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Measurement of prolactin (PRL), growth hormone (GH), insulin-like growth factor-I (IGF-I), cortisol and insulin in gilthead seabream *Sparus auratus*

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Abstract

The role of GH, IGF-I, PRL, insulin and cortisol is very important in fish growth and metabolism. The aim of this pilot study was to use of human diagnostic kits, based on the method of chemiluminescence, to determine the highly conserved hormones in teleost fish seabream (*Sparus auratus*) farmed in floating marine cages. Blood was sampled from six adult male individuals. Total protein levels exhibited values from 2.9 to 4.2 g/dl. Cholesterol mean value (mv) in plasma was 292 mg/dl vs 267 mg/dl in serum. Glucose mv was higher in plasma (97 mg/dl) than in serum (76 mg/dl). Insulin levels ranged from 1.24 to 1.40 μIU/ml, PRL was undetectable. GH levels range 0.033-0.046 ng/ml. IGF-I mv was measured 29.16 ng/ml in plasma and 12.83 ng/ml in serum, Cortisol mv was 9.8 and 7.2 μg/dl in plasma and serum respectively. No significant differences were observed between fed and fasting condition, similarly with anesthetized and non anesthetized individuals. These preliminary data are so far to be thought conclusive. However, the results are indicative of the fact that human kits, based on the method of chemiluminescence, properly modified, could be used for hormonal evaluation in fishes.

Keywords: *Sparus auratus,* Prolactin, Growth hormone, Insulin-like growth factor-I, Cortisol, Insulin

Introduction

The welfare status of cultured fishes is well known as topic of great interest. Numerous studies have investigated the aquaculturing conditions in order to develop new technologies and practices in this area (North et al. 2006; Roncarati et al. 2006). The implicated factors are stocking density, food preparations, water salinity and temperature, photoperiod, handling procedures, anesthesia conditions and other environmental stressors (Imsland et al. 2008; Di Marko et al. 2008; Montero et al. 2001).

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All these are involved in fish growth, reproduction and metabolism. Hormones named growth hormone (GH), insulin-like growth factor-I (IGF-I), cortisol, insulin, prolactin (PRL), are considered as sensitive indicators (Funes et al. 2006; Laiz-Carrión et al. 2009). The above mentioned highly conserved hormones have been widely studied in various teleosts species, like *Dicentrarchus labrax, S. auratus, Solea senegalensis, etc* (Duan 1998; Funes et al. 2006; Varsamos et al. 2006). The gilthead sea bream (*S. auratus*), is used as a Mediterranean fish model for growth endocrinology studies.

Specific radioimmunoassays (RIAs), used for hormones measurement, have been described in literature. Some of them are commercially available (Dyer et al. 2004; Varsamos et al. 2006). However, for every day practice, RIAs have been replaced by other less time consuming and harmful methods, as enzymatic or chemiluminescent immunoassays. Taking into account the high sensitivity and specificity of the latter, we have tried to evaluate hormones blood levels in *S. auratus* by chemiluminescence using human kits.

Materials and methods

The study was conducted on *S. auratus* farmed in floating marine cages in Itea bay, Greece, more specifically in Continova bay (N 38°24'40,36'' and 22°23' 42,44'') under natural conditions of sunlight, sea water temperature 21 °C, dissolved oxygen 80%, pH 8.14 and salinity 35.5‰. The initial fish population loaded in the cages in the end of March 2008 was 62000 individuals with an average weight of 6 g.

Blood was sampled from six adult male individuals (mean weight: 400-410 g, 18 months old). Two of them were anesthetized with Finquel (MS-222) manufactured by Argent Laboratory while the other four were not. Blood was sampled under different dietary conditions (fish fed and non). Biochemical parameters (glucose, cholesterol, total proteins) were measured by a colorimetric assay in an auto analyzer (P800 subunit, Hitachi-Modular, Roche). Insulin, cortisol and PRL, were measured with electrochemiluminescent immunometric assays in ELECSYS 2010 analyzer, Roche. GH and IGF-I were measured by a chemiluminescent immunometric assay in IMMULITE 1000 analyzer, Siemens. Successive dilutions have been done in GH, IGF-I and PRL assays. Mann Whitney test ($P < 0.05$) was applied in order to examine the difference between fed *vs* fasting, anesthetized *vs* non anesthetized and serum *vs* plasma measurements using SPSS 16.

