Aquaculture 465 (2016) 287–295

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

The effect of sustained swimming exercise on the growth performance, muscle cellularity and flesh quality of juvenile qingbo (*Spinibarbus sinensis*)

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ARTICLE INFO

Article history:
Received 10 September 2015
Received in revised form 6 September 2016
Accepted 12 September 2016
Available online 12 September 2016

Keywords:
Sustained swimming exercise
Growth
Muscle cellularity
Flesh quality
Spinibarbus sinensis

ABSTRACT

This study was undertaken to investigate the effects of sustained swimming exercise on the growth performance, muscle cellularity and flesh quality of juvenile qingbo fish (*Spinibarbus sinensis*). Experimental fish were exercised under four water velocities, nearly still (control, 3 cm s^{-1}) and 1, 2 and 4 body lengths (bl) s^{-1}, for eight weeks at 25 °C. Then, growth performance indicated by the specific growth rate (SGR), white muscle cellularity suggested by the diameter and density of white muscle fiber, flesh quality evaluated by some textural mechanical properties (hardness, springiness, chewiness, cohesiveness) and physico-chemical parameters (pH, color, moisture, ash, protein, fat, amino acids, and fatty acids) were measured. Sustained swimming at moderate velocities (1 and 2 bl s^{-1}) resulted in a significantly higher SGR and similar white fiber diameter and density compared with the controls. However, the fish trained at a water velocity of 4 bl s^{-1} displayed a similar SGR, smaller white fiber diameter and higher white fiber density compared with the controls. Sustained swimming resulted in significant increase in the values of pH and all textural traits but showed no significant effect on the color of the flesh for juvenile *S. sinensis*. Fish swimming under moderate water velocities exhibited higher levels of the protein content of the muscle, total essential amino acids ($\sum$EAA) (at 2 bl s^{-1}) and total amino acids ($\sum$AA) (at 1 and 2 bl s^{-1}) compared with control fish. However, the lowest levels of total amino acids ($\sum$AA) and total n-6 poly-unsaturated fatty acids ($\sum$n-6 PUFA) were observed in fish swimming at water velocity of 4 bl s^{-1}. These data suggest that (1) moderate swimming exercise (1 or 2 bl s^{-1}) improved growth performance, which could not be attributed to changes in white muscle cellularity; (2) sustained swimming showed a positive effect on texture characteristics, which was partly due to the higher white fiber density and flesh pH compared with controls; and (3) moderate swimming exercise (1 or 2 bl s^{-1}) was beneficial for improving the nutritional quality of the flesh, whereas high-intensity swimming (4 bl s^{-1}) resulted in an impairment of the nutritional quality of the flesh in juvenile *S. sinensis*.

Statement of relevance: We declare that the experiments complied with the current laws of the country in which the experiments were performed and that our paper complied with the laws of commercial aquaculture.

1. Introduction

Post-natal muscle growth in fish involves hypertrophy (increase in muscle fiber size) and hyperplasia (increase in muscle fiber numbers) of muscle fibers (Weatherley et al., 1988; Rowlerson and Veggetti, 2001). The wide range of muscle fiber numbers and diameters observed in flesh is referred to as muscle cellularity and is heavily influenced by many factors such as genetic characteristics (Johnston and McLay, 1997; Valente et al., 1999), diet composition (Alami-Durante et al., 2010), water temperature (Martins et al., 2014) and light (Johnston et al., 2003) in fish species. Swimming is an important physiological function and is primarily powered by skeletal muscle in fish. Numerous studies have found that sustained swimming exercise training is a powerful stimulus for muscle hypertrophy and hyperplasia (Davison and Goldspink, 1977; Sänger, 1992; Martin and Johnston, 2005; Ibarz et al., 2011). White skeletal muscle accounts for the majority (approximately 95%) of muscle tissue, representing >50% of body weight in many fish species (Palstra and Planas, 2011). Therefore, many researchers believe that significant changes in white muscle fiber are an important reason for the differences in growth between exercised and non-exercised fish (Totland et al., 1987; Gruber and Dickson, 1997; Martin and Johnston, 2005). However, this positive correlation between white muscle fiber and body growth has not been found in a number fish species subjected to a continuous water flow, which could be mainly due to the different species, training regimes and tissue sampling sites

