



## Research Paper

# Regional asymmetry of metabolic and antioxidant profile in the sciaenid fish shi drum (*Umbrina cirrosa*) white muscle. Response to starvation and refeeding



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## ABSTRACT

The objective of the present study is to characterize the metabolic and antioxidant profile of white muscle of shi drum in two sites of the body, anterior dorsal (AM) and posterior dorsal (PM) portions. In addition, it will be analyzed the possible effect of starvation and a subsequent refeeding, with two different protocols, pair feeding and ad libitum. Activities of key enzymes of intermediary metabolism and of antioxidant enzymes, as well as lipid peroxidation, as an index of oxidative stress, were evaluated. The results indicate the existence of a regional asymmetry of the metabolic capacities of the white muscle of shi drum, which is likely related to the different contribution to swimming of the body regions examined. Starvation induces a metabolic depression that is more marked in those activities that support burst swimming in PM, while those activities supporting maintenance requirements are conserved. The greatest energy demands during starvation appear to lie in AM, which showed the highest oxidative metabolism rate. The increased use of fatty acids as energy source for AM leads to oxidative stress. A period of more than four weeks of refeeding for full restoration of metabolic capacities in AM is needed, probably related to the higher muscle mass located in this region. On the contrary, all enzyme activities in PM returned to control levels in both refeeding protocols, but pair feeding seems to be advantageous since compensatory growth has been taking place without signs of oxidative stress. This work was addressed to gain knowledge on the physiology of a promising fish species in aquaculture like shi drum. The results displayed here show how the starving and further re-feeding events could generate oxidative stress situations characterized by high lipid peroxidation levels which may influence negatively on the quality of the edible part of the fish. This study opens an interesting field on this fish species which deserves being investigated in the future.

## 1. Introduction

The main aspect that affects the profitability of the fish farms is the cost of food. For this reason, to improve food efficiency is among the main objectives of aquaculture industry. Intensive aquaculture allows farmers to enhance farm production by employing practices that manipulate the biology of their fish stocks. Protocols based on starvation-refeeding cycles have been reported to induce compensatory growth, a phase of accelerated growth when favourable conditions are restored after a period of growth depression [1]. To use compensatory growth in aquaculture represents a unique opportunity to boost profit margins. Starvation is a situation that fish experience in its natural

environment and by this is rather tolerated by many species [14,18,20,24,27,29,30,4,6]. Is for this capacity to withstand a long period without food than aquaculture industry uses this tool to induce compensatory growth and to control overproduction [1].

Either in natural environments or in fish farms effects of starvation affects fish physiology, although the specific response will be species-dependent [23,35,8]. In general, high tolerance of fish to starvation occurs because are capable of allocate their energy reserves, from muscle and liver, towards processes of maintenance and survival, often at the cost of metabolic and locomotive scopes [19]. These changes in energetic status will modify many aspects of swimming performance, which reflects the general response of fish to food deprivation consist-

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ing in three phases: the stress phase in which locomotive activity for food searching increases for a short period (24 h approximately), the transition phase characterized by a decline in both swimming activity and energy expenditure, and the adaptation phase in which a low activity and metabolic rate is maintained until food is available again [36]. In many fish, acquisition of food depends on the anaerobic white muscle activity, which will be the most affected under such circumstances of fasting and refeeding.

Starvation induces a reduction in glycogen deposits in white muscle, which usually takes place in the short term. Simultaneously, a marked reduction in fat stores occurs. Finally, if fasting is extended in time, fish will begin to mobilize skeletal muscle proteins. Such mobilization of energy reserves imposes metabolic adaptations that involve the major pathways of intermediary metabolism [21,23].

Starvation has been reported to have pro-oxidant effects in fish due to both the inadequate neutralization of ROS generated by sustained aerobic metabolism and the reduced level of antioxidant defenses [21]. Regarding white muscle, there are few studies evaluating the impact of starvation on antioxidant defenses and they report that muscle-specific activity of antioxidant enzymes are maintained [11, 25 26].

It is well established a regional heterometry of muscle metabolic capacities in fish that, at least in part, is based in regional differences in the contribution of muscle during swimming [11,15,17,33]. It has been reported that metabolic consequences of starvation vary depending on the body region considered [11,17].