Results and discussion

Proteins levels exhibited values ranging from 2.9 to 4.2 g/dl (kit measurement range [kmr]): 0.2-15.0 g/dl. Cholesterol mean value (mv) in plasma was 292 mg/dl vs 267 mg/dl in serum, kmr: 3-800 mg/dl. Glucose mv was higher in plasma than in serum (97 mg/dl and 76 mg/dl, respectively), kmr: 2-750 mg/dl. Insulin levels ranged from 1.24 to 1.40 μIU/ml, kmr: 0.2-100 μIU/ml. PRL was undetectable, kmr: 0.047-470 ng/ml. GH levels were also very low (0.033-0.046 ng/ml), kmr: 0.01-40.0 ng/ml. IGF-I mv was measured 29.16 ng/ml in plasma and 12.83 ng/ml in serum, kmr: 20-1600 ng/ml. Cortisol mv was 9.8 and 7.2 μg/dl in plasma and serum respectively, kmr: 0.018-63 μg/dl. In all cases no significant differences were observed between fed and fasting condition, except for the cases of insulin (P= 0.06) and GH (P= 0.06) where a borderline significance was revealed.

Moreover, non significant differences were appeared comparing the anesthetized and non anesthetized individuals, except borderline cortisol value (P= 0.06). Between the plasma and serum values only IGF-I, showed P= 0.05. (Table 1).

Table 1. Results of Mann whitney tests (P-values)

	Cortisol	GH	Insulin	IGF-I	GLU	CHOL	TP
Fed *vs* fasting	1.00	0.06	0.06	0.35	0.64	0.35	0.81
Anesthetized *vs* non	0.06	1.00	0.16	0.35	0.16	0.35	0.81
Plasma *vs* serum	0.82	0.82	0.51	0.05	0.27	0.51	0.82

GH: Growth Hormone, IGF-I: Insulin-like Growth Factor-I, GLU: Glucose, CHOL: Cholesterole (total), TP: Total Protein.

It was clear that the same no statistically significant results were found using the independent samples *t*-test. Glucose, cholesterol, triglycerides and total proteins concentrations are changing under different physiological or acute stress conditions in S. auratus (Montero et al. 1999, 2001).

Seasonal variations of gonadal steroids and haematological parameters have been reported for *Dicentrarchus labrax* (Kavadias et al. 2003, 2004). In our study cholesterol and glucose levels were higher in plasma than in serum, as was expected. Insulin levels have been found at low concentrations, as well as GH levels, almost near to kit sensitivity value. The determination of GH and PRL, by RIAs, sometimes with solvent extraction steps or the use of combined radioenzymatic techniques, reveal their critical role in growth, metabolism, osmoregulation and reproduction. PRL gene has been isolated from S. auratus in addition with very low blood levels of GH and PRL measured (Astola et al. 2003).

According to our knowledge specific RIA for PRL in *S. auratus* is not available. In response to a variety of stressors (salinity changes, overloading and food-deprivation), pituitary CH and PRL may decrease or increase (Laiz-Carrión et al. 2009; Company et al. 2001; Sangiao-Alvarellos et al. 2006). References for PRL in blood are minimal, probably due to the extremely low levels of PRL.

Several studies concerning IGF-I show that its precise effects on teleosts have not been clarified. Specific RIA has been developed and validated, with parallel production of recombinant peptides and antibodies for confirmation of IGF-I and GH changes to stress (Vega-Rubín de Celis et al. 2004; Dyer et al. 2004).

Cortisol is the principal corticosteroid hormone in fishes and its function as both glucocorticoid and mineralocorticoid hormone, is important in the regulation of glucose biosynthesis by the liver (gluconeogenesis), and in adaptation to hypersaline environment. Rapid increase in cortisol levels occur in response to a wide range of stressors (Marino et al. 2001). Cortisol also rapidly decreases the release of PRL by nongenomic action. Prolonged stressor induced elevation of cortisol can also impair reproductive and immune functions, and negatively influence growth by stimulating protein catabolism.

In conclusion, although measurable levels of several important hormones have been found by using human diagnostic kits, further studies are necessary on a considerable number of cage farmed fishes (with known age and feeding conditions of the specimens). In such a case the control group should be tested through the established specific RIA methods in order to validate the human (electro) chemiluminescent immunometric assays for fish hormone determination. Prevention is necessary for the rational exploitation of a fish farm since it assures the robustness of the cultured species. The reliable measurement of the influence of both anthropogenic and environmental stress on the fish, by using a simple method, like the one proposed, can lead to prompt diagnosis and quick intervention so that the desirable results in the production can be achieved. These results firmly bind with the welfare of the cultured species.

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