MASCULINIZATION OF TILAPIA BY IMMERSION IN TRENBOLONE ACETATE: DETECTION OF TRENBOLONE ACETATE IN WATER AFTER TREATMENT

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ABSTRACT

In a previous experiment in which Nile tilapia fry were successfully masculinized, we investigated how the concentration of trenbolone acetate (TA) in the immersion water changed before and after treatment. The results from that experiment indicated that the concentration of TA before and after treatment of Nile tilapia fry was highly variable and below the expected levels. Therefore, we decided to corroborate those results by running two experiments in which fry were not present and by testing different water sources. These new experiments confirmed our previous findings, indicating that independently of the source of water, the concentration of TA is highly variable and below the expected levels.

INTRODUCTION

Previous experiments in our laboratory have demonstrated that short-term immersions in synthetic steroids such as 17α-methyldihydrotestosterone (MDHT) and trenbolone acetate (TA) are effective masculinizing treatments for Nile tilapia fry (Contreras et al., 1997, 1999, 2000; Gale et al., 1999). Recently, Bart et al. (2000) have found that ultrasound treatment enhances masculinization when short-term immersions are used. However, short-term immersions are far from being used on a commercial scale. One of the major criticisms to this method is that the concentration used is higher than the amount of 17α-methyltestosterone (MT) used in feeding trials. However, the immersion protocol presents advantages over feeding treatment for hormone control and disposal, as well as fewer risks of environmental contamination.

The use of TA in the cattle industry for growth enhancement has created expectations in the aquaculture industry. This steroid can be used for androgenic purposes and may have anabolic effects while fry are under masculinization treatment. It has been reported that TA administered in the food successfully masculinized channel catfish (Galvez et al., 1995) and blue tilapia (Galvez et al., 1996). However, Davis et al. (2000) found that the catfish treated with TA were not functional males but infertile organisms after three years of growth.

Research is needed to understand how much steroid is available to the fry during immersion treatment. Because of their hydrophobic properties, steroids may be very unstable in the water treatment, form precipitates, or bind to the walls of the containers. To address some of these questions, we performed experiments to determine the concentration of TA in dechlorinated water and distilled water at different times.

METHODS AND MATERIALS

Experiment 1: Steroid Solutions without Fry Immersed

Following an experiment reported on in Contreras et al. (2001) involving examination of TA solution following immersion of fry, a similar experiment with no fry immersion was planned. This experiment consisted of three 3.8-l glass jars containing 1 l of well water. To each replicate 500 µl of TA were added, mixed thoroughly, and maintained at 28 ± 1°C under constant aeration. Samples were collected at 0, 3, and 30 h at the surface, middle, and bottom of the jar (samples taken at 30 hours at middle depth were lost during processing).

Experiment 2: Short Trial in Borosilicate Tube

Ten ml of double-distilled water were placed in a borosilicate tube, to which 100 µl of TA were added and thoroughly mixed

by vortexing. Samples of 1 ml were collected at time 0 (from the middle of the tube) and at 1 h (from the surface, middle, and bottom). Once all samples were collected, the water remaining in the tube was vortexed, and the tube was emptied and washed with 1 ml of ether to assay for TA attached to the tube walls.

TA Detection

All samples were stored frozen (–20°C) until processed for TA detection. From each sample, 2.0 ml were extracted in 8 ml of diethyl ether. The organic phase of each sample was collected in a new tube after the aqueous phase was snap-frozen in liquid nitrogen. The extraction procedure was repeated, and the ether extracts were pooled for each sample and dried down in a SpeedVac. Each dried extract was reconstituted in 1 ml of methanol. Aliquots of the reconstituted extracts were removed to 150-ml glass inserts for determination of TA concentration by High Performance Liquid Chromatography (HPLC). The HPLC methods followed the procedure outlined in Huang et al. (1983) and modified by Feist et al. (1990). The HPLC analysis was performed using a Waters System consisting of a 600 controller, 717 autosampler, 996 photodiode array detector, a Dell Dimension V400c computer, Millenium PDA software, and a reverse phase C18 column (flow rate 0.4 ml min^{-1}). We used an isocratic mobile phase of water:methanol:acetonitrile: isopropanol (62:28:5:5) followed by a linear gradient (3.3% min^{-1}) of water:methanol:butanol (35:45:20) for 30 min monitored at a wide variety of wavelengths but specifically analyzed at 254, 280, and 340 nm. This system allows for the separation of 19 steroid standards with detection limits of 3 ng for each steroid. Each sample was analyzed once.

RESULTS

Experiment 1

Concentrations of TA in well water were variable at all times and at all sampling points (surface, middle, and bottom). At time 0, levels of TA in the surface water were lower than the expected value (mean = 300.7 µg l^{-1}, SD = 87.5). Initial values of hormone concentration range from 211.2 to 372.4 µg l^{-1}. Similar patterns were observed at 3 and 30 h after addition of the steroid. This trend was also observed in samples taken at the middle point and bottom of the jars (means = 279.5 and 271.8 µg l^{-1}, respectively). Concentration values of TA showed no consistent patterns (Figure 1).

Experiment 2

Concentration of TA at time 0 was 8,731.5 µg ml^{-1} (the expected value was 10,000 µg ml^{-1}). No significant changes were observed in the samples taken at the surface, middle, or bottom of the tube after one hour of mixing of the steroid with water (Figure 2). TA was detected in the ether used for rinsing the glass tube (1,887.7 µg l^{-1}). The estimated total amount of TA detected in the borosilicate tube after adding the concentration rinsed from the glass accounted for 92.4% of the total amount of TA added to the tube.

