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Influence of fasting on muscle composition and antioxidant defenses of market-size *Sparus macrocephalus*[*]

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Abstract: The study was conducted to investigate fasting effects on flesh composition and antioxidant defenses of market-size *Sparus macrocephalus*. Two hundred fish (main initial weight 580 g) were divided into two groups (control and fasted) and reared in 6 cages. After two weeks of adaptation, group I fasted for 28 d; group II was fed normally as a control. In 3, 7, 14, 21 and 28 d, 6 fish per group were sampled for proximate flesh composition, liver antioxidant enzyme activities and malondialdehyde flesh content analyses. In fasted fish, the reduction of lipid content in muscle occurred after day 3, and, compared to controls, the content of protein decreased from day 14, the activities of liver antioxidative enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) increased from day 3, and flesh malondialdehyde levels increased from day 21. Flesh fat reduction shows that fasting may be used as a technique to reduce flesh lipid content in *Sparus macrocephalus*. However, considering flesh protein loss and the subsequent oxidative stress, the fasting technique should be used with precautions.

Key words: Antioxidant defenses, Fasting, Muscle composition, *Sparus macrocephalus*
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INTRODUCTION

Under normal feeding situations, fish grow and store energy reserves, while metabolic demands to maintain life processes in fasted fish are met by mobilizing body nutrient stores leading to alterations in muscle proximate composition (Collins and Anderson, 1997; Guderley *et al*., 2003; Paul *et al*., 1995; Power *et al*., 2000). Unlike mammals, fish may survive long periods of starvation, so it is possible to use fasting as a technique to improve product quality by reducing flesh lipid content (Einen and Thomassen, 1998; Rasmussen *et al*., 2000).

Studies in mammals showed that reactive oxygen species generated by fasting could not be adequately removed when the antioxidant defenses were overcome by pro-oxidant forces. The increased generation of ROS caused pro-oxidant effects, resulting in aging, lipid peroxidation and other body damages (Di Simplicio *et al*., 1997; Robinson *et al*., 1997; Bhat *et al*., 2007; Vogt and Richie, 1993). Lipid peroxidation is directly linked to rancidity in fish, which has a large influence on flesh quality (Gatta *et al*., 2000; Ruff *et al*., 2003). However, studies regarding the influence of fasting on antioxidant defenses and flesh lipid oxidation in fish are scarce (Bastrop *et al*., 1992; Blom *et al*., 2000; Guderley *et al*., 2003; Pascual *et al*., 2003; Viganò *et al*., 1993), and it is important to study the effect of fasting on flesh quality from this respect.

The aim of the present study was to investigate the effects of food deprivation on flesh composition and antioxidant defenses in market-size *Sparus macrocephalus*. This was performed to evaluate the

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possibility of using the fasting technique to manipulate the end product quality of *Sparus macrocephalus* and to establish the suitable duration of fasting.

MATERIALS AND METHODS

Fish and rearing conditions

Sparus macrocephalus weighting (580±32) g were purchased from a private fish farm (Xiangshan Port, Zhejiang Province, China). Two hundred fish collected from a single net-cage were placed in 6 cages of 3 m^3 (1.5 m×1 m×2 m) in size and divided into 2 groups (three replicates each group). During adaptation to experimental conditions for 2 weeks, all fish were fed daily with a natural diet (trash fish) to apparent satiation. Following the adaptation period, one group was continuously fed (the control group) and another group fasted for 28 d. During the experimental period, water temperature and salinity were 22~29 °C and 38 g/L, respectively.

Sampling

On days 3, 7, 14, 21 and 28, 6 *Sparus macrocephalus* per group (2 fish randomly captured from each replicate) were sampled and dissected in a box containing sea water and ice. After removal, liver samples were carefully cleaned of adhering then stored in liquid nitrogen prior to analysis for hepatic antioxidant defense enzyme activities. Each fish was filleted from the left side after discarding the skin. Four pieces of fillet from different part of the fish [upper and lower (belly flap side)] were obtained. Flesh samples were stored in liquid nitrogen until analyses for proximate composition and malondialdehyde (MDA) content.

