ABSTRACT

All-male tilapias are desirable because they manifest superior growth characteristics compared to females. The synthetic steroid, 17α-methyltestosterone (MT) has been commonly used to sex-reverse tilapia but because of its potential health and environmental hazards, the use of phytochemicals as alternative means to affect sex differentiation has to be explored. We addressed objective 1 by using the high performance liquid chromatography (HPLC) technique to determine MT concentrations in the water and in fish. We determined the rate of excretion/degradation of MT following dietary uptake. We addressed objective 2 by examining the dietary intake of quercetin, genistein, and complex of flavonoids in the form of maca meal or “propolis”. The phytochemicals that we tested in this study showed potential for affecting sex ratios although their potency was lower compared to MT. Further studies are needed to evaluate other phytochemicals that contain flavonoids and/or isoflavonoids and how these substances are metabolized.

INTRODUCTION

In tilapia aquaculture, all-male populations are desirable because males demonstrate superior growth characteristics compared to females. Moreover, culture of monosex populations prevents reproduction and results in a uniform fish size. The synthetic steroid, 17α-methyltestosterone (MT) is a derivative of a male specific hormone commonly used to masculinize tilapia juveniles (Green et al., 1997; Abucay and Mair, 1997; Gale et al., 1999). The effect of MT is dependent on various factors such as dose, timing and duration of treatment, and mode of administration (Mirza and Shelton, 1988). A problem associated with the use of MT is that, at high doses or prolonged treatment, MT induces gonadal intersexuality and paradoxical feminization (Goudie et al., 1983; Solar et al., 1984; Van den Hurk et al., 1989; Blasquez et al., 1995; Rinchard et al., 1999; Papoulias et al., 2000). Piferer and Donaldson (1989) suggested that paradoxical feminization might be due more to aromatization than to inhibition of in vivo synthesis of androgens. However, these authors stressed that in some species both aromatization and inhibition of in vivo synthesis of androgens could be the cause. Use of synthetic steroids in fish culture is associated with potential release to environment and contamination. This alerts the public and causes concern as to the safety of the product. Therefore, alternative methods and new, safe chemicals to produce monosex populations should be considered.

17α-alkyl anabolic synthetic steroids are popular because alkylation at the 17-position prevents the rapid oral inactivation that occurs with other anabolic steroids and therefore, eliminates the need to inject the drug (Stanley et al., 1997, Gonzalo-Lumbreras and Izquierdo-Hornillos, 2003). The uptake and depletion of MT have been reported in several species of teleost fish, including cichlids (Fagerlund and Dye, 1979; Goudie et al., 1986; Curtis et al., 1991; Cravedi et al., 1993; Rinchard et al., 1999). As MT administered orally is readily metabolized, research on the fate of MT and on its metabolites need to be addressed from human and environmental safety perspective. Chronic exposure of humans to MT can cause adverse health effects such as hepatotoxicity. Therefore, ingestion of MT residue in treated fish may be a potential hazard to human consumers. The quantity of MT residue in fish tissue will depend on its dosing history and its pharmacokinetics characteristics (Vick and Hayton, 2001).

Several methods have been reported to analyze MT and its many hydroxylated metabolites. Most researchers use the gradient elution with retention times for MT greater than 30 min followed by an additional, between-run, re-equilibration. Some of these methods require complex eluent gradient systems that often cause significant changes in the baseline that affect
resolving and detection of later-eluted peaks (Testino Jr et al., 1999). Such problems have focused the analysis of steroids and metabolites by complex techniques based on combined detection methods such as HPLC-MS (high pressure liquid chromatography-mass spectrophotometry) (Stanley et al., 1997; Tsai and Wang, 1999; Clouet-Dumas et al., 2000; Williams et al., 2000; Lagana et al., 2001). We considered that with modifications of the existing detection techniques described in the literature it will be possible to detect 17α-methyltestosterone by using exclusively an HPLC technique.

