Effect of Starvation on Kidney Melano-macrophage Centre in Sub-adult Rock Bream, *Oplegnathus fasciatus* (Temminck and Schlegel)

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We conducted a histological analysis to investigate the influence of nutritional changes on melano-macrophages (MMs) accumulation in the kidney of sub-adult rock bream (*Oplegnathus fasciatus*). Four experimental groups were established (initial control, control, fed and starved), and fed commercial feed amounting to 1-3% of their body weight for 2 weeks prior to initiation of experiments. Kidney MMs with dark brown pigment were randomly observed in the kidneys of starved fish, increasing rapidly after 4 weeks, while deposition levels remained low throughout the experiment in the control and fed groups. These results suggest that catabolic tissue breakdown is a major factor contributing to the formation of pigments within MMs. Results also suggest that the degree of MMs deposition in the kidney can be used as alternative indicators in identifying starvation in wild and cultured rock bream.

Key words: Rock bream, Starvation, Kidney, Melano-macrophages (MMs), *Oplegnathus fasciatus*

Introduction

Most fish undergo periods of fasting or starvation due to wintering, spawning, migration or regional decreases in food resources (Hur et al., 2006). Fish can overcome starvation using biochemical, physiological and behavioural strategies. Endogenous energy from basic metabolic accumulations in the body is spent as fish consume their own tissues to remain alive during starvation (Weatherley and Gill, 1987; Lee et al., 1999; Woo, 2005).

In fish, melano-macrophages (MMs) are normally located in the stroma of the haemopoietic tissue within the spleen and the kidney. However, in amphibiams, reptiles, and some fish, they are also found in the liver (Agius and Roberts, 2003). MMs, which are similar to human macro-phages, microscopically metabolize toxic substances and perform various immune functions in the kidney (Roberts, 1975; Agius, 1979a; 1979b; Agius and Roberts 2003).

Melano-macrophages usually contain a variety of pigments (melanins, lipofuscin and haemosiderin) which increase in range and volume in older fish or in the presence of cachectic disease (Agius and Roberts, 2003). Moreover, MMs increase in number in response to various pathological and physiological conditions such as vitamin E deficiency (Palmer et al., 1992), consumption of human-based feed (Blazer and Wolke, 1983), starvation (Agius and Couchman, 1986; Micale and Perdichizzi, 1990; Hur et al., 2006) or aging (Brown and George, 1985).

Melano-macrophages are reported to differ in shape, number, size and content (Agius and Roberts, 1981). Furthermore, researchers have recently examined the possibility of using morphological changes in MMs, such as number and melanin content, as a biomarker in aquatic environments (Macchi et al., 1992; Wolke, 1992). Increase in the number of MMs in the kidney induced by starvation has been studied in rainbow trout (*Oncorhynchus mykiss*), plaice (*Pleuronectes platessa*), swordtail (*Xiphophorus*

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helleri), tilapia (*Tilapia zillii*), masu salmon (*O. masou*) and olive flounder (*Paralichthys olivaceus*) (Agius and Roberts, 1981; Mizuno et al., 2002; Hur et al., 2006).

The rock bream (*Oplegnathus fasciatus* Temminck et Schlegel) belongs to the family Oplegnathidae, and is one of the most economically important fisheries species in Korea. It is widely distributed in the Pacific Ocean, including the southern parts of the Korean Peninsula, Japan, Taiwan, and Hawaii. They are marked with five dark vertical bars on a grayish body when young, although the bars may fade in adulthood. Large adults tend to be charcoal gray with thick black skin on the face. A mature female spawns serially in sandy/gravel substrate from May to July (Kim et al., 2005; An et al., 2006).

The aim of this study is to provide methods, based on histological observations that can easily be applied in the aquaculture industry to estimate rock bream condition, and to extend our knowledge of histological changes that occur in sub-adult rock bream under starvation. Specifically, we investigated histological changes in MMs accumulation in the kidney caused by long-term starvation. These data were used to determine nutritional indices for rock bream.

Materials and Methods

Specimens

Sub-adult rock bream (average body length and weight in all groups were 159 ± 4.8 cm and 165 ± 10.7 g, respectively) was obtained from Sebo Fishery Company (Tongyeung, Korea) and transferred into the Fishery Genetics and Breeding Science Laboratory of the Korea Maritime University in Busan, Korea. Before the initiation of experiment, fish were acclimated to experimental conditions for 1 month. The starvation experiment began in August 2007.