DISCUSSION

Our earlier findings (Contreras et al., 2001) indicate that the target dose for TA immersion is rarely achieved. The surprisingly low levels of TA found in our previous report have forced us to validate these results by conducting further experiments. However, we have found that the patterns are maintained independently of the source of water used (dechlorinated versus well water) or the location at which the samples are collected (surface, middle, or bottom of jars). Our first hypothesis focused on the precipitation of TA out of solution after mixing with water. However, the data from the experiments reported here indicate that samples from the bottom of the jar have similar patterns to those observed in the surface water. Another hypothesis for explaining the low levels of steroid in the water is that the steroid could be binding to the walls of the jars (which may be porous). The data obtained from a borosilicate tube (used for assays because of its low-binding properties) indicate that about 2% of the TA added binds to the glass. Therefore, it can be expected that binding to jars is higher. More research is needed to determine if the glass employed is trapping a significant amount of steroid, decreasing the efficacy of the immersion technique.

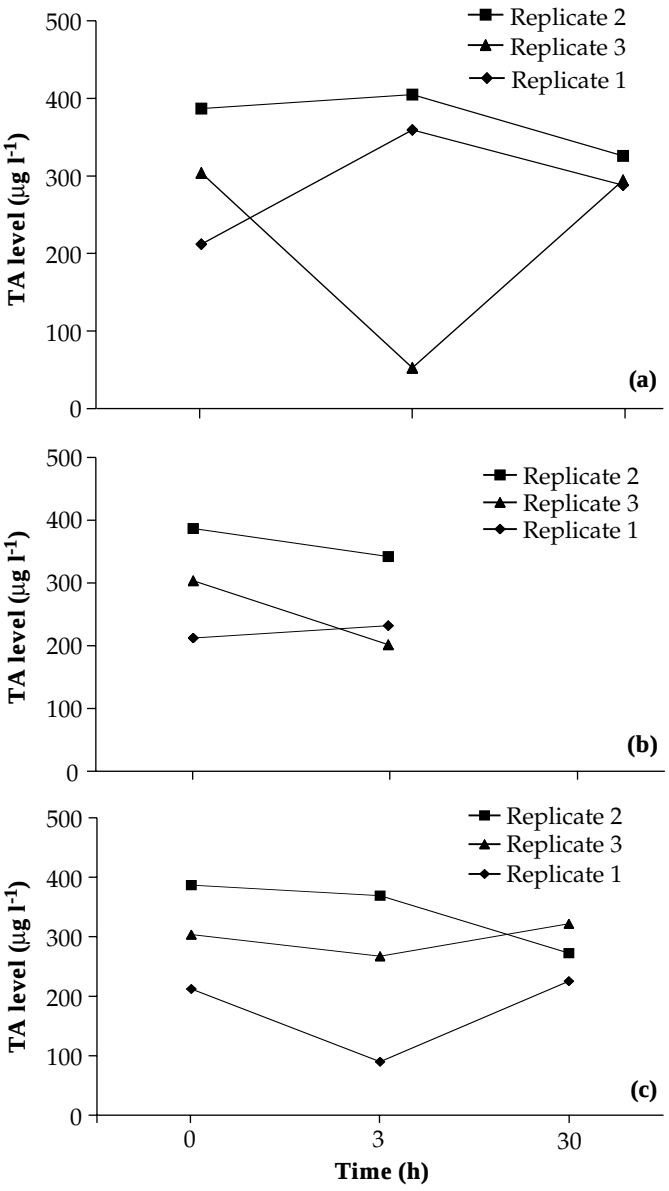

Figure 1. Trenbolone acetate (TA) levels in well water through time in experimental jars containing 500 µg l^{-1} of steroid with no fish added. Samples were taken at the surface (a), middle (b), or bottom (c) of the container.

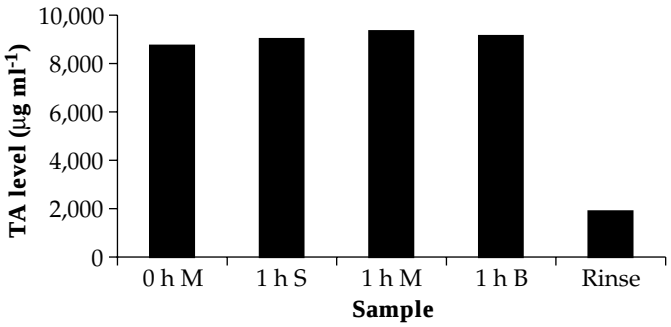

Figure 2. Trenbolone acetate (TA) levels in double-distilled water mixed in a borosilicate assay tube containing 1,000 μg ml^{-1} of steroid. Samples were taken from the middle (M) at 0 and 1 h, and from the surface (S) and bottom (B) at 1 h. Sample named "Rinse" was collected once the tube was emptied and rinsed with ether to determine if TA was binding to the glass.

ANTICIPATED BENEFITS

Masculinizing Nile tilapia fry by immersion can be a good alternative to feeding the fry with hormone-impregnated food. However, the amount of variability observed in the concentration of the steroid used during treatment indicates that this technique requires refinement to obtain more consistent results.

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