Proximate composition analysis

The proximate composition analysis of flesh was carried out according to the AOAC International (1999) and reported on a wet weight basis.

Tissue preparation for analytic procedure

Livers and fillets were rapidly thawed and manually homogenized, using a potter homogenizer with a glass pestle, in 9 volumes of ice-cold normal saline (0.86%). All procedures were performed on ice. Homogenates were frozen at −20 °C for 5 min and centrifuged for 15 min at 4000×g (Hitachi CPWX centrifuge, Hitachi Koki Co., Ltd., Japan), and the resultant supernatants were collected for biochemical assays.

Detection of superoxide dismutase (SOD)

The activity of SOD was assayed by the method of Oyanagui (1984). Superoxide anion radical, produced in the reaction of xanthine with O_2 catalyzed by xanthine oxydase, reacts with hydroxylamine producing nitric ion. After nitric ion reacting with naphthalene diamine, sulfanilic acid produces a colored product. The concentration of this mixture is proportional to the amount of produced superoxide anion radical. Increase of absorbance is assayed at 550 nm. Liver SOD activity is expressed in unit per milligram of protein (U/mg protein). One unit (U) means 50% of inhibition by SOD of nitric ion production in this condition.

Detection of glutathione peroxidase

The activity of GPX was determined by quantifying the rate of H_2O_2-induced oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSH). A yellow product which had absorbance at 412 nm could be formed as GSH reacted with dithiobisnitrobenzoic acid (Xia and Zhu, 1987). One unit (U) of GPX was defined as the amount that reduced the level of GSH by 1 μmol/L in 1 min per milligram of liver protein. Liver GPX activity was expressed in milliunit per milligram of protein (mU/mg protein).

Detection of lipid oxidation

The level of lipid peroxidation was indicated by the content of MDA in muscle tissue. Thiobarbituric acid reaction (TBAR) method was used to determine the MDA which can be measured at the wavelength of 532 nm by reacting with thiobarbituric acid (TBA) to form a stable chromophoric production (Ohkawa *et al*., 1979). MDA content was expressed as nanomoles per milligram of protein (nmol/mg protein).

Statistical analysis

The SPSS 10.0 (SPSS Inc., Chicago, IL, USA) package was used for the statistical analysis. A multiple comparison (Student-Neuwman-Keuls) was used to determine the difference within groups. The differences between groups were evaluated with a Paired-Samples *t*-test.

RESULTS

Fasting had a notable influence on flesh proximate composition (Table 1). In fasted fish, the flesh moisture increased significantly (P<0.05) after day 14 and remained high to the end. The content of protein in muscle decreased significantly (P<0.05) from day 14 to day 28. A significant (P<0.05) reduction of lipid content occurred from day 3 in fasted fish and kept low levels to the end.

Hepatic activities of SOD in fasted group significantly (P<0.05) increased from day 3 and kept high to the end of the experiment compared to the fed group (Fig.1). Hepatic activities of GPX in fasted fish also increased from day 3 and kept high to day 28 compared to the controls. At day 21 the GPX activity in fasted group was higher than that in the fed group, but the difference was not significant (P=0.134) (Fig.2). Within the fasted group, hepatic activities of SOD and GPX did not vary significantly at different days of sampling.

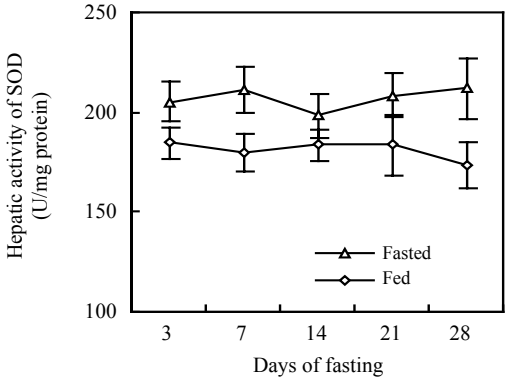

Fig.1 Hepatic activity of superoxide dismutase (SOD) in *Sparus macrocephalus* during the experiment (mean±*SD*)

