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Time course deposition of conjugated linoleic acid in market size rainbow trout (*Oncorhynchus mykiss*) muscle

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Abstract

Previous studies clearly suggested that conjugated linoleic acid (CLA) could be successfully incorporated up to 1% in rainbow trout diets contributing to the production of a functional food. The determination of the time course deposition of CLA has never been evaluated in fish. Hence, homogenous groups of 43 rainbow trout (*Oncorhynchus mykiss*) with an average initial body weight of 216.5 ± 19.6 g were randomly distributed among 6 square fibre glass tanks (250 l), in an open flow-through system. Triplicate groups of fish were fed commercial extruded diets containing 0% CLA (control group) or 1% CLA, by hand to apparent satiety, for 12 weeks. Every 2 weeks, 18 trout from each treatment were sampled to evaluate whole body composition, muscle fatty acid profile and sensory properties. At the end of the experiment all groups of fish weighted more than 400 g and no significant differences were detected in growth performance, feed conversion, nutrient or energy utilization and body composition among treatments. In general terms, the muscle total saturated, monounsaturated and polyunsaturated fatty acids did not vary among dietary treatments, despite the increasing concentration levels of CLA. The muscle incorporation of the two biological active isomers increased gradually during the 12 weeks of feeding CLA reaching the maximum level (2.7% of total fatty acids) after 12 weeks. Nevertheless, after 8 weeks of feeding, the observed value (2.2% of total fatty acids) was not significantly different from the final. At every sampling point, sensory data indicated no significant differences between animals fed control and CLA diets. The present results suggest that feeding market size rainbow trout with 1% CLA, during an 8-week period, is enough to attain muscle CLA levels similar to those observed at 12 weeks. Moreover, a fast accumulation of CLA isomers in the muscle was registered, reaching 1.3% of total fatty acids just after 2 weeks of supplementation, which reinforces the potential of aquaculture fish to supply those bioactive fatty acids, becoming functional foods.

Keywords: Rainbow trout; Conjugated linoleic acid (CLA); Sensory analysis; Muscle fatty acid profile; Functional food

1. Introduction

Food quality is presently receiving more and more attention and aquaculture products are no exception. The consumer begins to realize the influence of diet on his health and the benefits of some food components, searching for new and healthier products. Dietary formulations in aquaculture tend to increase the lipid content in diets to spare protein and to decrease the amount of waste produced by fish (Cho, 1992; Cho et al., 1994; Kaushik, 1998; Medale et al., 1998; Company et al., 1999). However, these diets alter body composition and slaughter quality, particularly through an increase of lipid deposition (Cowey, 1993; Hillestad and Johnsen, 1994; Vergara et al., 1999). Additionally, the fish oil

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substitution by vegetable sources in fish diets, an actual tendency that will continue to grow, is accompanied by a reduction of valuable $n-3$ highly unsaturated fatty acids (HUFA) in edible products (Arzel et al., 1994; Bell et al., 2003; Izquierdo et al., 2003, 2005; Regost et al., 2004) that can induce detectable sensory alterations, since lipids, and fatty acids in particular are the major responsible for fish characteristic flavour and texture (Arzel et al., 1994; Sérot et al., 2001, 2002; Regost et al., 2004). The deleterious aspects of feeding either high-energy diets or vegetable oil diets, which are indispensable for the sustainability of the activity, can be compensated by the inclusion of functional dietary components that present beneficial effects for human health. Among the components that can receive such designation are conjugated linoleic acids (CLA), a generic designation for geometric and positional isomers of linoleic acid with conjugated double bonds. These conjugated fatty acids are receiving great attention by the scientific community, especially *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers. Several studies relate their involvement in health-promoting actions (reviewed by Belury, 2002; Rainer and Heiss, 2004), specially the anti-carcinogenic activity (Ha et al., 1990; Ochoa et al., 2004; Kuniyasu et al., 2006) and the modulating of body fat, reducing lipogenesis and fat accumulation (recently reviewed by Park and Pariza, 2007) in laboratory (Park et al., 1997; Sisk et al., 2001) and farm animals (Szymczyk et al., 2001; Ostrowska et al., 2003). In humans the results concerning the body fat decrease are limited and contradictory (Blankson et al., 2000; Gaullier et al., 2004; Terpstra, 2004).

