fish in contact with external environment (Gad and Yacoub, 2009). In the present investigation, enzymatic antioxidant activities in gills subjected to starvation were represented in Table 1-4. SOD activity of both *C. striatus* and *C. marulius* decreased on 2nd week of initial starvation and increased during further starvation, whereas CAT values decreased throughout the starvation period and increased during re-feeding. GPx, GST, GR and TRG values of *C. striatus* increased during initial starvation period. GPx, GST and TRG values of *C. marulius* showed a decreased after second week of starvation. In the initial re-feeding period of 2 weeks, GPx and GST of *C. marulius* and GST of *C. striatus*, in-

Figure 1. Level of non- enzymatic anti-oxidants GR and TRG in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

Figure 1a. Level of GR in the liver of CS and CM

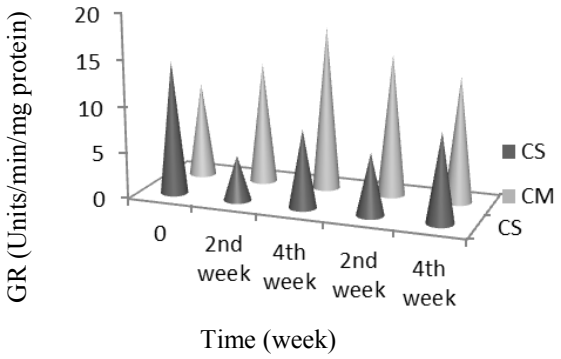


Figure 1b. Level of TRG in the liver of CS and CM

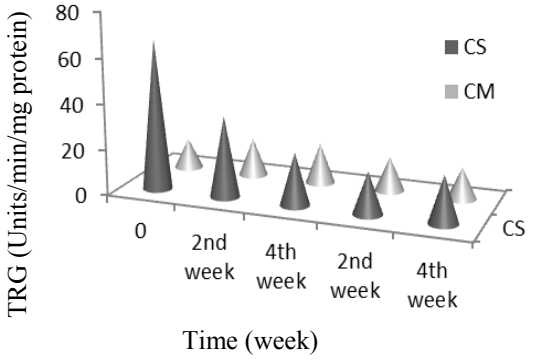


Figure 1c. Level of GR in the muscle of CS and CM

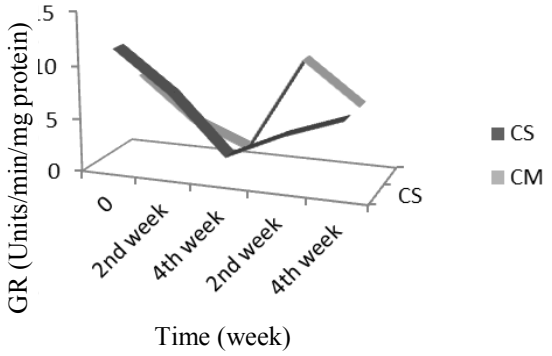


Figure 1d. Level of TRG in the muscle of CS and CM

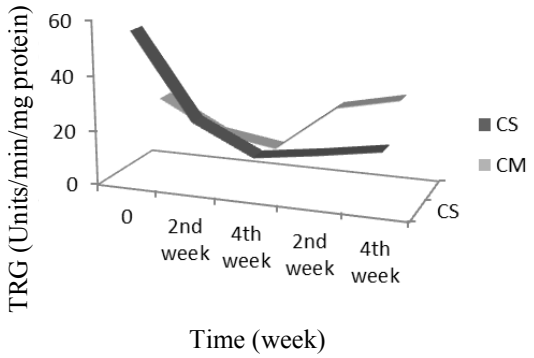


Figure 1e. Level of GR in the gills of CS and CM

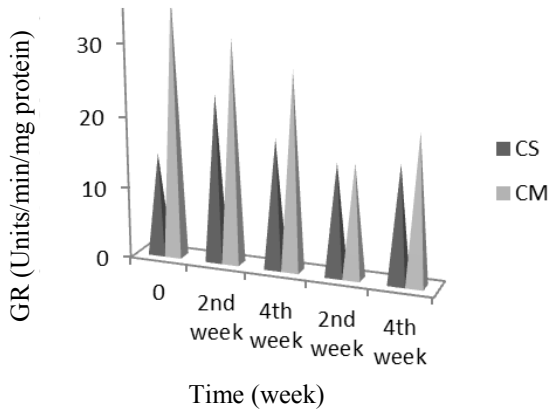
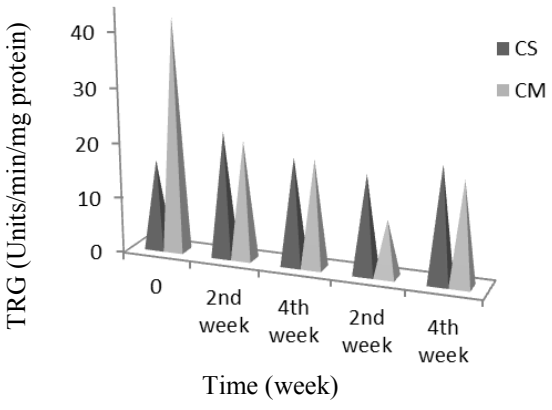


Figure 1f. Level of TRG in the gills of CS and CM



creased slightly, whereas GPx of *C. striatus* decreased. GR and TRG values of both the species showed a gradual decrease during initial re-feeding and then increased during the 3rd and 4th week of re-feeding as illustrated in Figure 1.