Experimental procedure and rearing

Four experimental groups were established; initial control, control, fed and starved. All fish were fed daily with commercial diet (E-Wha oil & Fat Ind. Co., Busan, Korea: consisting of about 50% crude protein, 8% crude fat, 4% crude fiber, 15% crude ash, 1.5% calcium, 1.7% phosphorous, and 3% mineral on a dry basis) at 1-3% of their total body weight for 2 weeks prior to initiation of experiments in August 2007. The fed and control groups were hand-fed two times daily at 4 hr feeding intervals (the first feeding occurred between 08:00 and 10:00 hours, the second between 17:00 and 19:00 hours). The control group received feed *ad libitum*, while the starved group was subjected to fasting throughout the experiment.

Fishes were reared in a recirculating system. Twenty fishes were placed in each 1.1 tonne fibre glass, reinforced plastics, circular tank (118 cm diameter×100 cm depth); each experimental group consisted of two tanks of fish. Light was provided by four 40 W (5400 K) fluorescent bulbs controlled by an electric timer, which kept the photoperiod at a 12:12 h light/dark cycle. No light was used during the dark period. Water temperature was controlled automatically and held at $22.1\pm0.6°C$ during the experimental period.

The dissolved oxygen concentration of seawater during the experiment ranged from 5.7 to 8.0 mg/L (Table 1). The water exchange rate was 30 times the water volume, and involved replenishing two-third of the water volume with natural seawater once a week. Table 1 shows other water conditions during the experimental period. Each tank was covered with a net to prevent fish from jumping out, and survival was recorded throughout the experiment.

Table 1. Values of water parameters (i.e., temperature, salinity, dissolved oxygen, and hydrogen potential) used during the course of this experiment. These values were monitored for 16 days. COD: chemical oxygen demand; TKN: total Kjehldahl nitrogen; T-N: total nitrogen; T-P: total phosphorus

Parameters	Values
T-N (mg/L)	1.9-4.1
TKN (mg/L)	1.0-1.2
NH_4^+-N (mg/L)	0.9-1.1
NO_3^--N (mg/L)	0.9-2.9
T-P (mg/L)	0.02-0.78
PO_4^{3-}-P (mg/L)	0.004-0.351
COD_{Mn} (mg/L)	2.0-11.1
Water temperature (°C)	19.4-22.4
Salinity (‰)	34.0-37.0
Dissolved oxygen (ppm)	4.7-7.0
Potential of hydrogen (pH)	6.5-7.8

MMs deposition

Ten fishes were removed from the initial control group at the start of the experiment and from each of the other three groups on a 2 weekly basis during the 8-week experimental period. Fishes were euthanized within 2 hrs of sampling using an overdose of lidocaine-HCl (300 mg/L at 22°C; Park et al., 1988) and immediately dissected on an ice-cold cutting board for observation of kidney MMs accumulation. The kidneys were removed and tissue samples were fixed in 10% neutral formalin solution (100 mL formalin, 6.5 g $Na_2HPO_4 \cdot 12H_2O$, 4.5 g KH_2PO_4, 900 mL DW) for 5 days. The samples were then refixed in

Boun's solution for 24 hrs. Samples were prepared in 6 μm thick paraffin sections, placed on slides, stained with haematoxylin and eosin – phroxine B and observed under a microscope (Axioskop, Zeiss, Oberkochen, Germany). Photographs of tissues were also taken. The mean MM area for each photograph was calculated using an NIH IMAGE (Ver. 1.57) system to determine the rate of accumulation.

Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the effect of feeding and starvation on change in MM deposition in the kidney ($P>0.05$), followed by Student-Newman-Keuls multiple range tests using the SPSS statistics package (SPSS 9.0, SPSS Inc., USA).

Results

After 8 weeks, the starved group rapidly lost vitality, and as a result the experiment was terminated. Accumulated survival was $80.1 \pm 2.55\%$ in the initial group, $89.2 \pm 3.47\%$ in the fed group and $91.4 \pm 2.59\%$ in the starved group in each duplicated tanks. Figure 1 shows changes in MMs deposition during the experimental period. At the start of the experiment figures showed $0.11 \pm 0.03\%$, $0.12 \pm 0.01\%$ and $0.11 \pm 0.04\%$ in the control, fed and starved groups respectively ($P>0.05$). Melano-macrophages, recongizable as dark brown pigments were randomly distributed throughout the kidney in the control group (Fig. 2a).

