Detection of MT in Pond Water after Treatment with MT Food

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Abstract

The objective of the study was to determine if methyltestosterone (MT) can be detected in the treatment environment and, if so, for how long after treatment. *Oreochromis niloticus* fry with a mean initial length of 9.5 mm were stocked into adjacent cages in an earthen pond at Auburn University, Alabama, at 2,000 fry cage^{-1} and fed for 28 days a feed containing 60 mg MT kg^{-1} or a non-treated feed. At the end of the controlled feeding period both sets of fish were harvested, and growth and survival were determined. Fish were returned to their respective hapas and fed a non-hormone treated feed for additional growth until a mean total length of approximately 5 cm was reached. A sample of 100 fish from each hapa was preserved in 10% formalin and the sex was determined. Water and soil samples from the treatment pond were taken prior to, during, and after the hormone administration period. Water samples (collected approximately 10 cm below the surface) were taken weekly from within the cage receiving hormone-treated feed and the cage receiving non-treated feed. At the same time intervals water samples were taken at 2, 5, and 10 m from the cage receiving hormone-treated feed. Soil samples were taken of the upper 5 cm of soil at the same locations at which water samples were taken; samples were collected from under the cages and from the pond bottom at the distances specified above. Soil and water samples were analyzed at Oregon State University. There was no evidence that MT altered the sex ratio of non-target tilapia held in the same pond as and confined near fish receiving MT. The treated population was 91% male, 5% female and 4% intersex. Fish held in an adjacent cage approximately 50 cm away and fed a non-hormone-treated diet had a sex ratio of 48% males and 52% females. Mean MT concentration in the water sampled within MT-treated or non-treated cages did not differ ($P = 0.14$). Pretreatment MT concentration in the water column was 8.0 ± 5.7 pg g^{-1}, and values within the treatment cage were similar except for one sample during the treatment period. The radioimmunoassay when used with soil cross-reacted with other materials in addition to MT. Pretreatment soil samples from the pond, which had no previous history of MT administration, had a concentration of 875 ± 147 pg g^{-1}. The highest concentration of MT indicated (1,417 pg g^{-1}) was from a soil sample beneath the cage receiving the non-treated feed.

Introduction

The use of all-male populations of tilapia for culture offers several important advantages, including enhanced growth (males grow faster and larger than females) and prevention of unwanted reproduction, which diverts energy away from somatic growth. Treatment with 17α-methyltestosterone (MT)-impregnated food has been shown to be an effective means of producing all-male tilapia populations. However, there exists the possibility of steroid loss into the treatment environment from uneaten or unmetabolized food and a risk of unintended exposure of fish or other non-target organisms.

Budworth and Senger (1993) reported on fish-to-fish testosterone transfer in a two-tank recirculating-water system. MT-exposed non-target fish had significantly higher plasma testosterone concentrations than non-exposed control fish during weeks 1 to 3, and no significant difference was detected between MT-exposed and control fish during week 4. In another recirculating system study, common carp (*Cyprinus carpio*) in one tank were fed an androgen-treated diet, while fish in another tank within the same system were fed an androgen-free diet. Both sets of fish developed skewed sex ratios (Gomelsky et al., 1994). It is clear that unmetabolized MT and metabolites of MT, both excreted by the target fish, can accumulate in the water of recirculating systems. The degree of accumulation appears to depend on the frequency and dose of MT administered to the target fish.

The fate of MT in other settings is less clear. In an outdoor tank setting, Phelps et al. (1992) used small hapas placed approximately 30 cm apart in a static water 20-m^2 tank to hold fish given an MT- or fluoxymesterone-treated feed or a non-treated feed. The treatments were randomly assigned within the tank. There was no evidence that hormone leaching affected the sex ratio of non-treated fish. Soto (1992) used a similar experimental setup with eighteen 0.12-m^2 hapas distributed in a 20-m^2 tank to evaluate the androgenic potential of mestanolone to sex-reverse *O. niloticus*. Non-hormone treated fish did not have a skewed sex ratio even though they were surrounded by sets of fish that had been given the hormone-treated feed and were successfully sex-reversed. Abucay et al. (1997) found that reusing static water aquaria that had been used to hold tilapia fry that were given an MT-treated feed for 25 days could alter sex ratios. When a second group of fish were stocked into such aquaria and given a non-hormone-treated feed, the sex ratio was skewed. They also found that when an all-female set of fry were stocked into a cage in an aquarium and an MT-treated feed was added to the bottom of the aquarium where the fish had no access to it, the sex ratio became skewed to males.