The shi drum, *Umbrina cirrosa* (Linnaeus, 1758), is a member of the sciaenidae family (Nelson, 1994) and has been considered a very promising candidate species for Mediterranean aquaculture, since it has a high growth rate and good capacity of adaptation to the culture conditions [22]. The objective of the present study is to characterize the metabolic and antioxidant profile of white muscle of shi drum in two sites of the body, anterior dorsal and posterior dorsal portions. In addition, it will be analyzed the possible effect of starvation and a subsequent refeeding, with two different protocols, pair feeding and ad libitum. The comparison between anterior and posterior muscle will allow to appreciate if a differential response depending on muscle location exists. On the other hand, the use of two refeeding protocols could provide information on the best strategy to follow in order to improve production.

## 2. Material and methods

### 2.1. Experimental design

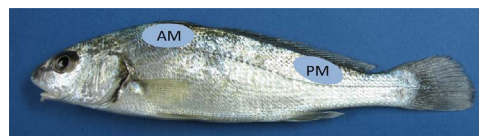
This experiment was directed by trained scientists (following the Federation of Laboratory Animal Science Associations, FELASA, category C recommendations) and was conducted according to the European Union Directive 2010/63/EU on the protection of animals for scientific purposes. Both, University of Granada and Spanish Institute of Oceanography possessed all required licenses from the Animal Research Ethic Committees.

Sexually immature shi drum (*Umbrina cirrosa*) were obtained and raised at the facilities of the Marine Culture Experimental Plant of the Spanish Institute of Oceanography in Mazarrón (Murcia, Spain).

The fish (initial weight of  $206.3 \pm 1.8$  g) were distributed in 9 tanks 2000 L each, 60 fish per tank. The average water temperature was of  $14.2 \pm 0.6$  °C (37‰ salinity). The photoperiod was 12 h L/12 h O and the average dissolved oxygen was no lower than 90% saturation level.

The experiment lasted for 8 weeks; during this time 3 tanks of fish were fed ad libitum (twice a day) with a commercial diet (control lots, C); fish of the 6 remaining tanks were starved (starvation lots, S) during the 4 first weeks. Immediately afterward, fish previously fasted were re-fed the remaining 4 weeks in two different ways: ad libitum twice a day (Ra lots) and pair fed to the control lots (lots RP).

C, Ra, and RP lots were fed with a commercial diet for scianidae, whose composition in dry matter basis was 47.2% protein, 19.9% fat,



**Fig. 1.** Scheme of white muscle sampling of shi drum (*Umbrina cirrosa*). AM, anterior muscle. PM, posterior muscle.

6.1% ash and 23.0% of MELN.

### 2.2. Sampling

Fish were sampled at the end of both starvation and refeeding periods (4th and 8th weeks). Fish sampled from control tanks at each sampling time were used as controls of starvation (C) and refeeding (CR), respectively. At each sampling, three fish from each tank (nine per treatment) were taken at random and anaesthetized by immersion in a 1:2000 (v/v) 2-phenoxyethanol solution and subsequently slaughtered. Samples of white muscle were taken at two sites of the dorsal left-side avoiding contamination by pink and red fibers. The first sample was taken in the anterior region behind the head (AM) and the second in the caudal region (PM) (Fig. 1). Samples were immediately frozen in liquid nitrogen and kept at  $-80$  °C until use.

### 2.3. Analytical determinations

Tissue samples were homogenized in nine volumes of ice-cold 100 mM Tris–HCl buffer containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100, pH 7.8. All procedures were performed on ice. Homogenates were centrifuged at  $30,000 \times g$  for 30 min at 4 °C and the resultant supernatants were kept in aliquots and stored at  $-80$  °C for further enzyme assays.

Fructose 1,6-bisphosphatase (FBPase; EC 3.1.3.11), glycerol kinase (GyK; EC 2.7.1.30), pyruvate kinase (PK, EC 2.7.1.40), lactate dehydrogenase (LDH, EC 1.1.1.27), citrate synthase (CS; EC 4.1.3.7),  $\beta$ -hydroxyacyl CoA dehydrogenase (HOAD; EC 1.1.1.35), glutamate pyruvate transaminase (GPT; EC 2.6.1.2), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), and glutamate dehydrogenase (GDH; EC 1.4.1.2) were determined as previously described by [7].

Superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), and glutathione reductase (GR, EC 1.6.4.2) were determined according to [28].