In the starved group, MMs deposition increased with time, going from 0.20 ± 0.04 at 2 weeks, to
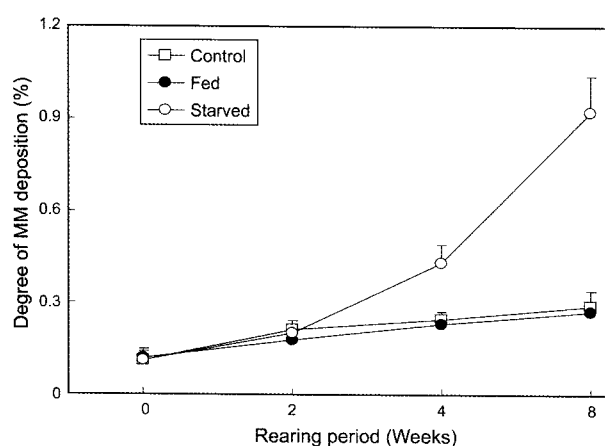

Fig. 1. Changes in melano-macrophage (MM) centres deposition during the experiment in the control, fed, and starved groups in rock bream, *Oplegnathus fasciatus*. Astrick indicate significant difference from the starved and control group values ($P<0.05$).

0.06% at 4 weeks and $0.92 \pm 0.12\%$ at 8 weeks. However, MM deposition ranged from $0.21 \pm 0.03\%$ to $0.29 \pm 0.05\%$ in the control group and 0.18 ± 0.03 to $0.27 \pm 0.07\%$ in the fed group over the experimental period, and did not differ significantly from corresponding values at the start of the experiment ($P>0.05$, Fig. 1). Although no significant difference was observed among the three groups during the first 2 weeks, the degree of MM deposition was significantly higher in the starved group than in the control and fed groups at 4 and 8 weeks ($P<0.05$, Fig. 1).

Under the optical microscope, MMs appeared as small to large round or oval structures. Figure 2b showed kidney tissue from the fed group at 8 weeks. Little MMs deposition was observed in either the fed or control groups (Fig. 2). In contrast, MMs deposition in the starved group at 12 weeks was significantly higher than in the fed group ($P<0.05$, Fig. 2).

Discussion

Under the optical microscope, MMs appear as small-to-large round or oval structures which are easily distinguished from surrounding lymphatic tissue, whereas under the electron microscope they appear as groups of macrophages (Agius and Agbede, 1984). Melano-macrophages are observed in normal fish, but are more numerous in physiologically abnormal states such as disease or stress. Moreover, in these states, the number, size and shape of MMs vary (Agius, 1979b; Agius and Agbede, 1984; Agius and Roberts, 2003; Hur et al., 2006).

The cytoplasm of MMs contains abundant melanin, hemosoderin, lipofuscine (fat-free lipochrome) and ceroid, which react positively to PAS and Ziehl-Neelson reactions. Melano-macrophages vary in colour from yellow to black, mainly because of tissue catabolism (Micale and Perdichizzi, 1990). The colour intensity depends on the type of fish, its age and health status (Agius and Roberts, 1981; Kranz, 1989). Increased number of MMs are related to detoxification and destruction in response to endogenous or exogenous toxins, inflammatory response and circulation (Wolke, 1992). Melano-macrophages increase in the kidney of some teleosts, such as rainbow trout, during starvation (Agius and Roberts, 1981).