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http://dx.doi.org/10.1016/j.aquaculture.2016.09.021
0044-8486/

involved (Davison and Goldspink, 1977; Stickland, 1983; Martin and Johnston, 2006; Ibarz et al., 2011). Moreover, there are scarce studies in the scientific literature investigating the relationship between muscle cellularity and body growth in warm-water cyprinid fish species (Davison and Goldspink, 1978; Sänger, 1992).

The flesh quality of fish is increasingly subject to consumer concerns as total fishery production increases year by year. Flesh quality is the result of a combination of features involving both exogenous characteristics (e.g., freshness indicators, fin damage and indirectly through exercise-inducible fish growing parameters like condition factors and specific growth rate) and endogenous characteristics (e.g., texture, color, proximate composition and amino acid and fatty acid profiles) in fish species (Rasmussen et al., 2013). These traits could dependent on the strain and sexual maturation and are strongly influenced by a variety of extrinsic factors, such as temperature, feeding, pre- and post-slaughter handling procedures and storage procedures (Ginés et al., 2004; Aussanasuwannakul et al., 2011; Digre et al., 2011; Shi et al., 2013). Significant difference in white muscle cellularity between exercised and unexercised fish has been reported in many publications (Davison and Goldspink, 1977; Totland et al., 1987; Gruber and Dickson, 1997; Martin and Johnston, 2005; Ibarz et al., 2011). Additionally, some studies have found that the diameter and density of white muscle fiber are closely related to texture attributes in sea bass (*Dicentrarchus labrax*, L.) and Atlantic halibut (*Hippoglossus hippoglossus*, L.) (Periago et al., 2005; Hagen et al., 2007). Therefore, some researchers believe that sustained swimming may be an effective way to improve texture attributes in fish (Johnston, 1999; Bugeon et al., 2003). In addition, because swimming exercise is a process that increases the overall energy expenditure, fish must take in more fuel and use body energy substances to compensate for the increased energy expenditure during long-term swimming (Palstra and Planas, 2011). Therefore, sustained swimming activity had a significant effect on the pattern of energy deposition and chemical composition (fat content, fatty acid profile, protein content and amino acid profile) in fish species (Davison and Goldspink, 1978; Kiessling et al., 2005; Ibarz et al., 2011; Song et al., 2012; Li et al., 2013). There is growing evidence that the induction of swimming exercise could represent a potential tool for controlling and improving the flesh quality of fish (Bjørnevik et al., 2003; Bugeon et al., 2003; Palstra and Planas, 2011; Song et al., 2012; Rasmussen et al., 2013). Moreover, it has been suggested that whether swimming exercise shows positive (or negative) effects on flesh quality is dependent on the fish species and exercise intensity (Totland et al., 1987; Tachibana et al., 1988; Bjørnevik et al., 2003; Rasmussen et al., 2011).

In the present study, juvenile qingbo (*Spinibarbus sinensis*) a warm-water cyprinid fish species, was selected as the experimental animal because it is an important cultured fish species in the middle and upper reaches of the Yangtze River of China that typically inhabits areas with flowing water (Duan et al., 2002). A previous study in juvenile *S. sinensis* showed that prolonged swimming exercise improved growth performance and increased the protein and fat contents in the whole body at a water velocity of 2 bl s^{-1} (body lengths per second) (Li et al., 2013). The aims of present study are to (1) investigate whether sustained swimming exercise has effects on the structure of white muscle and its possible relationship with growth performance and (2) test whether sustained swimming exercise can improve flesh quality at different water velocities in juvenile *S. sinensis*. To achieve these aims, we assessed the specific growth rate (SGR), diameter and density of white fibers, texture traits, color, pH, proximate composition and amino and fatty acid composition of fillets after sustained swimming exercise in juvenile *S. sinensis*.