A different approach to the use of methyltestosterone for sex reversal in fish may involve the use of isoflavonoids, flavonoids and saponins, which are natural estrogenic/androgenic compounds derived from soy, tea, fruits, and vegetables that present an anti-estrogenic activity. The known mechanisms include inhibition of several steroid metabolizing enzymes such as aromatase, a cytochrome P-450 hemoprotein that catalyzes the conversion of androgens, androstenedione, and testosterone via three hydroxylation steps to estrone and estradiol and other enzymes such as 5α-reductase and 17β-hydroxysteroid dehydrogenase (Brodie et al., 1999; Griffiths et al., 1999; Eng et al., 2001). Aromatase is an enzyme of particular interest in sexual differentiation in fish since inhibition of aromatase action mimics the sex-reversal effects of androgen treatments in some fish species (Kwon et al., 2001).

Flavonoids have been found to act as phytoestrogens since these compounds have structures that are recognized as estrogen mimics for the estrogen receptor. They can compete with endogenous estrogens for binding to the estrogen receptor; therefore, they can act as antiestrogens or weak estrogens. Given that estrogen is the product of aromatase, it is not unexpected that some of these compounds can behave as inhibitors of aromatase, TCM them estrogen biosynthesis in cells (Geahlen et al., 1989; Pelissero et al., 1996; Eng et al., 2001).

Several experiments where phytoestrogens have been used either to inhibit aromatase or as steroid receptor antagonists are described in the literature. Isoflavonoids such as genistein act as estrogen agonists via estrogen receptors in cultured cells and also manifest estrogen-like effects in the female reproductive system (Miksicke, 1995; Santell et al., 1997). Some flavonoids, such as chrysin, are natural aromatase inhibitors (Chen et al., 1997) and may be used to boost low levels of testosterone in aging males. Aromatase affinity for flavonoids is generally lower than it is for steroidal derivatives (Seralini and Moslemi, 2001). 7-hydroxyflavone and apigenin used in microsomes of human placenta after normal full-term delivery were the most effective aromatase and 17β-hydroxysteroid dehydrogenase inhibitors. Le Bail et al. (1998) experiments showed that flavonoids with 7-methoxy or 8-hydroxy groups in the ring A showed an important anti-aromatase activity, thus there is some of the structure-activity relations implied.

In contrast, results of the use of other non-steroidal aromatase inhibitors, such as tamoxifen, in tilapia are controversial. Hines and Watts (1995) are the only authors to report a masculinizing effect of this compound (100 mg kg-1 of food) in a hybrid tilapia. Guigue et al. (1999) has demonstrated that tamoxifen has no effect on the masculinization of all female tilapia or rainbow trout. New experimentation is required to validate the possibility of the use of phytoestrogens as sex reversal agents in tilapia, in order to ensure that they are as effective as the common technique to produce monosex populations that involves the use of steroid hormones such as MT. We intend to develop a synthetic steroid-free method for producing all-male populations of tilapia using specific phytochemicals, including genistein, quercetin, and a complex mixture of flavonoids provided by propolis and maca (Lepidium meyenii) meal. We hypothesize that feeding fish with a diet containing those natural substances will affect the sex ratio of the tilapia populations.

<table>
<thead>
<tr>
<th>Group (genotype)</th>
<th>Dietary treatment</th>
<th>Feeding time</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-male</td>
<td>Control</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>All-male</td>
<td>Genistein 500 mg/kg</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>All-female</td>
<td>Control</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>All-female</td>
<td>Genistein 500 mg/kg</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>All-female</td>
<td>17α-MT 60 mg/kg</td>
<td>6 Weeks</td>
</tr>
</tbody>
</table>

**Methods and Materials**

**Objective 1:** Determination of methyltestosterone metabolites concentration in tilapia flesh and water

1a. Determination of 17α-methyltestosterone (MT) accumulation in tilapia flesh

MT accumulation in whole body tissue was conducted at the completion of the first feeding experiment of genetically all-female tilapia Oreochromis niloticus (see Ohio State University feeding trials 2) examining the dietary impact of synthetic steroids and phytochemicals. Fish were assigned randomly into three tanks (150 fish/tank) with semi-recirculating conditions and fed for 45 days. MT was dissolved in ethyl alcohol and incorporated into a semi-purified gelatin-casein diet at a dose of 60 mg/kg (Contreras-Sanchez et al., 2001). After 30 days of feeding, fish were sampled (n=10) every 15 days, from each tank and frozen at −80 °C for further analysis. MT in fish tissues was analyzed using HPLC (Feist et al., 1990) following extraction as described below in HPLC analysis section.