Melano-macrophages deposition ranged from 0.21% to 0.29% in the control group and from 0.18 to 0.27 in the fed group during the 8 week experiment. This result suggests that there is no MMs difference according to food quantity. Mizuno et al. (2002)
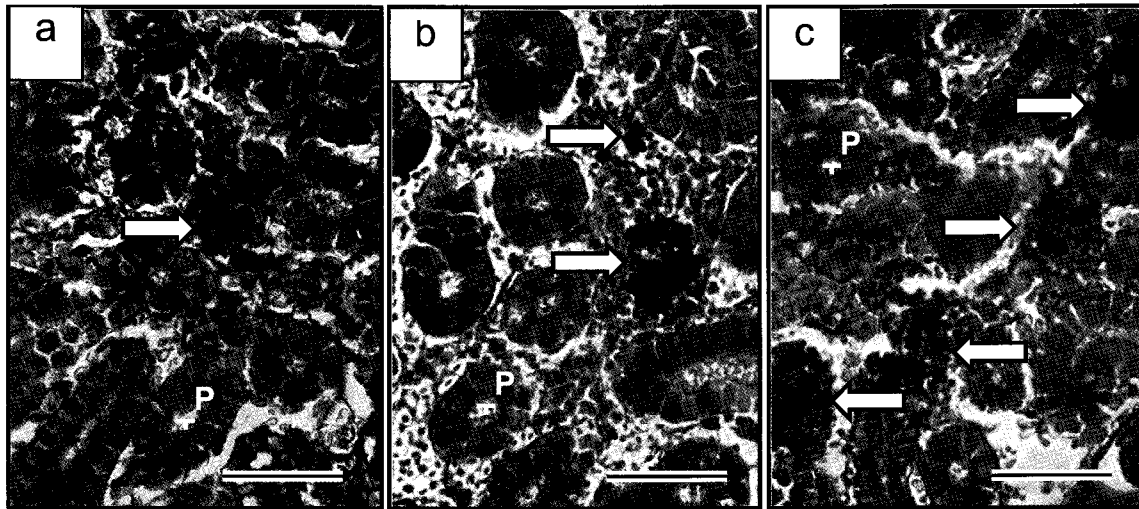
Fig. 2. Histological observations of melano-macrophage (MM) centres on the kidneys of (a) control, (b) fed and (c) starved group in rock bream, *Oplegnathus fasciatus*. The arrows indicate increase in degree of MM deposition during starvation. Scale bars are 20 μm. PT, proximal tubule.

reported that MMs deposition in wild type and hatchery-reared masu salmon was 0.1-0.2% and 0.1-0.7%, respectively, which is similar to that observed in the control and fed groups of sub-adult rock bream. No significant changes were observed histologically in the fed group during the experimental period. However, MMs deposition increased significantly in the starved group similar to results reported for plaice, rainbow trout, swordtail, tilapia (Agius and Roberts, 1981) and blue tilapia (*Oreochromis aureus*) (Agius and Couchman, 1986), sea bream (*Diplodus annularis*) (Micale and Perdichizzi, 1990), masu salmon (Mizuno et al., 2002) and olive flounder (Hur et al., 2006). Rates of MMs deposition ranged from 0.20% to 0.92% with starvation from week 2 to week 8. These results agree with the 0.1-0.9% increase in MMs deposition observed in masu salmon by Mizino et al. (2002).

The rate of MMs deposition due to starvation was directly related to mortality, which agrees with observations of masu salmon after 60 days of starvation (Mizuno et al., 2002). After 60 days of starvation, mortality was 20% (0.9% MMs deposition) in the starved group and 15% (0.7% MMs deposition) in the fed group (Mizuno et al., 2002). Comparatively, in the olive flounder after 12 weeks of starvation, mortality was 22.5% (1.10% MMs deposition), 10.8% (0.29% MMs deposition) and 10.0% (0.28% MM deposition) in the starved, control and fed groups, respectively (Hur et al., 2006). Similarly in rock bream, mortality by the end of the experimental period was 19.9%, 10.8% and 8.6% and the respective MM deposition was 0.92%, 8.6% and

10.8% in the starved, control and fed groups, respectively.

The distribution of MMs deposition, and their shape, size and number increase with various pathological and physiological conditions (Palmer et al., 1992; Agius and Roberts, 2003) such as vitamin E deficiency, humus-based feed (Blazer and Wolke, 1983) and starvation (Agius and Couchman, 1986; Micale and Perdichizzi, 1990). Mortality and MMs deposition increased with starvation in rock bream, suggesting that starvation presents a significant physiological burden to fish.

Increased MMs deposition owing to starvation has been observed in various organs of different species such as dogfish (*Schliorhinus canicula*), rainbow trout, plaice and tilapia (Agius and Roberts, 1981; Hur et al., 2006). Agius and Roberts (1981) reported that this increase in MMs deposition is mainly due to catabolic tissue destruction. Changes in MM deposition are also induced by feed, water conditions, water temperature, age and seasonality (Blazer et al., 1987). Therefore, further studies are needed to establish the possibility of using different morphological changes, such as the number of MMs expressed, and their size and degree of deposition, as biological and physiological marker (Macchi et al., 1992; Wolke, 1992).

Acknowledgements

References

Agius, C. 1979a. The role of melano-macrophage centres in iron storage in normal and diseased fish. J. Fish Dis., 2, 337-343.

Agius, C. 1979b. Aspects of the melano-macrophages centres in fish. PhD Thesis, University of Stirling, vi+, 165.

