The influence of acute handling stress on some blood parameters in cultured sea bream (Sparus aurata Linnaeus, 1758)

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Abstract

The effect of acute handling stress on haematological profile, blood glucose and lactate (secondary stress markers) in cultured sea bream Sparus aurata was evaluated. Sixty six Sparus aurata were used and equally divided into two groups (A and B). Group A was not subjected to stress, Group B was subjected to acute handling stress. From each fish, biometric data and blood samples were collected to evaluate haematological profile, blood glucose and lactate. Unpaired t-test Student was applied to evaluate possible differences in parameters between the two groups. Red blood cells, haematocrit, haemoglobin, white blood cells (WBC), glucose and lactate showed an increase (P<0.05) in Group B compared with Group A, while mean corpuscular volume decreased (P<0.05) in Group B. The results highlight the role of studied parameters in monitoring the stressful conditions of aquaculture production which affect animal welfare and fish products quality.

Introduction

Acute or chronic stressors, unavoidable consequences of normal hatchery practices in aquaculture, strongly affect fish physiology including growth, reproduction and welfare (Eslamloo et al., 2014). Management practice has been recognised as a key to profitable and sustainable fish farming. This has led to the application of manipulation strategies to maximise fish production in the culture environment. Some aquaculture procedures commonly used, such as acclimation and handling, affect the stress response of fish (Ashley, 2007). Fish’s responses to stress are known to cause alteration of blood characteristics, reduction of growth rate and reproductive process, suppression of immune system, disruptions in metabolic activities and alteration of fish products quality (Wells and Pankhurst, 1999). The primary stress response in fish involves the release of catecholamines and the activation of the hypothalamic-pituitary-interrenal. Hypothalamic-pituitary-interrenal activation results in energy source mobilization, depletion of glycogen stores, and an increase in plasma levels of glucose, along with high muscle activity, anaerobic glycolysis and an increase in plasma lactate. Therefore, the levels both of glucose and lactate in plasma are often used to assess stress levels (Arends et al., 1999; Acerete et al., 2004).

Haematological parameters are important to assess the physiological status of fish and to monitor stress and pathological changes (Fazio et al., 2012b). A basic knowledge of the haematology represents a valuable guide to assess the condition of aquatic organisms and it is widely used as indicator of environmental stress. Haematological and haematochemical responses to a particular stress factor are quantitatively related to the severity and longevity of the stress.

The aim of the present study was to evaluate the effect of acute handling stress on haematological profile, blood glucose and lactate (secondary stress markers) in cultured sea bream Sparus aurata.

Materials and Methods

A total of 66 adult sea bream (Sparus aurata) in excellent health status, taken from an onshore aquaculture system located on the South-Eastern coast of Sicily, was used in this study. Water physico-chemical parameters measured in the tank using a multiparametric probe C203 (Hanna Instruments, Woonsocket, RI, USA) were the following: temperature=21°C, salinity=38‰, dissolved oxygen=5 ppm.

The following experimental protocol was used: fish were divided into two groups (Groups A and B), of 33 fish each. During the experimental protocol the two groups were transferred in two tanks, respectively, equipped with aerators. The tanks were in a flow-through system. Fish of Group A were not subjected to stress (control group), while those of Group B were subjected to acute handling stress (3 times at 10 min intervals for 30 min).

From each fish biometric data and blood samples were collected. Fish of each group were not anaesthetised prior to blood sampling. Blood samples were collected immediately after capture (Group A) and immediately after the last stress administration (Group B) by venipuncture from caudal vein using a sterile plastic syringe (2.5 mL). Blood samples were transferred into a tube (Miniplast 0.5 mL; LP Italiana Spa, Milan, Italy) containing ethylenediamine tetraacetic acid (1.26 mg/0.5 mL) as an anticoagulant agent to evaluate haematological profile, blood glucose and lactate levels.

Haematological profile was assessed within 2 h from collection using a blood cell automatic counter HeCo Vet C (SEAC, Florence, Italy) and blood lactate and glucose levels were assessed immediately after blood sampling using the portable blood glucose (ACCU-Chek Active; Roche Diagnostics, Basel, Switzerland) and blood lactate analyser (Accusport, Boehringer, Germany). Unpaired t-test Student was applied to evaluate the differences of parameters studied between the two groups.

Results

No significant difference in biometric indexes between the two groups was found (Table 1). Red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), white blood cells (WBC), glucose and lactate levels (P<0.05) showed a significant increase in Group B in comparison with Group A, while mean corpuscular volume (MCV) statistically decreased (P<0.05) in Group B with respect to Group A (Table 2).
Discussion

Aquaculture practices including intensive handling,crowding during most of the catching protocols, pre-slaughter and slaughter, could be a very traumatic time for the farmed fish, involving the onset of a stress status which can compromise the organoleptic, marketable quality and sanitary quality of the final product. Stress is linked to a reduction of flesh quality and this is associated mainly with the pre-slaughter distress. In fact, the relative endocrine responses imply alterations before death starting processes of recall and intense consumption of glucose reserves which cause modifications of normal post-mortem processes and higher susceptibility to microbial attack. Stress during harvesting and death times plus relative endocrine response can hardly influence post-mortem biochemical processes such as the adenosine triphosphate degradation rate, rigor mortis onset and release, and freshness involution rate (Conte, 2004; Ashley, 2007; Poli, 2009).