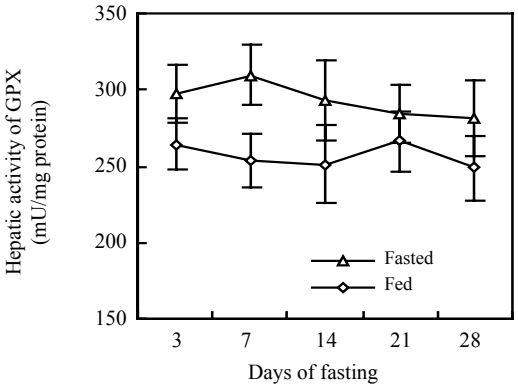

Fig.2 Hepatic activity of glutathione peroxidase (GPX) in *Sparus macrocephalus* during the experiment (mean±*SD*)

Fasting had a notable influence on lipid peroxidation in the flesh of *Sparus macrocephalus* (Fig.3). Fasting did not affect the level of MDA on day 3, 7, or 14. The levels of MDA were significantly (P<0.05) higher in fasted group than those in the fed group on days 21 and 28. The highest MDA levels were found in fasted fish at day 28, reaching level of (19.9±2.5) nmol/mg protein.

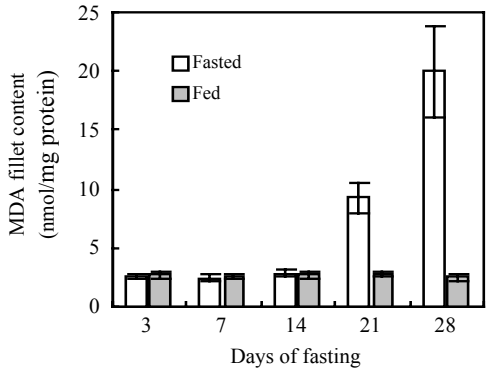

Fig.3 Levels of malondialdehyde (MDA) in flesh during the experiment (mean±*SD*)

Table 1 Effect of food deprivation on proximate fillet composition in *Sparus macrocephalus* (g/kg wet weight)

Days of fasting	Moisture		Lipid		Protein	
	Group I	Group II	Group I	Group II	Group I	Group II
3	754.0±6.9^{aA}	748.0±8.1^{aA}	42.5±1.9^{aA}	48.4±2.6^{aB}	179.1±7.0^{aA}	180.2±5.8^{aA}
7	758.5±9.8^{aA}	751.3±7.7^{aA}	32.7±4.5^{bA}	50.1±4.3^{aB}	176.1±4.9^{aA}	175.7±6.9^{aA}
14	769.2±10.9^{bA}	755.7±4.6^{aB}	18.9±5.2^{cA}	48.2±2.7^{aB}	168.9±5.0^{aA}	177.9±4.3^{aB}
21	772.3±5.6^{cA}	747.2±8.3^{aB}	12.4±2.1^{cA}	47.5±3.1^{aB}	159.4±6.6^{bA}	182.8±7.6^{aB}
28	778.8±8.6^{cA}	743.5±7.4^{aB}	10.3±2.4^{dA}	51.4±3.6^{aB}	156.9±5.8^{bA}	187.0±7.7^{aB}

Group I was fasted group; Group II was fed group. Means in the same column with different lowercase superscript were significantly different (P<0.05) in the same group at different sampling dates. Means in the same row with different uppercase superscript were significantly different (P<0.05) at the same sampling date between two groups (n=6)

DISCUSSION AND CONCLUSION

There are remarkable differences in flesh quality, especially the flesh lipid content, between reared and wild fish (Bergstrom, 1989; Rueda *et al*., 1997; Stroud and Dalgarno, 1982). In comparison with intensively reared fish, wild fish are usually considered as non-fatty fish. Higher lipid level in cultured fish is a general phenomenon observed for a variety of species studied (Ackman and Takeuchi, 1986; Aubourg *et al*., 2007; George and Bhopal, 1995; Grigorakis *et al*., 2002). The high content of lipid in reared fish is highly susceptible to oxidation, one of the major problems of flesh quality postslaughter (Baker, 2001; Ruff *et al*., 2003; Waagbø *et al*., 1993). Fasting can lead to alterations in muscle proximate composition and fat depots, and this technique has been already proved effective in end product improvement of the salmonids (Einen and Thomassen, 1998; Rasmussen *et al*., 2000) and gilthead sea bream (Grigorakis and Alexis, 2005). However, the energy utilization differs among aquatic species and fasting technique is not always effective. For example, perivisceral adipose tissue was utilized first in rainbow trout during the starvation (Jezierska *et al*., 1982).

