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Steroid bioaccumulation profiles in typical freshwater aquaculture environments of South China and their human health risks via fish consumption☆

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ABSTRACT

More attention was previously paid to adverse effects of steroids on aquatic organisms and their ecological risks to the aquatic environment. So far, little information has been reported on the bioaccumulative characteristics of different classes of steroids in cultured fish tissues. The present study for the first time provided a comprehensive analysis of the occurrence, bioaccumulation, and global consumers' health risks via fish consumption of androgens, glucocorticoids and progestanges in typical freshwater cultured farms in South China. The numbers and total concentrations of steroids detected in the tissues of five common species of the cultured fish were in the order of plasma > bile > liver > muscle and plasma > bile, muscle > liver, respectively. The field bioaccumulation factors for the detected synthetic steroids ranged from 450 to 97,000 in bile, 450 to 65,000 in plasma, 2900 to 16,000 in liver, and 42 to 2600 in muscle of fish, respectively. This data suggests that steroids are bioaccumulative in fish tissues. Mostly important, 4-androstene-3,17-dione (AED) and cortisone (CRN) were found to be reliable chemical indicators to predict the levels of steroids in plasma and muscle of the inter-species cultured fish, respectively. Furthermore, the maximum hazard quotients (HQs) of testosterone and progesterone were 5.8×10^{-4} and 9.9×10^{-5}, suggesting that human health risks were negligible via ingestion of the steroids-contaminated fish.

1. Introduction

In recent years, more and more public attentions have focused on the occurrence and fate of steroids in the environment. It has been confirmed that wastewater treatment plants (WWTPs) and livestock farms are two main sources of steroids in the environment (Fan et al., 2011; Liu et al., 2012b). The total estimated contribution of steroids via WWTPs, swine farms and cattle farms in China were up to 140, 66 and 61 t/yr, respectively (Liu et al., 2012b). Furthermore, aquaculture is also an important source of steroids in the aquatic environment (Kolodziej et al., 2004). In the breeding process of aquaculture, steroids come mainly from two ways. One is that fish could excrete metabolized steroids into water like livestock not only via urine, but also via gill or bile (Ellis et al., 2004; Scott and Ellis, 2007; Liu et al., 2012b). The other is that some natural or synthetic steroids were added into the aquaculture environment directly or into the feed to prevent or treat diseases, promote growth, or produce monosex populations (Beardmore et al., 2001).

Although the amount of steroids discharged from a typical aquaculture farm was equal to several hundred animals or a

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wastewater treatment plant serving several thousand people (Kolodziej et al., 2004), little is known about occurrence and bioaccumulation of steroids in aquaculture. Only few studies paid their attention on the occurrence and bioaccumulation of estrogens in fish tissues from pond, reservoir, river, lake, and WWTP effluents (Liu et al., 2012a; Huang et al., 2013; Yang et al., 2014). The concentrations of estrogens including estrone (E1), 17β-estradiol (E2), 17α-ethynylestradiol (EE2) and estriol (E3) in these aquatic environments were always at very low levels (ng/L) which could still be detected in fish tissues with the bioaccumulation factors up to 40,000 (Liu et al., 2012a; Yang et al., 2014). For other steroids, such as androgens, glucocorticoids and progestagens, there is only one study recently reported the bioaccumulation and human dietary exposure risk of these steroids in marine fish muscle by our group (Liu et al., 2015a).

China is the largest freshwater aquatic product farming country in both production and farming area. In 2015, the output of aquaculture in China was up to 67 million tons (MAPRC, 2016), in which 4.1 million tons of aquatic products were exported to Japan, Association of Southeast Asian Nations, the United States, the European Union, Korea, Hong Kong, Taiwan and other regions (IEAP, 2016). Pond culture is the most common culture mode in China, accounting for 70% of the total output of freshwater aquaculture (Yu, 2015). Most of pond cultured farms are traditionally managed on small scales without effective management measures. According to the guidelines of General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, synthetic steroids including methyl testosterone, 17α-trenbolone, 17β-trenbolone and megestrol acetate are forbidden to use for any purposes in all food-producing animals in China (QSIQ, 2002). Previous studies have confirmed that synthetic steroids were still being illegally used in the livestock and marine aquaculture farms in China (Liu et al., 2012b, 2015a). Thus, our hypotheses are 1) there might be illegal usage of steroids in feed samples in freshwater pond cultured farms in China, 2) steroids are bioaccumulative in fish tissues and there are linear correlations between single steroid and other individual steroid/total steroids in different tissues, 3) the residual steroids in fish muscle might bring human health risks.