The development of methods for monitoring metabolic indicators of stress in fish has obvious potential for improving husbandry protocols and post-harvest product quality. Haematological parameters as well as blood glucose and lactate levels are routinely used for the evaluation of physiological environmental and husbandry stressors in fish (Gabriel et al., 2011). In the present study significant higher RBC, Hct, Hb, WBC, glucose and lactate levels were found in Group B, while lower MCV levels was found in Group B compared to Group A. Higher RBC, Hct, and Hb levels found in the stressed group may be due to splenic contraction. In fact, it is known that the spleen is a major storage organ for blood cells and it is known to contract in teleost fish during acute stress (Ruanu et al., 2006). Contraction of the spleen results in the release of blood cells into the circulation and may account for the increase in erythrocytes. An enhancement in RBC, Hct and Hb levels following an acute stress was reported in most previous study (Olsen et al., 2008; Suskii et al., 2007) and it was described as a strategy to improve blood capacity to carry oxygen under the high energy demand condition (Eslamloo et al., 2014). The MCV decrease in stressed group may indicate a release of smaller immature erythrocytes due to splenic contraction. According to several studies on stressed fish response (Puisford et al., 1994; Gabriel et al., 2011), the WBC increase in Group B confirms a stress condition and may also be caused by their migration from spleen to the blood circulation (Gabriel et al., 2011). Although glucose and lactate levels’ increase have been shown in fish exposed to chronic stress (Barcellos et al., 2009), the higher blood glucose and lactate levels found in this study in the stressed group could be caused by repeated acute stress affecting fish as chronic stress (Eslamloo et al., 2014). Higher level of plasma glucose following stress generally occurs in response to the release into the circulation of stress-induced hormones, mainly epinephrine and norepinephrine, triggering muscle or liver glycogenolysis and releasing glucose for the increased energy demands during and after stress (Eslamloo et al., 2014). Thus, the increase of glucose found in this study may be explained by a high demand of glucose by tissues of fish faced with repeated stress. The increased energy demands during and after stress also led to the increase in blood lactate. The increase of lactate is caused by anaerobic activity of muscles (Wang and Richards, 2011) and may be experienced in hypoxic stress conditions triggering instant glycolysis (Eslamloo et al., 2014). Plasma lactate has also been found to increase in other fish species 1 and 3 h after a single acute stress (Costas et al., 2011; Olsen et al., 2008; Suskii et al., 2007).

The increase of anaerobic glycolysis in muscle due to higher energy mobilisation and utilisation likely to occur under stress condition implies an increase of lactate and a related decrease of muscle pH (Poli, 2009). When recovery of fish is not possible, for example when fish are slaughtered in a short time, the muscle pH will remain low and will further decrease due to the post-mortem glycolytic activity, thus compromising the quality of fish products (Poli, 2009).

Conclusions

The results of this study show that acute handling stress affects some haematological parameters and secondary stress markers of Sparus aurata and suggest that the changes of these parameters are particularly useful for monitoring the stressful conditions of aquaculture production which affect not only animal welfare but also the quality of fish products.

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Table 1. Mean valuesstandard deviation of biometric parameters obtained in Groups A and B.

| Biometric parameters | Sparus aurata (Linnaeus, 1758) |
|----------------------|--------------------------------|
|                      | Group A (n=33) | Group B (n=33) |
| Fork length (cm)     | 19.64±0.95    | 18.96±1.10    |
| Weight (g)           | 182.30±29.75  | 189.60±27.42  |
| Condition factor (g/cm²) | 2.42±0.39    | 2.88±0.97    |

Table 2. Mean valuesstandard deviation of haematological, glucose and lactate data obtained in Groups A and B.

| Blood parameters | Sparus aurata (Linnaeus, 1758) |
|------------------|--------------------------------|
|                  | Group A (n=33) | Group B (n=33) |
| RBC (×10⁹/μL)    | 2.74±0.13     | 3.35±0.40*    |
| Hct (%)          | 49.83±2.51    | 56.98±3.40*   |
| Hb (g/dL)        | 9.07±0.44     | 10.78±0.20*   |
| WBC (×10⁹/μL)    | 45.73±2.82    | 48.10±2.44**  |
| TC (×10⁹/μL)     | 104.75±38.73  | 110.23±30.11  |
| MCV (FL)         | 181.60±1.99   | 171.20±6.78** |
| MCH (pg)         | 33.05±0.74    | 32.38±2.11    |
| MCHC (g/dL)      | 18.34±0.61    | 18.92±0.87    |
| Glucose (mg/dL)  | 124.60±32.45  | 254.00±34.45* |
| Lactate (mmol/L) | 5.04±0.90     | 7.51±1.17*    |
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