1b. Evaluation of possible differences in the absorption and excretion of 17α-methyltestosterone between all-female and all-male tilapia with varying dietary histories.

Five experimental treatments with three replicates (n=25 fish per replicate), selected from the previous experiment under Objective 2, were used for this trial. Table 4 summarizes the characteristics of experimental groups used in the experiment. Fish were kept in fifteen, 35 L tanks provided with water of constant temperature (26 ± 1 °C) in two recirculation systems (one for each genotype). Fish were starved for 24 hours and fed for two consecutive days with the same diets described in Table 4, supplemented with 60 mg kg-1 of 17α-methyltestosterone (Sigma) dissolved in ethanol. Fish were fed at 6% of fish biomass in each tank. Total food consumption in each tank was recorded.
After the second feeding (1:30 h), fish were moved into 7.5 L plastic containers filled with 3 L of dechlorinated water and provided with constant aeration. The containers were placed floating in the 35 L tanks to maintain a stable temperature. Whole body samples were frozen at -80°C before feeding, and at 1:30 h and 48 h after second feeding (n=3 per replicate) for analysis of 17α-MT and its metabolites in the body tissues by HPLC. Water samples (25 ml) were also collected and frozen at -80°C at different times from the 35 L tanks before the second feeding (T0), and 90 minutes after feeding (T1), to evaluate the loss by leaching of 17α-MT in the diets in all tanks. Water samples (25 ml) from the containers where the fish were moved after the second feeding were sampled and frozen at -80°C to evaluate the excretion rate of 17α-MT and its metabolites at different times. Tissue and water samples were extracted and analyzed by HPLC using the procedure described by Feist et al. (1990) with modifications described below.

1c. HPLC analysis

Water and tissue samples were extracted by liquid-liquid extraction. 2 ml of water were extracted in 8 ml of diethyl ether, the organic phase was removed after snap freezing in liquid nitrogen, the procedure was repeated, and extracts were pooled and dried using nitrogen gas. For MT extraction from whole body tissue, each fish was homogenized in 1.5 ml of distilled water (Omni Int. model 5000 homogenizer), and extraction was performed as described above for water samples. In both cases, dry extracts were reconstituted in 0.5 ml of methanol HPLC grade, vortexed for 30 seconds and filtered through a 13 mm nylon disk syringe filter to 0.45 μm (Phenomenex) prior to injection to HPLC, 20 μl of each reconstituted sample were injected into the HPLC system for each analysis.

The HPLC analysis was performed by means of a modified procedure described by Feist et al. (1990), using a Beckman System consisting of two 110B solvent delivery pumps, 166 system gold detection module, 406 system gold analog interface module, a IBM 433 DX/Dc computer, a Peaksimple chromatography data system and software, and a Phenomenex reverse phase C18 column 150X4.50 mm (flow rate 1.0 ml min⁻¹). Two mobile phases were used, mobile phase A, water:methanol:acetonitrile:isopropanol (62:28:5:5), and mobile phase B, water:methanol:butanol (35:45:20), both mobile phases at a constant flow rate of 30% or 70% mobile phase A: mobile phase B; MT concentration was analyzed at a wavelength of 254 nm. The standard curve was calculated with a range of concentrations from 0.02 µg ml⁻¹ to 20 µg ml⁻¹. The detection limit established corresponds to the lowest concentration. Extraction efficiencies were estimated at 70 ± 16% for water samples and 55 ± 5% for whole body fish tissues samples (n=5).

Objective 2: Evaluation of potential action of phytochemicals on sex differentiation of tilapia

2a. Experimental design for feeding experiments.