All enzyme assays were carried out at 25 °C and changes in absorbance were monitored to determine the enzyme activity using a PowerWaveX microplate scanning spectrophotometer (Bio-Tek Instruments, USA). The optimal substrate and protein concentrations for the measurement of maximal activity for each enzyme were established by preliminary assays. The millimolar extinction coefficients used for NADH/NADPH, DTNB, and  $H_2O_2$  were 6.22, 13.6, and  $0.039 \text{ mM}^{-1} \text{ cm}^{-1}$ , respectively.

Lipid peroxidation was determined based on malondialdehyde (MDA) levels. In the presence of thiobarbituric acid, MDA reacts producing colored thiobarbituric acid reacting substances (TBARS) that were measured as previously described by [28]. Results were expressed as nmol MDA per gram of fresh tissue, calculated from a calibration curve.

Soluble protein concentration in tissue homogenates was analyzed using the method of [5], with bovine serum albumin used as a standard.

Materials (chemicals and tools) were provided by the laboratory of Nutrition of the Department of Zoology (Animal Physiology Unit) of the University of Granada. The chemicals were obtained in Sigma-Aldrich Co. (St. Louis, MO).

**Table 1**

White muscle specific activity of intermediate metabolism enzymes of shi drum. Control, C. Starved, S. AM, anterior muscle. PM, posterior muscle.

	AM		PM	
	C	S	C	S
FBPase	8.60 ± 0.49	6.7 ± 0.34 <sup>#</sup>	10.26 ± 0.45 <sup>†</sup>	8.25 ± 0.35 <sup>#*</sup>
GyK	687.3 ± 19.5	660.1 ± 33.0	652.6 ± 16.0	658.5 ± 15.5
PK	2634 ± 70	1341 ± 63 <sup>#</sup>	1996 ± 62 <sup>†</sup>	1433 ± 55 <sup>#</sup>
LDH	6490 ± 291	5985 ± 332	9458 ± 372 <sup>†</sup>	7253 ± 265 <sup>#*</sup>
CS	24.4 ± 1.4	28.5 ± 1.0 <sup>#</sup>	29.1 ± 1.0 <sup>†</sup>	24.4 ± 0.7 <sup>#*</sup>
HOAD	2.90 ± 0.19	4.35 ± 0.23 <sup>#</sup>	4.42 ± 0.27 <sup>†</sup>	4.25 ± 0.17
GOT	194.5 ± 8.0	124.6 ± 2.5 <sup>#</sup>	186.3 ± 7.9	171.1 ± 6.3 <sup>#</sup>
GPT	29.9 ± 2.2	28.2 ± 1.7	18.0 ± 0.5 <sup>†</sup>	16.2 ± 0.5 <sup>#*</sup>
GDH	37.2 ± 1.7	33.8 ± 2.4	33.2 ± 0.5	24.6 ± 0.6 <sup>#*</sup>

Enzymatic activities are expressed as mU mg prot<sup>-1</sup>.

Values are means ± SEM (n=9).

<sup>#</sup> Indicates significant differences between control and starved shi drum either in anterior as in posterior muscle (P < 0.05).

<sup>†</sup> Indicates significant differences between anterior and posterior muscle in each experimental treatment (P < 0.05).

#### 2.4. Statistical analysis

Results are presented as means ± pooled standard error of mean (SEM).

The statistical package IBM SPSS Statistics v.20 for Windows was used for data analysis. After testing for homogeneity of variances using Levene's test, analysis of significance were made. Student's *t*-test was used for comparison values between two groups (C vs S; AM vs PM, etc). Comparisons after refeeding period (CR, Ra, RP groups) were performed using ANOVA test and Tukey's HSD multiple comparison test. The level of confidence considered for the comparisons was 95% (P < 0.05).

### 3. Results and discussion

#### 3.1. Regional asymmetry

Results obtained in control groups indicates a longitudinal variation in white muscle metabolic capacities, which is likely related to the different contribution to swimming of the body regions examined (Table 1). LDH, CS and HOAD activities were significantly higher in PM, which indicates a higher capacity for ATP generation in the caudal region of the body. Similar results have been reported for cod (*Gadus morhua*) [15] which like shi drum also presents a subcarangiform locomotive pattern. Regarding antioxidant enzymes (Table 2), higher SOD and G6PDH activities, and a non significant tendency for increased GPX and CAT activities were observed in PM, which may be related with the higher aerobic capacity of this body region. In spite of this apparent increased ROS generation in the caudal white muscle, lipid peroxidation levels were similar in both muscle regions, which seems to indicate that antioxidant defenses are effective in ROS scavenging.

