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Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*)

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ABSTRACT

Two stunning/slaughtering methods, ice asphyxia and carbon dioxide narcosis, as common capture procedures in commercial farms, were compared with asphyxia without ice in order to assess their suitability as stunning methods for European sea bass. In this study we have analyzed the stunning effects on the stress response related to fish welfare, and on the meat quality. Sampling was performed 0, 2, 9, 24, 48, 72 and 96 h after death, and for all sampling times, *rigor mortis* assessment, muscle pH, eye refraction index and sensory evaluation were determined as quality parameters. Moreover, osmolality, glucose, lactate and cortisol were determined as stress indicators. The muscle pH values were significantly lower in CO_2 narcosis group from the first 9 h until the end of the study in comparison to the respective zero time. If we compared muscle pH values between each slaughtering method with the asphyxia group through sampling time, we observed statistically higher values in CO_2 and ice asphyxia throughout the study. The sensorial evaluation showed a progressive decrease in the three groups analysed versus the respective zero time, although the comparison with the control group showed statistical differences only at zero and nine hours sampling time. Regarding stress indicators, all of them showed significant higher levels after both slaughtering methods compared with the undisturbed group and significant lower levels if compared with the asphyxia group. Our results also show that asphyxia produced a five-fold increase in glucose levels whereas other methods studied only produced a 1.5–1.7-fold increase. Cortisol levels increased 8-fold after asphyxia whereas the other slaughtering methods provoked a 5-fold increase. In summary, the results indicate that both methods are suitable to process fish although the CO_2 treatment showed lower lactate and cortisol response compared to asphyxia in ice. In terms of quality, differences between methods are minor.

1. Introduction

European sea bass, *Dicentrarchus labrax* is a popular and also valued species for consumers in the Mediterranean area, where it is mainly produced as well as in other parts of Europe. This acceptance is based on the traditional consumption and in its characteristic taste quality. As the production of the European sea bass rose to 88.531 tones per year in 2006 (according to the Federation of European Aquaculture Producers), the interest of maintaining the quality characteristics has been an increasing concern for the aquaculture industry. In addition, as well as the overall animal production sector, the aquaculture industry is also concerned with the ethical and welfare issues. Recently, the Scientific Panel on Animal health and welfare of the European Food safety authority has issued an opinion related to the animal welfare during stunning and killing procedures in farms, recommending further investigations on the mechanisms and effects of different stunning/killing methods (EFSA Journal, 2004).

It is important to obtain information on how the usual husbandry methods could affect welfare and quality in cultured fish and to propose alternative procedures. One of the critical steps that affect both quality and welfare is the stunning/slaughtering procedure.

One way to minimize the suffering during slaughter is to use methods that make fish less sensitive to stressful procedures prior to the actual death. Recent reviews on slaughtering methods for fish (Robb et al., 2000) presently address questions about the possibility of ethical considerations in industry procedures for slaughtering (Lambooij et al., 2002). Various anaesthetizing methods meet this demand and, besides, do not negatively affect the final quality of fish (Ribas et al., 2007). Of course, those based on the use of chemicals, should be rejected considering the potential risk for human consumption. Today it is important to continue the research on stunning methods alternative to chemical anesthetics, and especially stunning/killing methods, as this will simplify commercial procedures.

Besides the ethical considerations and therefore to avoid fish suffering, there are also economical and commercial reasons, since

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quality meat is reduced in badly treated fish. To assess the quality traits in fresh fish, several indicators have been used by different authors (Parisi et al., 2002; Lougovois et al., 2003). Amongst the biochemical indicators, some are more related to flesh deterioration, such as concentrations of amines, ammonia, trimethylamine, dimethylamine, and lipid peroxidation compounds. A good indicator of freshness is the degradation of nucleotides expressed as indicators such as the *K* value or the relative ATP/IMP content ratio (258/250 nm absorbance ratio of perchloric extracts) (Huss et al., 1992). Other indicators could be used for shorter time effects on quality. Amongst the physical indicators the measurement of muscle pH, texture, eye liquor refraction index and muscle water retention capacity have been used (Poli et al., 2005). It is well known that inadequate handling before the slaughtering may lead to faster flesh deterioration (Lowe et al., 1993; Sigholt et al., 1997). Both the capture and sacrifice methods have an influence on the post-mortem beef muscle degradation, causing changes in *rigor mortis* onset and resolution, drop of muscle pH and loss of water retention capacity, protein denaturation, lipid oxidation and increase of microbiological flora (Tornberg et al., 2000). Similar effects have been found for different fish species, i.e. common seabream, *Pagrus auratus* (Lowe et al., 1993), rainbow trout, *Oncorhynchus mykiss*, common carp, *Cyprinus carpio*, european eel, *Anguilla anguilla* (Marx et al., 1997) and Atlantic salmon, *Salmo salar* (Skjervold et al., 1999).