The experiments were conducted on first feeding tilapia Oreochromis niloticus (genetically all-female Fish-Gen Phillipines and genetically all male TilTech Aquafarm USA at OSU laboratory, and a population of undetermined sex at UJAT Mexico). In all cases fish were randomly distributed into glass aquaria at a density of 150 fish per aquarium with three replicates per treatment. Fish were fed at 10-15% body weight ratio for 8

### Table 1. Summary of mean final individual weight (±SD), weight gain and specific growth rate of tilapia from each dietary treatment reared in a recirculating system at the Aquaculture Laboratory of The Ohio State University.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Individual Weight (g)</th>
<th>Weight Gain (g)</th>
<th>SGR (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.16 ± 0.74</td>
<td>8.2</td>
<td>5.2</td>
<td>36.7</td>
</tr>
<tr>
<td>Quercetin 1%</td>
<td>7.96 ± 2.66</td>
<td>8.0</td>
<td>5.2</td>
<td>38.7</td>
</tr>
<tr>
<td>Vit C 1000 mg/kg</td>
<td>7.91 ± 1.77</td>
<td>7.9</td>
<td>5.2</td>
<td>40.9</td>
</tr>
<tr>
<td>Quercetin 1% + vit C 1000 mg/kg</td>
<td>6.87 ± 0.84</td>
<td>6.9</td>
<td>5.0</td>
<td>38.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Individual Weight (g)</th>
<th>Weight Gain (g)</th>
<th>SGR (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.14 ± 0.19</td>
<td>1.12</td>
<td>8.28</td>
<td>55.75</td>
</tr>
<tr>
<td>17α-MT</td>
<td>0.74 ± 0.26</td>
<td>0.73</td>
<td>7.52</td>
<td>52.72</td>
</tr>
<tr>
<td>Quercetin 1%</td>
<td>1.17 ± 0.06</td>
<td>1.16</td>
<td>8.33</td>
<td>58.18</td>
</tr>
<tr>
<td>Genistein 500 mg/kg</td>
<td>1.21 ± 0.32</td>
<td>1.20</td>
<td>8.40</td>
<td>57.87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Individual Weight (g)</th>
<th>Weight Gain (g)</th>
<th>SGR (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69 ± 0.15</td>
<td>0.68</td>
<td>8.28</td>
<td>38.97</td>
</tr>
<tr>
<td>Maca</td>
<td>0.50 ± 0.06</td>
<td>0.50</td>
<td>7.73</td>
<td>52.72</td>
</tr>
<tr>
<td>Propolis</td>
<td>0.50 ± 0.14</td>
<td>0.49</td>
<td>7.70</td>
<td>58.18</td>
</tr>
<tr>
<td>Genistein 500 mg/kg</td>
<td>0.73 ± 0.22</td>
<td>0.72</td>
<td>8.38</td>
<td>57.87</td>
</tr>
</tbody>
</table>
ph concocted diets were formulated to avoid contamination with natural steroids (Feist and Schreck, 1990). In order to determine the effects of phytochemicals on sex differentiation, four casein-gelatin based diets were prepared for each experiment. Growth performance was evaluated in terms of the final individual body weight, survival (%), specific growth rate (SGR, %), and weight gain (%). The process of sex differentiation was followed by histological analysis of the gonads by hematoxilin-eosin technique at different intervals for each experiment. The sex was also determined by microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guiguen et al., 1999).

weeks. Semi-purified diets were formulated to avoid contamination with natural steroids (Feist and Schreck, 1990). In order to determine the effects of phytochemicals on sex differentiation, four casein-gelatin based diets were prepared for each experiment. Growth performance was evaluated in terms of the final individual body weight, survival (%), specific growth rate (SGR, %), and weight gain (%). The process of sex differentiation was followed by histological analysis of the gonads by hematoxilin-eosin technique at different intervals for each experiment. The sex was also determined by microscopic analysis of gonadal squashes (Guerrero and Shelton, 1974; Guiguen et al., 1999).

2b. Ohio State University feeding trials.

Three experiments were performed at OSU, two with all male tilapia and one with all female tilapia.