Murrels are carnivorous and can withstand fast-

Figure 2. LPO values in liver, muscle and gills of *C. striatus* (CS) and *C. marulius* (CM)

Figure 2a. Level of LPO in the Liver of CS and CM

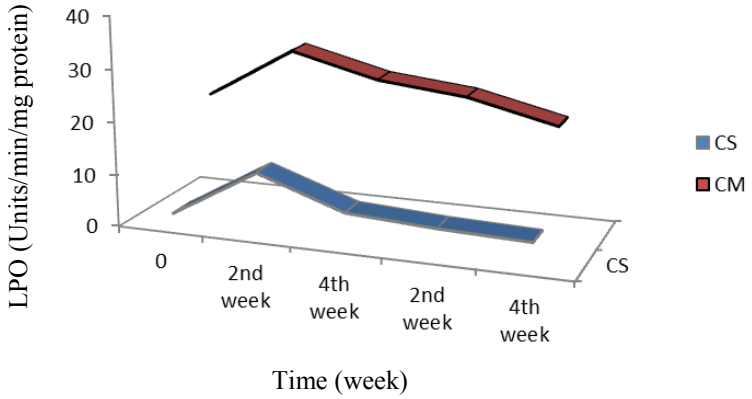


Figure 2b. Level of LPO in the muscle of CS and CM

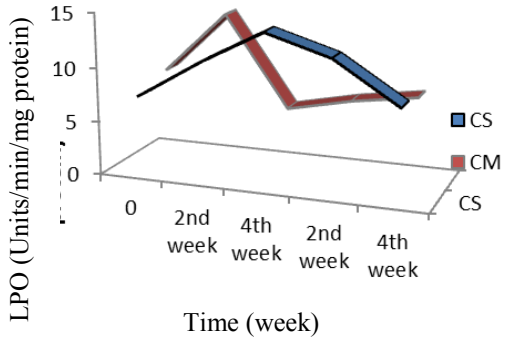
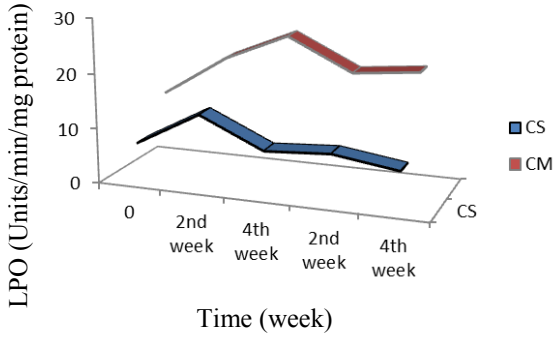


Figure 2c. Level of LPO in the gills of CS and CM



ing state while waiting for the next meal. During acclimating process, even when food is surplus, fishes undergo the state of starvation for a period of time (Sridee and Boonanuntanasarn 2012). Under stress, animal cells produce reactive oxygen species (ROS), such as O_2, H_2O_2 and hydroxyl radicals, as well as ozone. Other molecules normally included as ROS are derived from the reaction of carbon-centered radicals (alkyl radicals R) with molecular oxygen (alkoxyl radicals [RO]; peroxyl radicals [ROO] and organic hydroperoxides [ROOH]) (Halliwell 2000). At the same time, antioxidant defences, such as NADH/NADPH, glutathione (GSH), protein sulphydryl (–SH) groups and free radical scavenging enzymes (such as SOD, CAT and GPX) remove ROS (Winston and Giulio, 1991).Oxidative stress is produced when there is an imbalance between production and removal of ROS (Tocher *et al*., 2002). The bio-membranes of the fish tissues which are rich in n-3 Polyunsaturated Fatty Acids (PUFA) are highly susceptible to oxidation by ROS (Sargent *et al*., 1999).

The level of the antioxidants may be high or low depending on the on the intensity and duration of stress applied as well as susceptibility of exposure species (Gad, 2011). SOD is responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water (Oost *et al*., 2003). Also SOD is the metabolizing superoxide radical and its levels are directly related to CAT activity. Halliwal (1994) reported that an increase in SOD is followed by a parallel increase in CAT, since both enzymes are linked functionally and in tandem. In fish, the existing data in the literature is controversial. The increase of antioxidative enzymes may be a physio-

logical adaptation for the elimination of ROS generation.

Toxicity biomarkers, such as malondialdehyde (MDA), have been also proposed to reflect the oxidative status of exposed species (Sole *et al*., 1996) and as marker of oxidation of membrane phospholipids through lipid peroxidation. An increase in MDA levels can be related to degradation of an environmental site by decreasing the water quality (Charissou *et al*., 2004).