Agius, C. and S.A. Agbede. 1984. An electron microscopical study on the genesis of lipofuscin, melanin and haemosiderin in the haematopoietic tissues of fish. J. Fish Biol., 24, 471-488.

Agius, C. and W. Couchman. 1986. Induction of alkalin phosphates activity in the melano-macrophage centres of Oreochromis aureus (Steindachner) through starvation and vaccination. J. Fish Biol., 28, 87-92.

Aguis, C. and R.J. Roberts. 1981. Effects of starvation on the melano-macrophage centres of fish pathology. J. Fish Biol., 19, 161-169.

Agius, C. and R.J. Roberts. 2003. Melano-macrophage centres and their role in fish pathology. J. Fish Dis., 26, 499-509.

An, H.S., J.W. Kim and J.Y. Park. 2006. Microsatellite DNA loci in the rock bream Oplegnathus fasciatus. Mol. Ecol., 6, 44-46.

Blazer, V.S. and R.E. Wolke. 1983. Ceroid deposition, retinal degeneration and renal calcium oxalate crystals in cultured clown fish, Amphiprion ocellaris. J. Fish Dis., 8, 135-138.

Blazer, V.S., R.E. Wolke, J. Brown and C.A. Powell. 1987. Piscine macrophage aggregate parameters as health moniters: effect of age, sex, relative weight, season and site quality in largemouth bass (Micropterus salmoides). Aquat. Toxicol., 10, 199-215.

Brown, C.L. and C.J. George. 1985. Age dependent accumulation of macrophage aggregates in the yellow perch, Perca fluviatilis (Mitchell). J. Fish Dis., 8, 135-138.

Hur, J.W., S.R. Woo, J.H. Jo and I.S. Park. 2006. Effects of starvation on kidney melano-macrophage centre in olive flounder, Paralichthys olivaceus (Temminck and Schlegel). Aquacult. Res., 37, 821-825.

Kim, I.C., Y. Choi, C.L. Lee, Y.J. Lee and B.J. Kim. 2005. Illusted book of Korean fishes. Kyo-Hak Publishing Co., Seoul, Korea, 615.

Kranz, H. 1989. Changes in splenic melano-macrophage centres of the dab Limanda limanda during and after infection with ulcer disease. Dis. Aquat. Org., 6, 167-173.

Lee, K.K., Y.H. Kim and I.S. Park. 1999. Effect of starvetion in some nutritional parameters in Rhynchocypris oxycephalus. 1. Characteristics of the histological and biochemical changes. Korean J. Ichthyol., 11, 33-41.3

Macchi, C.J., L.A. Romano and H.E. Christiansen. 1992. Melano-macrophage centres in the whitemouth croaker, Micropognis furnieri, as biological indicators of environmental changes. J. Fish Biol., 40, 971-973.

Micale, V. and F. Perdichizzi. 1990. A quantitative and histochemical study on melano-macrophage centres in the spleen of the teleost fish Diplodus annularis L. J. Fish Biol., 37, 191-197.

Mizuno, S., N. Misaka, Y. Miyakoshi, K. Takeuch and N. Kasahara. 2002. Effects of starvation on melano-macrophages in the kidney of masu salmon (Oncorhynchus masou). Aquaculture, 209, 247-255.

Palmer, R., R.H. Soutar, E.J. Branson, P.J. Southgate, E. Drivan, R.H. Fichards and R.O. Collins. 1992. Mortalis in the Atlantic salmon, Salmo salar L. associated with pathology of the melano-macrophage and haemopoietic systems. J. Fish Dis., 15, 207-210.

Park, I.S., J.M. Kim, Y.H. Kim and D.S. Kim. 1988. Influence of lidocaine as an anesthetic for marine fishes. J. Fish Pathol., 1, 123-130.

Roberts, R.J. 1975. Melanin-containing cell of teleost fish and their relation to disease. In: W.E. Ribelin and G. Migaki (eds), The Pathology of Fishes, University of Wisconsin Press, Madison, WI, USA, 399-428.

Weatherley, A.H. and H.S. Gill. 1987. The biology of fish growth. In: 4. Protein, Lipid and Caloric Contents. Academic Press, London, UK, 139-146.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. Annu. Rev. Fish Dis., 2, 91-108.

Woo, S.R. 2005. Effect of starvation on parameters in olive flounder, Paralichthys olivaceus (Temminck et Schlegel). Master Thesis, Korea Maritime University, Korea, 76.