#### 3.2. Starvation

Weight of fasted fish decreased with respect to the control fish, being the instantaneous growth (%/day) in controls 0.17 ± 0.01 and -0.28 ± 0.01 in starved fish.

Starvation induces a virtually generalized metabolic depression in AM and PM (Table 1, Fig. 2). However, some activities are conserved and/or increased. Namely, HOAD activity remained unchanged in PM and increased in AM. These results indicate that muscle energy requirements after four weeks of starvation would be provided by the mobilization of fat reserves, showing the AM the highest rate of fatty acid oxidation leading to the significant increase in CS activity. Given

**Table 2**

White muscle specific activity of antioxidant enzymes, and biomarker for lipid oxidative damage (MDA) of shi drum. Control, C. Starved, S. AM, anterior muscle. PM, posterior muscle.

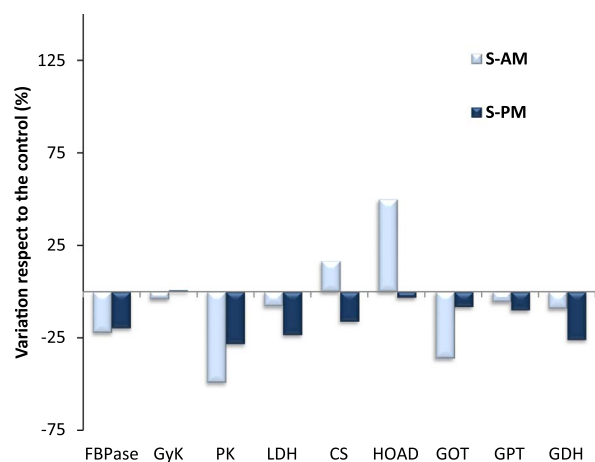
	AM		PM	
	C	S	C	S
SOD	28.9 ± 1.1	36.3 ± 1.6 <sup>#</sup>	33.3 ± 1.4 <sup>†</sup>	29.2 ± 2.2 <sup>†</sup>
CAT	0.18 ± 0.02	0.33 ± 0.03 <sup>#</sup>	0.17 ± 0.02	0.19 ± 0.02 <sup>†</sup>
GPX	2.19 ± 0.18	2.17 ± 0.16	2.51 ± 0.13	2.16 ± 0.26
GR	1.18 ± 0.04	0.89 ± 0.06 <sup>#</sup>	0.97 ± 0.05 <sup>†</sup>	0.81 ± 0.06 <sup>#*</sup>
G6PDH	0.21 ± 0.01 <sup>b</sup>	0.10 ± 0.01 <sup>#</sup>	0.28 ± 0.03	0.13 ± 0.01 <sup>#*</sup>
MDA	10.6 ± 2.1	25.0 ± 3.3 <sup>#</sup>	15.6 ± 3.0	10.7 ± 1.8 <sup>†</sup>

Enzymatic activities are expressed as mU mg prot<sup>-1</sup>, except SOD and CAT activities that are expressed as U mg prot<sup>-1</sup> and MDA levels as nmol g tissue<sup>-1</sup>.

Values are means ± SEM (n=9).

<sup>#</sup> Indicates significant differences between control and starved shi drum either in anterior as in posterior muscle (P < 0.05).

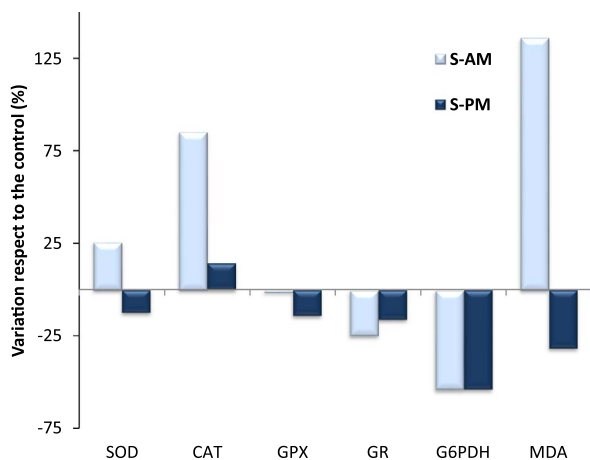
<sup>†</sup> Indicates significant differences between anterior and posterior muscle in each experimental treatment (P < 0.05).