CLA, mainly *cis*-9, *trans*-11, are found naturally in ruminant lipids, being intermediary products of ruminal biohydrogenation of linoleic acid to stearic (Pariza et al., 2001; Fukuda et al., 2005). In dairy products, CLA *cis*-9, *trans*-11 level range from 0.4 to 0.8% of fat (Lin et al., 1995) and in ruminant meats, from 0.1 to 1.9% of total fatty acids, but is usually lower than 1% (Schmid et al., 2006). The interest of CLA enhancing or supplementation in food products is clear and an actual challenge in several animal production sectors (Bessa et al., 2000; Ostrowska et al., 2003; Cherian and Goeger, 2004; Raes et al., 2004) including aquaculture. In pork meat, Lauridsen et al. (2005) reported CLA levels of 0.44% of fatty acids (80% *cis*-9, *trans*-11 and 20% *trans*-10, *cis*-12) after 0.5% of dietary CLA supplementation. In broilers fed 0.5 to 1.5% CLA for 42 days, Szymczyk et al. (2001) observed CLA levels in meat from 3.1 to 9.8% of fatty acids (approximately 35% consisted of the two functional isomers, like in the diet). Raes et al. (2002) reported a mean level of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA of 3.4% of yolk fatty acids, after feeding laying hens with 1% CLA for 28 days. In fish, several experiments tested increasing dietary CLA levels: rainbow trout (0.5 to 2% of CLA, Bandarra et al., 2006; Valente et al., 2007b), Atlantic salmon (0.5 to 4% of CLA, Berge et al., 2004; Kennedy et al., 2005; Leaver et al., 2006), hybrid striped bass (0.5 to 1% of CLA, Twibell et al., 2000), Atlantic cod (0.5 and 1% of CLA, Kennedy et al., 2007a), channel catfish (0.5 and 1% of CLA, Manning et al., 2006), yellow perch (0.5 and 1% of CLA, Twibell et al., 2001), European sea bass (0.5 to 2% of CLA, Valente et al., 2007a) and common carp (2.5 and 5% of CLA, Schwarz et al., 2002). Those trials (8 to 14 weeks, but mainly 12) resulted in no decrease of muscle lipid level, but in a successful incorporation of CLA in muscle, contributing to the production of a functional food. The sum of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA levels in flesh ranged from 1.8% of fatty acids in European sea bass fed 0.5% (Valente et al., 2007a) to 8.4% of fatty acids in Atlantic salmon fed 4% CLA (Leaver et al., 2006), being superior to values observed in natural sources of CLA, and similar or higher than those reported in pork or chicken meat where CLA content was enhanced (Szymczyk et al., 2001; Lauridsen et al., 2005). These results indicate that the ability of fish to incorporate CLA varies among species, but the developmental stage of the fish and the dietary lipid content also seems to affect the pattern of lipid metabolism. Previous studies with market size rainbow trout clearly showed that CLA could be incorporated up to 1% in high-energy diets, contributing to the production of a functional food with 2.1% of CLA (sum of *cis*-9, *trans*-11 and *trans*-10, *cis*-12) in fillet fatty acids, after 12 weeks supplementation (Valente et al., 2007b).

The objective of the present experiment was to determine the minimal administration period of dietary CLA to obtain the desirable deposition levels of CLA in the muscle. The time course effects of supplementation market size rainbow trout with 1% CLA on whole body composition, CLA deposition levels, and sensory properties of fillets were evaluated over 12 weeks.

2. Materials and methods

2.1. Experimental diets

A commercial extruded diet for rainbow trout was supplied by Sorgal S. A. (Ovar, Portugal). The CLA mixture (containing 70% CLA) was offered by Bioriginal Food and Science Corp., Saskatoon SK, Canada. Before oil coating, the pellets (5 mm of diameter) were analyzed for fat composition and then coated with 21.9% oil containing the different CLA levels (0, control or 1%). The CLA supplement was added to the diet at the expense of fish oil to maintain a constant lipid (27% DM) and energy (25–26 kJ/g DM) level among dietary treatments. Ingredients and proximate composition of the experimental diets are presented in Table 1 and the fatty acid profiles of the diets in Table 2.

2.2. Growth trial

The trial was conducted in the University of Trás-os-Montes and Alto-Douro (UTAD, Vila Real, Portugal) rearing facilities, with market size rainbow trout (*Oncorhynchus mykiss*) acquired from a trout farm. Fish were acclimated to the experimental conditions for a period of 2 weeks and fed the control diet until the beginning of the experiment. Six homogenous groups of 43 fish with an average initial body weight of 216.5 ± 19.6 g (mean ± S.D.) were randomly distributed among 6 square fibre glass tanks (250 l), in an open flow-through system. Triplicate groups of fish for each treatment were hand-fed to apparent satiety, two times a day (08:30 and 17:00 h) for 12 weeks. The pH, ammonia, nitrites, nitrates and phosphates were monitored during the entire trial and maintained at levels compatible with the species. The daily water temperature was 12 ± 1 °C and fish were exposed to natural photoperiod.

A pooled sample of 6 fish from the initial stock at the beginning of the experiment was taken and stored at −20 °C for subsequent whole body