Fish experiencing severe stress develop a stress response in which hormonal, biochemical, osmoregulatory, immune and energetic alterations take place (Tort et al., 1996; Tort, 1998; Barton, 2002). That is why we think that a reliable assessment of animal welfare and quality requires the study of stress indicators. Many physiological changes are involved in such a stress response including hematology (Dethloff et al., 1999) osmolality (McDonald and Milligan, 1997), hormone release, and energetic metabolism (Carragher and Rees, 1994; Barton and Iwama, 1991). One of the most accepted primary responses to stress is the increase in plasma cortisol (Barton, 2002). European sea bass is considered more sensitive to stress than other species such as gilthead sea bream, *Sparus aurata* (Rotllant et al., 2001), because the cortisol levels of unstressed fish are in the upper range across fish species (Cerdá-Reverter et al., 1998; Roche and Bogé, 1996 Planas et al., 1990). On the other hand, previous work on the mechanisms of cortisol release in the sea bass demonstrated a continuous production of cortisol under control conditions, but lower reactivity to adrenocorticotropin hormone (ACTH) pulses (Rotllant et al., 2003).

Although some works have shown quality changes after slaughtering in European sea bass (Poli et al., 2001; Parisi et al., 2002), this paper describes in addition the relationship of the quality indicators with the degree of stress caused by the different slaughtering methods.

So the present study aims to provide an integrated vision of the effects of different slaughtering methods regarding stress response and quality changes in the European sea bass (*D. labrax*). There are different stunning and slaughtering methods commonly used in fisheries or aquaculture in European farms for some of the most important cultured species, such as gill cut, CO_2 narcosis, electrical stunning, percussion, asphyxia, salt bath, ice plus water or ice only (Poli et al., 2005). We selected two stunning/slaughtering groups: cold shock or ice asphyxia, by immersing fish in an ice slurry, and CO_2 narcosis by immersing fish in a CO_2 supersaturated bath. These methods were compared with a typical *fishing* capture method that is asphyxia, which wouldn't comply with the present requirements of animal welfare for aquaculture, but still used in traditional fisheries. Several quality indicators were selected: (1) the dynamics of *rigor mortis* onset and resolution, considering that partially determines the tenderness of meat, (2) muscle pH as an indicator of texture, (3) the refraction index of eye liquor, as a measure of the dehydration of the fluid after death and (4) sensory evaluation to assess quality, as appreciated by consumers. Moreover, the ante mortem stress caused by the stunning procedure was assessed by measuring physiological stress indicators (plasma cortisol, plasma glucose, plasma lactate and plasma osmolality).

2. Materials and methods

2.1. Fish and slaughtering methods

A set of 250 immature European sea bass *D. labrax* (mean±SEM total body length of 31.90 cm±0.14; body weight 350 g±1.4) were obtained from a land based fish farm in the north-west Mediterranean coast (Base Viva, Conei Group). Prior to the experiments the fish were kept in 5000 L standard flow-through circular tanks under natural temperature (19.5 °C) and photoperiod, and they were fasted for 24 h, as a normal routine in the farm. Two methods of stunning/slaughtering were used: ice asphyxia (called ice in figures) by immersion of fish in chilled water (2–4 °C), water/ice ratio was 1:1; and CO_2 narcosis, by immersion of fish in CO_2 supersaturated seawater, 19.5 °C, obtained by flowing CO_2 through a diffuser. For comparison, a group of fish was left inside a bucket until death (asphyxia), simulating a procedure used today in some fisheries and formerly frequent in aquaculture. This group was considered as control group. For each method, 56 fish were subjected to the slaughtering method at the same time. For physiological stress indicators, we also used an additional undisturbed group of six animals as control group. In total, 174 out of 250 fish were sampled.

2.2. Sampling

Sampling was performed at 0, 2, 9, 24, 48, 72 and 96 h after death. Death was considered as complete stillness, without any opercula movement or gasping and without any reaction to manipulation. Time to death was also recorded for each stunning/slaughtering method. A number of 8 fish per group were used for each of the 7 sampling times and 3 methods. These 8 fish groups were kept in different porexpan boxes covered with ice in a cold chamber at 4.8±0.2 °C until evaluation was performed.

For all sampling times, *rigor mortis* assessment, muscle pH, eye refraction index and sensory evaluation were determined as quality parameters. Stress indicators were analysed from blood samples that were taken from the caudal vessels by puncture with a syringe. Blood was placed in heparinized tubes and centrifuged for 5 min at 4000 r.p.m. The sampling time for taken blood was immediately after stop of struggling movements. Then, quality indicators were measured. Plasma was separated and frozen at −20 °C for stress parameters analysis (plasma osmolality, plasma glucose, plasma lactate and plasma cortisol). In order to obtain resting values, blood from 6 fish was taken in less than 3 min after capture, without any other handling. This group was considered as control only for physiological parameters.

2.3. Analytical methods

Muscle pH was determined by direct reading in the dorsal muscle with a needle pHmeter Testo 320 (Testo GmbH & Co., Germany). Eye refraction index (RI, expressed as °Bx, degrees brix) was determined by extracting a few drops of aqueous ocular liquid with a syringe, placing a drop and measuring its refractometry (ATAGO N-1 refractometer, Japan) as previously described by Eskin (1990). Degrees brix gives information about the dehydration in ocular liquid which modifies light refraction. *Rigor mortis* development (RM) of whole fish, visible by stiffening of the body, was measured through the sag of the head while the fish was clamped in a vertical position by the tail in a "rigor meter" developed for European sea bass, following the method described by Korhonen et al. (1990). By measuring the angle of sag from the perpendicular to the vertical where the tail is clamped, the degree of stiffening (rigor mortis), and thus the stage of rigor, may be assessed. In this rigor meter the pre-rigor stage is shown by no stiffening, giving an angle lower than 30°, the