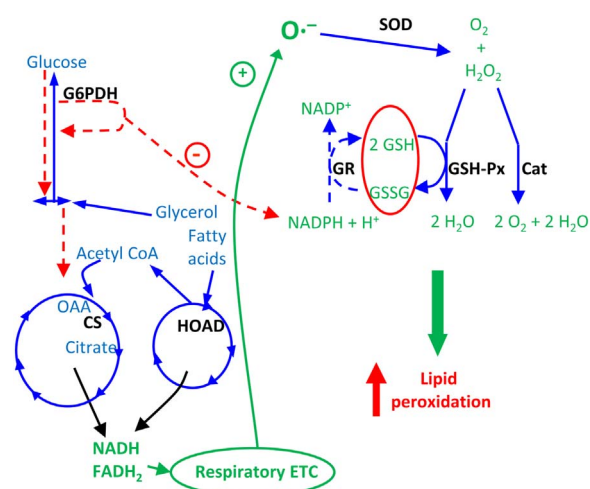
**Fig. 2.** Variations of enzymatic specific activities of starved (S) shi drum respect to the control (C) ones. Bars show % of change over control values. AM, anterior muscle. PM, posterior muscle.

that most of the muscle mass of shi drum is located in this region, this makes it the largest energy supplier, so this increase in oxidative metabolism would be expected, which would be responsible for the high lipid peroxidation level in AM, as described below. This preferential use of fat reserves should be also reflected in the GyK activity, which phosphorylates glycerol derived from triglyceride hydrolysis that is also conserved both in AM and PM. During starvation, fish goes through a transition state which leads to the adaptation, characterized by a decrease in locomotive activity, both burst- and steady-swimming. However, enzyme activities that support the episodes of burst swimming will be the most affected (PK, LDH) in the body region responsible for such activity (PM). Given that the fish must be kept a basal swimming activity, oxidative activities are less affected [3,10]. Previous studies on cod also reported that anaerobic metabolism in white muscle decrease more than aerobic one during prolonged starvation [11,16,17]. Regarding the activity of enzymes indicative of amino acid metabolism (GOT, GPT, GDH) no important changes were observed as consequence of starvation either in AM or PM. Given that GDH may provide an index of protein catabolism and nitrogen excretion, results seems to indicate that an increased protein hydrolysis from muscle is not yet taking place.

Regarding antioxidant enzymes (Table 2, Fig. 3), SOD significantly increases after starvation in AM, GPX and CAT activities remaining unchanged in both muscle regions, and G6PDH and GR significantly decreased in AM and PM of starved fish.



**Fig. 3.** Variations of antioxidant enzymatic specific activities and oxidative damage to lipids of starved (S) shi drum respect to the control (C) ones. Bars show % of change over control values. AM, anterior muscle. PM, posterior muscle.



**Fig. 4.** Schematic representation of the interconnection between intermediary metabolism and antioxidant defenses on anterior muscle of starved shi drum.

With respect to AM, SOD activity should increase in response to the higher aerobic activity previously reported, which would lead to an increased ROS generation. The increased amount of hydrogen peroxide generated by SOD activity should be scavenged by CAT and/or GPX that in the present study remaining unchanged. The analysis of MDA, indicative of lipid peroxidation, clearly show that prolonged starvation

led to oxidative stress in AM of starved fish, which would indicate a failure in antioxidant defenses. Responsible for this failure might be the depression in antioxidant defenses. Responsible for this failure might be the depression in antioxidant defenses found in the present study. G6PDH plays a crucial role in modulating the antioxidant defenses through the control of NADPH generation, since GR catalyzes a NADPH-dependent reaction and NADPH protects catalase from inactivation [9,13]. So, the reduced NADPH generation might be avoiding further increase in CAT and GPX activities leading to oxidative stress (Fig. 4). Studies on the impact of starvation on antioxidant defenses of fish white muscle are scarce, and most of them analyzed such activities in the more rostral region (AM). The results of these studies reported that antioxidant enzymes are spared during food deprivation avoiding increases in lipid peroxidation levels [2,26].

Regarding PM, SOD, CAT, and GPX remaining unchanged and G6PDH and GR are depressed with respect to controls. As reported previously, the reduced locomotive activity derived from adaptation to food deprivation would induce a more marked depression in most enzyme activities analyzed in this body region (Table 1, Fig. 2), including a significant reduction in CS activity. Such reduced rate of aerobic metabolism would lead to a lower ROS generation, thus being not necessary an increased response of antioxidant enzymes. In fact, contrary to the results for AM, lipid peroxidation levels in PM of starved shi drum were similar to those of controls. Similar results about the maintenance of white muscle antioxidant enzymes during starvation have been reported for cod [11].