In a laboratory setting, Fitzpatrick et al. (1999) detected MT in the water and soil of containers in which tilapia were fed an MT-treated feed during a treatment period of four weeks. Concentrations of MT in the water had decreased to within background levels by one week after the end of treatment, while measurable levels above background in the soil persisted for four weeks post-treatment.

MT is susceptible to breakdown when exposed to light or high temperatures (McEvoy, 1997). Both fungi and bacteria can metabolize exogenous steroids. Many different steroid metabolism reactions, including the metabolism of MT, are possible in bacteria (Schubert et al., 1972; Jankov, 1977), as well as metabolism of steroids to CO_2 and H_2O (Sandor and Mehdi, 1979). In an outdoor pond setting where fish are held in cages, the combination of light, temperature, and microbial degradation may result in a rapid breakdown of MT. This study evaluated the concentrations of MT in soil and water when tilapia were given a diet containing MT for a 28-day period when cultured in cages in an outdoor earthen pond. The effect on the sex ratio of non-treated tilapia held in an adjacent cage was also examined.

Methods and Materials

Oreochromis niloticus fry were obtained from a mass spawning of brooders in an earthen pond and graded to obtain fish with a mean initial length of 9.5 mm. Fish were stocked into adjacent cages in a 400-m^2 earthen pond at Auburn University, Alabama, at 2000 fry cage^{-1} and fed for 28 days a feed containing 60 mg MT kg^{-1} or a non-treated feed. Fish were fed at 25% body weight the first week; thereafter, feeding was reduced by 5% of body weight each week. Fish were sampled weekly during the feeding period, average weights were determined, and feeding rates were adjusted daily based on observed growth rates. At the end of the controlled feeding period both sets of fish were harvested, and growth and survival were determined. Fish were returned to their respective hapas and fed a non-hormone-treated feed for additional growth until a mean total length of approximately 5 cm was reached. A sample from each hapa of 100 fish was preserved in 10% formalin and dissected. Gonads were removed, lightly stained with fast green, squashed to facilitate microscopic examination, and the sex was determined. The entire gonad was examined and those containing both ovarian and testicular tissue were classified as intersex; gonads having only one form of tissue were classified as male or female, corresponding to the type of tissue observed.

Water and soil samples from the treatment pond were taken prior to, during, and after the hormone administration period. Water samples (collected approximately 10 cm below the surface) were taken weekly within the cage receiving hormone-treated feed and the cage receiving the non-treated feed. At the same time intervals, water samples were taken at 2, 5, and 10 m from the cage receiving hormone-treated feed. Soil samples were taken of the upper 5 cm of soil at the same locations at which water samples were taken; samples were collected from under the cages and from the pond bottom at the distances specified above. All soil and water samples were frozen soon after collection and held frozen for shipment.

Soil and water samples were analyzed at Oregon State University following the procedures of Fitzpatrick et al. (1999).

Results and Discussion

There was no evidence of MT altering the sex ratio of non-target tilapia held in the same pond and confined near fish receiving MT. Treatment of *O. niloticus* fry for 28 days with MT at 60 mg kg^{-1} of diet was effective in altering the sex ratio. The resultant population was 91% male, 5% female, and 4% intersex. The control group of fish held in an adjacent cage approximately 50 cm away and given a non-hormone-treated diet had a sex ratio of 48% males and 52% females. The lack of intersex fish in the non-treated fish is further evidence that MT was not released into the environment at a rate to alter sex ratios.