In this study, the significant reduction of flesh lipid content in fasted fish occurred from day 3 and kept low levels to day 28 compared to controls. The flesh protein content in fasted fish decreased significantly after day 14 in comparison with the controls, while no difference was found before day 7. This suggests that, in fasted *Sparus macrocephalus*, protein starts to mobilize after the fat depots. It seems that the energy utilization in *Sparus macrocephalus* is similar to the gilthead sea bream (*Sparus aurata* L.), but the gilthead sea bream saved protein for 3 weeks (Grigorakis and Alexis, 2005). However, the present results differ from findings in red porgy (*Pagrus pagrus* L.) that showed an initial protein mobilization during the first 14 d of fasting (Rueda *et al*., 1998). Thus, this fasting technique may not be fit for other farmed aquatic species. The results in the present study indicate that fasting technique is effective to decrease flesh lipid content in *Sparus macrocephalus*, thus improving end product quality. To avoid protein loss, the fasting duration ought to be controlled within 7 d.

To evaluate the effects of fasting on the antioxidant defenses in *Sparus macrocephalus*, we examined the hepatic activity of some antioxidative enzymes and the levels of flesh MDA. With respect to antioxidative enzymes, our results showed a significant increase of SOD activity (Fig.1) in the liver of fasted fish from day 3, which suggests that a rise in the O_2^- generation rate might have taken place. The increased SOD activity in fasted fish would result in a higher generation of H_2O_2. Actually, results of the H_2O_2-scavenging enzyme seem to confirm this fact. Hepatic activity of GPX (Fig.2) in fasted fish increased after day 3 and kept high to day 28. An increase of hepatic SOD activity had also been reported in sea bream (*S. aurata*) deprived of food for 46 d (Pascual *et al*., 2003). In *Dentex dentex* deprived of food for 5 weeks, the increases of hepatic SOD and GPX activities had also been reported (Morales *et al*., 2004). These results indicate that the fasting technique gradually generates oxidative stress.

MDA is a metabolite derived from lipid peroxidation and has been widely used as an indicator of oxidative damage and flesh quality (Frigg *et al*., 1990; Halliwell and Gutteridge, 1996; Winston and Di Giulio, 1991). The flesh MDA levels in *Sparus macrocephalus* (fasted and fed) are shown in Fig.3. Fasting did not affect the levels of MDA at the initial days, but started to increase after 21 d. Compared to the controls, the highest MDA levels were found in fish at day 28, where an eight-fold increase in MDA levels was observed compared to the control. These results show that the antioxidant defenses are overcome by pro-oxidant forces, and reactive oxygen species are not adequately removed from day 21. Partial food deprivation has been also reported to increase MDA levels in the livers of rainbow trout, *O. mykiss* (Hidalgo *et al*., 2002) and *S. aurata* (Pascual *et al*., 2003). Our results suggest that fasting enhanced reactive oxygen species generation in *Sparus macrocephalus* and that from day 21 the body defenses were not able to effectively scavenge these reactive oxygen species, thus leading to flesh lipid peroxidation. This lipid peroxidation phenomenon might have a large influence on flesh quality postslaughter, along with autolytic and bacterial spoilage (Watanabe *et al*., 1996); therefore, the fasting duration should be carefully controlled.

The present study demonstrated effects of fasting on fish flesh proximate composition, hepatic ac-

tivity of some antioxidative enzymes and the levels of flesh MDA in *Sparus macrocephalus*. The overall balanced data show that fasting can effectively decrease flesh lipid content during autumn (water temperature were 22~29 °C). Considering flesh protein losses and the flesh lipid peroxidation, we suggest the duration of fasting for *Sparus macrocephalus* should not be longer than 7 d.

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