### 3.3. Refeeding

After refeeding period, numerous significant differences in the AM of both Ra and RP with respect to controls were observed for metabolic activity (Table 3, Fig. 5), which indicate that this body region would have not recovered yet from effects of fasting. When food is available again, rate of protein synthesis, restoration of energy reserves, and metabolic capacities of muscle increases [12,34] in order to return to pre-starvation state. Probably, the presence of a greater muscle mass in the anterior body region would impose a longer period for recovery. A temporal priority of enzyme recovery in refeed fish has been already reported, with the level of enzymes recovering at different rates [1]. Independently of this transient metabolic status, the activity of antioxidant enzymes (Table 4, Fig. 6) in AM of Ra and RP fish was, in general, similar to controls except for GR that remained lower, however, lipid peroxidation decreased to control levels in both treatments.

Contrary to that reported for AM, metabolic activity in PM returned to control levels after refeeding, which would reflect the importance of restoring locomotive activity in order to capture food. In fact, activities responsible for such burst-swimming (LDH and PK) back to pre-

**Table 3**

White muscle specific activity of intermediate metabolism enzymes of shi drum. CR, control. Ra, refeeding ad libitum. RP, pair fed. AM, anterior muscle. PM, posterior muscle.

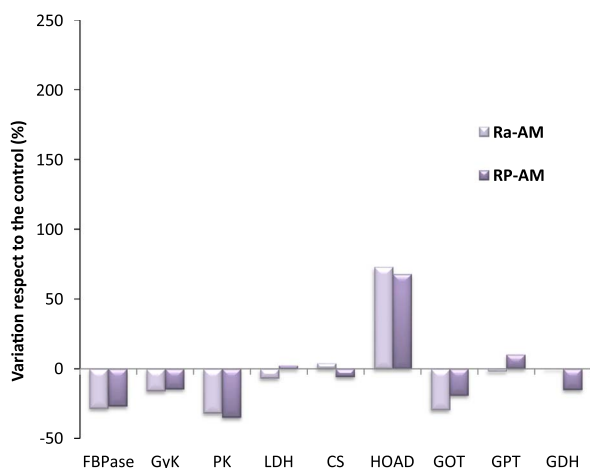
	AM			PM		
	CR	Ra	RP	CR	Ra	RP
FBPase	8.67 ± 0.58 <sup>b</sup>	6.21 ± 0.37 <sup>a</sup>	6.35 ± 0.48 <sup>a</sup>	9.21 ± 0.78 <sup>b</sup>	7.18 ± 0.66 <sup>a</sup>	8.36 ± 0.56 <sup>ab</sup> *
GyK	845 ± 39 <sup>b</sup>	710 ± 24 <sup>a</sup>	722 ± 18 <sup>a</sup>	631 ± 10 <sup>ab</sup> *	589 ± 18 <sup>a</sup> *	655 ± 11 <sup>b</sup> *
PK	2289 ± 169 <sup>b</sup>	1561 ± 69 <sup>a</sup>	1491 ± 54 <sup>a</sup>	1452 ± 53 <sup>*</sup>	1466 ± 60	1442 ± 32
LDH	6277 ± 397	5855 ± 168	6428 ± 329	8126 ± 856 <sup>*</sup>	7696 ± 353 <sup>*</sup>	7520 ± 445 <sup>*</sup>
CS	27.8 ± 1.3	28.9 ± 1.0	26.1 ± 0.8	26.9 ± 0.7	28.9 ± 1.7	26.5 ± 0.6
HOAD	3.16 ± 0.19 <sup>a</sup>	5.47 ± 0.40 <sup>b</sup>	5.31 ± 0.44 <sup>b</sup>	5.46 ± 0.25 <sup>*</sup>	4.92 ± 0.29	4.97 ± 0.42
GOT	193.1 ± 16.8 <sup>b</sup>	136.1 ± 4.5 <sup>a</sup>	156.4 ± 8.2 <sup>a</sup>	178.9 ± 6.5	177.3 ± 11.3 <sup>*</sup>	189.8 ± 11.8 <sup>*</sup>
GPT	18.3 ± 1.3	18.0 ± 1.0	20.2 ± 1.6	19.3 ± 1.4	18.4 ± 1.1 <sup>*</sup>	18.9 ± 0.9
GDH	38.7 ± 3.2	38.9 ± 4.3	32.9 ± 4.7	28.9 ± 1.1 <sup>*</sup>	29.1 ± 1.0 <sup>*</sup>	27.9 ± 1.5

Enzymatic activities are expressed as mU mg<sup>-1</sup> prot.