In the present study, non-enzymatic antioxidants like LPO was augmented during the initial period of starvation in both *C. striatus* and *C. marulius* in all the three tissues like liver, muscle and gills. In liver, LPO values increased after 2 weeks of starvation and gradually decreased during re-feeding in both the species. Muscle LPO of *C. striatus* reached a peak by the end of starvation and decreased during re-feeding, whereas in *C. marulius*, LPO values decreased by 4th week of starvation and increased during re-feeding. In *C. striatus* gills, LPO level decreased after 2 weeks of starvation and increased slightly during initial re-feeding and finally decreased, whereas in *C. marulius*, the LPO values recorded were in vice versa as in Fig. 2.

Lipid peroxidation is one of the main processes induced by oxidative stress. Lipid peroxides are the end products of oxidative deterioration of poly unsaturated lipids in membranes of cells and organelles. MDA, the bi -product of lipid peroxidation, is an indicator of increased concentration of cellular reactive oxygen species and cellular injuries (Gad and Yacoub, 2009). Tissue and cell membrane lipoperoxidation caused by ROS have been considered to be proportional to antioxidant content (Almeida 2002). The source of dietary energy (protein, lipid or digestible carbohydrate) has been shown to modulate lipid oxidation in muscle homogenates of rainbow trout and European sea bass (Alvarez *et al*., 1998; Lopez-Bote *et al*., 2001). The generation of oxidative stress determined as an increase in the LPO level, under starvation or restricted food condition has also been reported by other authors in the liver of rainbow trout (Hidalgo and Zamora, 2002), gilthead seabream (*S. aurata*) (Pascual *et al*., 2003) and of common dentex (*D. dentex*) (Morales and Hewitt, 2004).

Fish when they starve undergo lipid peroxidation. But the level of the antioxidant enzymes vary depending on the tissues tested. Bloom and Canning (2000) reported a decrease of roughly 35% in hepatic GR activity in rainbow trout after three weeks of starvation and this decrease was retained till 7 weeks of food restriction.

In Atlantic cod, *Gadus morhua,* observed high activity of certain antioxidant enzymes in liver, while in muscle these enzymes did not vary after 12 weeks of starvation (Guderley *et al*., 2003). Pascual *et al*. (2003) also studied oxidative stress parameters in the liver of gilthead seabream, *S. aurata*, after passing through food restriction period or starvation. Oxidation of glutathione and lipid peroxidation increased under both conditions. With food restriction, CAT activity decreased, while SOD, GR and GPx activities increased. In addition, new isoforms of the SOD enzyme were found during starvation. Common dentex (*Dentex dentex*), submitted to 5 weeks of starvation showed an increase in activity of the hepatic enzymes SOD, CAT and GPX, whereas a decrease in GR activity was recorded (Morales and Hewitt, 2004). Congleton and Wagner (2006) reported that in salmonid, starvation altered several enzymes including alkaline phosphatase, SGOT and SGPT depending on the species and rearing condition.

The antioxidant defences in fish can be influenced not just by extraneous elements, for example, the presence of poisons in the water, temperature changes and dissolved oxygen, or intrinsic factors, for example, age, phylogenetic position and feeding habits of the fish, in addition to pathogens or parasites (Martínez-Alvarez *et al*., 2005). Fish can adapt to starvation during seasonal food supply variation and spawning period in their natural habitat. Before marketing or slaughtering to improve preservation, food deprivation is a common and very old practice (Furné *et al*., 2012)

Nutrition influences oxidation and antioxidative defense mechanisms, and the composition of the diet should be evaluated in terms of its effect on the balance between ROS generation and breakdown. Besides, lipid and polyunsaturated unsaturated fats are associated in peroxidation, while exogenous dietary micronutrients like vitamin E, provides antioxidant protection (Tocher *et al*., 2002; Rueda-Jasso, 2004). Every one of the creatures require vitality for development, proliferation and for keeping up their basal digestion. Extra vitality is required for managing stress and if this is not given by means of the eating regimen, development and generation can be traded off (De Coen and Janssen, 2003). Overloading, an increased uptake of saturated fats and sugary nourishments are key dietary changes that have happened in late decades notwithstanding the rise of the obesity epidemic. Moreover, to the increase in fat storage, these dietary changes are joined by an elevation in mitochondrial macronutrient oxidation, prompting to free radical creation and thus, oxidative anxiety.

In both the species, the activities of certain antioxidant enzymes slightly increased and gradually decreased during later period of starvation or initial re-feeding period, which shows that the immune functions were triggered to protect themselves from the unfavourable condition of food deprivation. Few antioxidant activities did not return to normal even after re-feeding for four weeks, which shows that the study needs further extension until recovery. However, a lack of information about the physiochemical parameters affecting ROI's and the effects of starvation on the cellular structure of antioxidant defense system in murrels warrants further investigation.

CONCLUSION

In murrels, starvation as well as re-feeding induced an oxidative stress in liver, muscle and gills of both *C. striatus* and *C. marulius* suggesting the active involvement of the immune related responses. The broadening of the studies of this type, as well as going into depth regarding the different factors that interact in cultured animals, would contribute not only to a complete knowledge of fish physiology but also its optimization.

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