There was little to no evidence of MT escapement from the treatment cage environment. Mean MT concentrations in the water sampled within MT-treated or non-treated cages did not differ ($P = 0.14$). Pretreatment MT concentration in the water column was 8.0 ± 5.7 pg g^{-1}, and values within the treatment cage were similar during the treatment period with the exception of one sample. A concentration of 161 ± 28 pg MT g^{-1} was detected in this sample, and it is thought that this was caused by a particle of feed collected in the sample. MT concentrations in the water from the treatment cage for the 20-day post-treatment period averaged 9.7 ± 4.1 pg g^{-1}. The mean concentration of MT detected 2 to 10 m from the treatment cage was 9.7 pg g^{-1}. Concentrations of MT within the control cage averaged 9.2 pg g^{-1} for the study period. Concentration of MT in water or soil did not increase in relation to the increased MT input over time associated with feeding (Figure 1). On the last day of hormone treatment, 43.3 g of feed

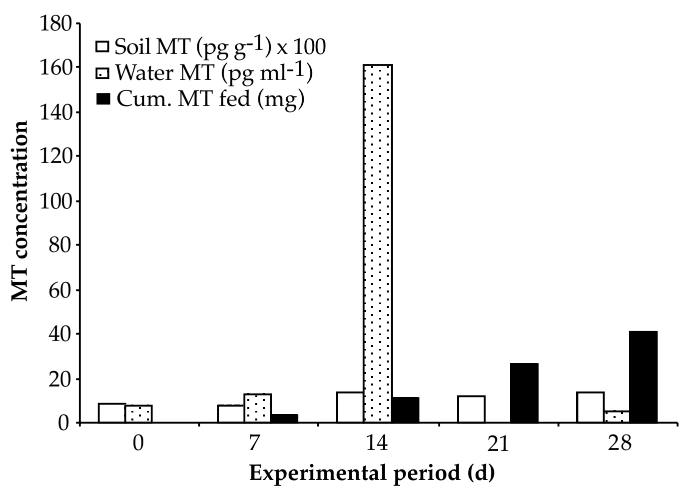

Figure 1. Concentration of methyltestosterone (MT) determined from weekly sampling of soil and water collected from below the treatment cage and within the cage, respectively, and cumulative quantity of MT added to the feed during the treatment period.

containing approximately 2.6 mg MT was given, but water from within the treatment cage contained less than 5 pg MT g^{-1}.

The radioimmunoassay used for soil samples cross-reacted with other materials in addition to MT. Pretreatment concentrations of soil from the pond, which had no previous history of MT administration, had a concentration of 875 ± 147 pg MT g^{-1}. The highest concentration of MT measured (1,417 pg g^{-1}) was from a soil sample collected beneath the cage of fish receiving the non-treated feed. The mean concentration of MT measured from soil samples beneath the cages was greater under the non-treated cage ($P = 0.007$).

The radioimmunoassay may have also cross-reacted with material in the water column since a pretreatment level of 8.0 pg g^{-1} was measured, and values within the control cage and up to 10 m from the treatment cage ranged up to 34.9 pg g^{-1}. The assay appeared to detect a general background level of approximately 9 pg MT g^{-1}. All assays, except for one sample within the treatment cage, gave values less than 40 pg MT g^{-1}.

The lack of accumulation of MT in the pond environment is to be expected. Tilapia are aggressive feeders and would consume all MT-treated feed offered under normal circumstances. MT ingested by the fish is metabolized, and MT and metabolites are excreted. If it is assumed that tilapia, like rainbow trout (Cravedi et al., 1993), excrete 14.8% of the administered MT dose as unmetabolized MT, then in this study the daily release of unmetabolized MT may have been as high as 192 mg l^{-1} on the last day of feeding, while the level detected in the water was < 5 mg l^{-1}. Fitzpatrick et al. (1999) found that MT concentrations in the water column returned to background levels within one week after hormone administration.

Both fungi and bacteria can metabolize exogenous steroids. The biodegradability of MT and its degradation by high light intensity and temperature contribute to the rapid decline of MT in the environment. In a pond setting the breakdown of MT is rapid with little to no effect on non-target fish.

ANTICIPATED BENEFITS

The environmental fate of steroids is a poorly understood area, but it is of concern in settings where non-target animals may be exposed. The use of methyltestosterone is of particular concern because it is a key component of a successful tilapia aquaculture industry. This study has shown that in a pond setting the life of the hormone is short. This study indicated that fish confined close to fish being treated with MT were not affected; concentrations of MT detected in the environment were not significantly above background levels. These data will be very useful for regulators assessing the impacts of producing male tilapia by MT administration. The study illustrates the difficulties associated with this field of study and the need for greater understanding of steroids and similar compounds in the environment before definitive statements can be made about any specific steroid.

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