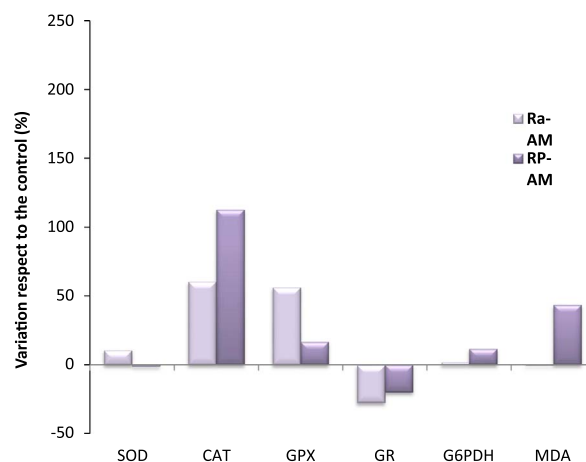
Values are means ± SEM (n=9).

Small letters in each line indicate significant differences (P < 0.05) induced by experimental treatments either in anterior as in posterior muscle.

\* Indicates significant differences between anterior and posterior muscle in each experimental treatment (P < 0.05).



**Fig. 5.** Variations of intermediate metabolism enzymatic specific activities in anterior muscle of refeeding shi drum respect to the control (CR) ones. Bars show % of change over control values. Ra, refeeding ad libitum. RP, refeeding pair fed.



**Fig. 6.** Variations of antioxidant enzymatic specific activities and oxidative damage to lipids in anterior muscle of refeeding shi drum respect to the control (CR) ones. Bars show % of change over control values. Ra, refeeding ad libitum. RP, refeeding pair fed.

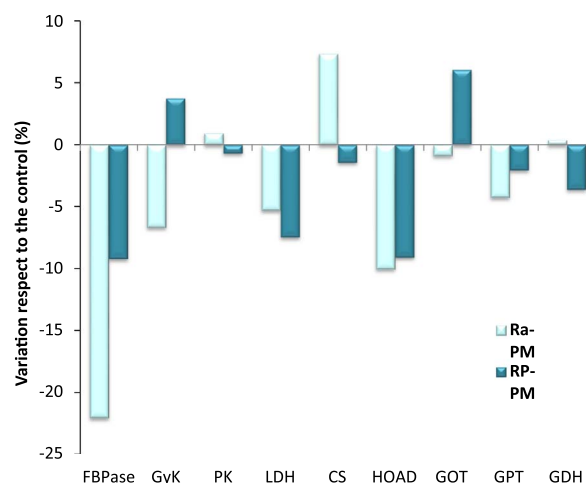
starvation levels in refeed fish, independently of refeeding protocol (Table 3, Fig. 7). Also the activity of antioxidant enzymes in Ra and RP groups were similar to that of controls but while in RP groups signs of oxidative stress are not observed, in Ra groups MDA values were significantly higher (Table 4, Fig. 8). For cod and pike (*Esox lucius*) it has been reported that rates of oxygen consumption following feeding to satiety are considerably higher than those imposed by maximal sustained exercise [31,32]. In this sense, in the present study food intake in Ra groups was a 30% higher than in RP. Such highest food intake and subsequent processing would lead to the increased levels of peroxidation in Ra group.

It must be noted that although food intake in Ra groups was higher than in RP, food efficiency was better in RP fish leading to similar instantaneous growth rates (%/day) in both Ra and RP groups ( $0.36 \pm 0.03$ , and  $0.29 \pm 0.02$ , respectively) that were significantly higher than in controls ( $0.19 \pm 0.02$ ). So, the pair feeding protocol seems to be advantageous since compensatory growth has been taking place without signs of oxidative stress.

**4. Conclusions**

The results indicate the existence of a regional asymmetry of the metabolic capacities in white muscle of shi drum, which is likely related to the different contribution to swimming of the body regions examined.

Starvation induces a metabolic depression that is more marked in those activities that support burst swimming in PM, while those activities supporting maintenance requirements are conserved. The



**Fig. 7.** Variations of intermediate metabolism enzymatic specific activities in posterior muscle of refeeding shi drum respect to the control (CR) ones. Bars show % of change over control values. Ra, refeeding ad libitum. RP, refeeding pair fed.

greatest energy demands during starvation appear to lie in AM, which showed the highest oxidative metabolism rate. The increased use of fatty acids as energy source for AM leads to oxidative stress.