Thus, the aim of this study was to investigate the bioaccumulation of steroids in the bile, plasma, liver and muscle tissues of fish raised in typical freshwater pond cultured farms and human health risks via fish consumption. Moreover, the potential chemical indicators of steroids in fish tissues were explored. To the best of our knowledge, no previous studies have reported the bioaccumulation of androgens, glucocorticoids and progestagens in freshwater pond cultured farms as well as the relationship between single steroid and the rest steroids in different fish tissues. Our study might provide a better understanding of the biological enrichment for different classes of steroids in fish tissues and have implications for appropriate management actions in the aquaculture environment.

2. Materials and methods

2.1. Chemicals and sample collection

Totally, 29 natural and synthetic steroids, including 11 androgens, 4 glucocorticoids, 14 progestagens, and 4 internal standards were selected in this study (Table S1). The detailed information on chemicals and materials is summarized in the Supplementary materials. Three typical freshwater cultured farms (F1, F2 and F3) in Guangzhou, one of the biggest aquatic products farming city as well as a huge aquatic products consumption city in China, were selected as the study areas. The total output of aquatic products in Guangzhou was 480,000 tons in 2015, and 82% of which was freshwater culture (GZSB, 2015). The daily consumption of aquatic products has been estimated up to 1600 tons in Guangzhou, only second to pork (GZRD, 2013). Farm F1 is managed in a swine-chicken-geese-fish polyculture mode including 30,000 tilapias. Farm 2 is managed in a mixed-fish-species pond culture mode, including 90,000 spotted scats and 18,000 tilapias. Both farm F1 and F2 are typically small-scaled aquaculture farms, which are the most common aquaculture breeding mode in South China. Farm F3 is a median-scaled farm with technical staff guidance, including 16,000 crucian carps, 3200 mullets and 1600 mud carps. All influents of these farms are from Zhujiang River, but only farm F3 has a sedimentation tank with 24 h retention time to treat the influent (F3-inf) (Fig. S1).

All samples including four water samples, four suspended particle samples, three sediment samples, three feces samples and five feed samples were collected from three farms in April 2015. Surface water samples were collected from the center of the pond to the pre-cleaned bottles immediately. Three replicate samples were mixed as a composited sample (5 L). To suppress bacterial activity, about 5% (v/v) of methanol was added into each composited water sample and its pH value was adjusted to 3.0 in the field (Zhao et al., 2015a). Surface sediments (0–20 cm, 200 g) were obtained using Peterson grab sampler and stored in glass bottles. Feeds used in the aquaculture farms (50 g) and feces produced there (100 g) were collected at the same time. All samples were transported back in coolers to the laboratory immediately. The collected water samples were then processed within 48 h. The solid samples (sediment, feed, feces and suspended particles) were freeze dried, ground and homogenized. In total, 37 fish including 6 from farm F1, 13 from farm F2 and 18 from farm F3 were collected from the three ponds in the selected farms. All fish collected in the study were adult and ready for sale. The collected fish species included tilapia (*Oreochromis* spp), spotted scat (*Scatophagus argus*), mullet (*Mugil cephalus*), mud carp (*Cirrhinus molitorella*), and crucian carp (*Carassius auratus*), which represent the most common freshwater aquaculture products in South China. Basic information on selected aquaculture farms and sampled fish are listed in Tables S2 and S3. All fish samples were kept alive in ice water aerated by portable air pumps, and sacrificed immediately upon arrival in the laboratory. A pre-rinsed sodium heparin syringe was used to collect the blood of the fish caudal vein, which was then stored in a 2 mL plastic vial for 8 h at 4 °C. The plasma was obtained from the supernatant in the vial which was centrifuged at 10,000 g for 10 min. The bile was obtained from the gall bladder by a syringe needle and stored in a 2 mL cryogenic vial. Approximately 2 g of muscle samples and 0.5 g of liver samples, both in wet weight, were dissected and kept in cryogenic vials independently. All tissues except the plasma were immediately stored at −20 °C until extraction.

2.2. Sample extraction and instrumental analysis

Sample extraction and instrumental analysis were carried out following previously established analytical methods (Liu et al., 2011a, 2014; Zhao et al., 2015a). The detailed method information can be found in the Supplementary materials (Tables S4 and S5). Briefly, water samples were extracted by the solid-phase extraction using Waters Oasis HLB cartridges (500 mg, 6 mL), while the solid samples were extracted with ethyl acetate by ultrasonication. The strong anion exchange/primary-secondary amine (SAX/PSA) cartridges (6 mL, 500 mg) and HLB cartridges (6 mL, 200 mg) were used firstly to remove bile acid from bile and lipid from both liver and muscle tissues, and finally to enrich the steroids in biota sample extracts, respectively. The plasma and bile samples were extracted by HLB cartridges and SAX/PSA-HLB tandem cartridges, respectively. The dissected liver and muscle samples were