2. Materials and methods

2.1. Experimental animals and training protocol

Juvenile *S. sinensis* (Cypriniformes: Cyprinidae) (Kong et al., 2007) were purchased from the Fisheries Hatchery of Hechuan Aquaculture School (Hechuan, Chongqing, China) and were acclimated for four weeks in a cement pit system with recirculating water (1200 L) before the experiment. During the acclimation period, the temperature of the de-chlorinated tap water in the system was maintained at 25.0 ± 0.5 °C, and the oxygen content was kept near saturation (approximately 8 mg L^{-1}). Fish were fed to satiation twice daily, at 09:00 and 18:00, on a commercial diet (Tongwei, China). The main composition of the diet consisted of 41.2 ± 0.9% protein, 8.5 ± 0.5% lipids, 25.7 ± 1.2% carbohydrates and 12.3 ± 0.4% ash. The photoperiod was 12 h light:12 h dark, with lights turned on and off at 09:00 and 21:00, respectively.

A self-made exercising system described by Li et al. (2013) was used in the present study. After the acclimation period, 144 fish of similar size (23.25 ± 0.03 g, 10.63 ± 0.02 cm) were slightly anesthetized (neutralized MS222, tricaine methane sulfonate, 50 mg L^{-1}) and randomly divided into four groups (control group, 1 bl s^{-1} training group, 2 bl s^{-1} training group and 4 bl s^{-1} training group). Thirty-six fish from each group were placed in a flume composed of three cells (12 fish per cell, three replicates). The fish in the control group swam at an average water velocity of 3 cm s^{-1}. Our pilot experiment showed that a water velocity of 3 cm s^{-1} was beneficial for water exchange and did not lead to an intense reaction in juvenile *S. sinensis*. The fish in the three training groups were exposed to three different water velocities: 1, 2 and 4 bl s^{-1} (approximately 11 cm s^{-1}, 22 cm s^{-1} and 44 cm s^{-1}, respectively), at the beginning of the experiment. There were no apparent differences in dissolved oxygen levels among the four flumes (approximately 7.0 mg L^{-1}). To reduce physiological stress, the water velocity was gradually increased over 4 days until the desired water velocity was reached for the first round of training (Davison and Goldspink, 1978). Water velocities were adjusted by controlling the different voltages of transducer power every other week after the body length of the fish had been measured. The exercise training sessions were carried out twice daily, from 12:00 h to 18:00 h and from 21:00 h to 09:00 h, for a total of 18 h per day for 8 weeks. The fish in all cells were fed twice daily to satiation at 09:00 and 18:00 with a commercial diet when the training procedure was ceased. The holding conditions for the experimental period were consistent with those of the acclimation period.

2.2. Experimental protocol

At the end of the experiment, all of the fish from the exercise system (three cells in each group) were fasted for 1 day and then killed by a sharp blow to the head, after which the mass and length of the fish were measured to the nearest 0.1 g or 0.1 cm. For the morphometrical and textural analyses (detail below), five individuals from each cell (total of 15 samples for each group) were sampled. Three muscle tissue blocks (0.5 cm × 0.5 cm × 0.5 cm) per fish were dissected for morphometrical analysis, from the anterior, middle and posterior regions of the dorsal muscle, just above the lateral line on the left side of the fish. Each sample was immediately mounted on a tablet package, covered with OCT embedding compound (Sakura Tissue Tek; Torrance, CA, USA), frozen in 2-methylbutane cooled to near its freezing point (−159 °C) in liquid nitrogen, and then stored in a −80 °C freezer until sectioning (Bjørnevik et al., 2003). White muscle samples (0.5 g) for pH determination were taken from the left fillet at a position just above the lateral line in front of the dorsal fin. The right fillets from fifteen fish per group were sampled and kept packed in closed polystyrene boxes with ice for subsequent analysis of texture and color. For the analyses of proximate composition, amino acids and fatty acids in muscle (detail below), seven additional individuals from each cell were sampled. All of the dorsal muscle (without skin) from above the lateral line was excised in the seven fish per cell, after which the samples were mixed and stored in a −80 °C freezer for subsequent biochemical measurements.