A period of more than four weeks of refeeding for full restoration of metabolic capacities in AM is needed, probably related to the higher muscle mass located in this region. On the contrary, all enzyme activities in PM returned to control levels in both refeeding protocols,

**Table 4**

White muscle specific activity of antioxidant enzymes, and biomarker for lipid oxidative damage (MDA) of shi drum. CR, control. Ra, refeeding ad libitum. RP, pair fed. AM, anterior muscle. PM, posterior muscle.

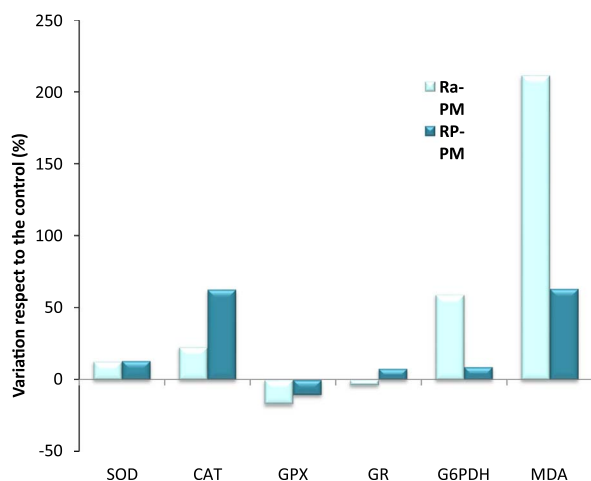
	AM			PM		
	CR	Ra	RP	CR	Ra	RP
SOD	34.6 ± 1.3	38.3 ± 1.5	34.2 ± 1.6	27.6 ± 1.5*	31.2 ± 1.6*	31.3 ± 0.7
CAT	0.18 ± 0.03 <sup>a</sup>	0.30 ± 0.05 <sup>ab</sup>	0.39 ± 0.04 <sup>b</sup>	0.21 ± 0.02	0.25 ± 0.04	0.34 ± 0.05
GPX	1.88 ± 0.29 <sup>a</sup>	2.94 ± 0.15 <sup>b</sup>	2.19 ± 0.13 <sup>a</sup>	2.50 ± 0.12	2.07 ± 0.13 <sup>†</sup>	2.23 ± 0.12
GR	1.33 ± 0.15 <sup>b</sup>	0.96 ± 0.04 <sup>a</sup>	1.06 ± 0.08 <sup>ab</sup>	0.96 ± 0.07 <sup>†</sup>	0.93 ± 0.08	1.04 ± 0.06
G6PDH	0.18 ± 0.02	0.18 ± 0.03	0.20 ± 0.03	0.20 ± 0.02 <sup>a</sup>	0.31 ± 0.01 <sup>b†</sup>	0.21 ± 0.03 <sup>a</sup>
MDA	9.53 ± 0.94	9.48 ± 1.98	13.66 ± 1.39	10.28 ± 1.08 <sup>a</sup>	32.04 ± 6.02 <sup>b†</sup>	16.78 ± 2.21 <sup>a</sup>

Enzymatic activities are expressed as mU mg prot<sup>-1</sup>, except SOD and CAT activities that are expressed as U mg prot<sup>-1</sup> and MDA levels as nmol g tissue<sup>-1</sup>.

Values are means ± SEM (n=9).

Small letters in each line indicate significant differences (P < 0.05) induced by experimental treatments either in anterior as in posterior muscle.

\* Indicates significant differences between anterior and posterior muscle in each experimental treatment (P < 0.05).



**Fig. 8.** Variations of antioxidant enzymatic specific activities and oxidative damage to lipids in posterior muscle of refeeding shi drum respect to the control (CR) ones. Bars show % of change over control values. Ra, refeeding ad libitum, RP, refeeding pair fed.

but pair feeding seems to be advantageous since compensatory growth has been taking place without signs of oxidative stress.

This work was addressed to gain knowledge on the physiology of a promising fish species in aquaculture like shi drum. Besides, few studies have been focused on the repercussions that diverse feeding strategies may have on the muscle as the edible part of the fish. The results displayed here show how the starving and further re-feeding events could generate oxidative stress situations characterized by high lipid peroxidation levels which may influence negatively on the quality of the edible part of the fish. This study opens an interesting field on this fish species which deserves being investigated in the future.

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