1. All-male tilapia larvae were fed one of four semi-purified diets: control, control plus 1000 mg kg⁻¹ vitamin C, quercetin 1% (Sigma), and quercetin 1% plus 1000 mg kg⁻¹ vitamin C (Table 1).
2. The effect of four experimental diets (supplemented with 1% quercetin or 500 mg kg⁻¹ genistein) on sex differentiation of all-female tilapia was evaluated along with the control diet. The control was free of hormones and phytochemicals containing 60 mg kg⁻¹ of the hormone 17α-methyltestosterone (Sigma) (Table 1).
3. The effect of four experimental diets on sex differentiation of all-male tilapia was carried out with two diets containing vegetal ingredients as potential sources of phytochemicals (maca, Lepidium meyenii, or propolis), a third diet contained genistein (pure form) at 500 mg kg⁻¹ (Sigma). These diets were evaluated against a control diet that was free of phytochemicals (Table 1).

Table 2. Summary of mean final individual weight (±SD), weight gain and specific growth rate of tilapia from each dietary treatment at the Aquaculture Laboratory of UJAT, Tabasco, Mexico. (* Indicates significant differences (P<0.05) in column values).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Individual Weight (g)</th>
<th>Weight Gain (g)</th>
<th>SGR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.72 ± 0.10</td>
<td>0.70</td>
<td>6.4</td>
</tr>
<tr>
<td>Quercetin 1%</td>
<td>0.65 ± 0.20</td>
<td>0.63</td>
<td>6.2</td>
</tr>
<tr>
<td>Vit C 1000 mg/kg</td>
<td>0.65 ± 0.12</td>
<td>0.63</td>
<td>6.2</td>
</tr>
<tr>
<td>Quercetin 1% + vit C 1000 mg/kg</td>
<td>0.88 ± 0.04</td>
<td>0.86</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Table 3. Summary of mean final individual weight (±SD), weight gain and specific growth rate of tilapia from each dietary treatment reared at the Aquaculture Laboratory of UJAT, Tabasco, Mexico. (* Indicates significant differences (P<0.05) in column values).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final Individual Weight (g)</th>
<th>Weight Gain (g)</th>
<th>SGR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.36 ± 0.24</td>
<td>0.35</td>
<td>6.15</td>
</tr>
<tr>
<td>17α-MT</td>
<td>2.02 ± 0.4 *</td>
<td>2.00*</td>
<td>9.56*</td>
</tr>
<tr>
<td>Quercetin 1%</td>
<td>0.62 ± 0.40</td>
<td>0.61</td>
<td>7.22</td>
</tr>
<tr>
<td>Genistein 500 mg/kg</td>
<td>0.47 ± 0.31</td>
<td>0.46</td>
<td>6.66</td>
</tr>
</tbody>
</table>

Two experiments were performed at UJAT. The first experiment with the control diet, control plus 1000 mg kg⁻¹ vitamin C, quercetin 1% and quercetin 1% plus 1000 mg kg⁻¹ vitamin C was performed (Table 2). In the second experiment, the possible effects of four experimental diets (control [casein-gelatin based diet], 60 mg kg⁻¹ 17α-methyltestosterone, 1% quercetin and 500 mg kg⁻¹ genistein) on the growth and sex differentiation of mixed sex tilapia were evaluated (Table 3).

RESULTS

Objective 1: Determination of 17α-methyltestosterone (MT) accumulation in tilapia flesh

The determination of 17α-methyltestosterone in whole body tissue in tilapia by HPLC technique using the modified procedure described by Feist et al. (1990) is an important improvement in the detection method. The time for analysis and the detection limit were considerably improved in comparison with the information available in the literature. Figure 6 demonstrates examples of chromatograms for water and tissue samples and validation by spiking the sample with a known amount of MT standard. The comparison of retention times and concentrations of the MT standards followed.

The rate of MT accumulation in whole body tissue is depicted in Figure 7; there were no significant differences between tanks in the concentration in µg fish⁻¹ or µg g⁻¹ of tissue at the same determination time (week). However, MT concentration tends to diminish per weight unit by the end of MT diet administration and remains at similar levels after 2 weeks of withdrawal of MT feed.

The amount of MT accumulated in whole body tissue in tilapia did not present differences among the different experimental treatments, in regards to sex and dietary history. However, in the group of all-female tilapia, MT exhibits a higher retention percentage 48 hours after feeding. Figure 8 exhibits the amounts of MT accumulated. The amounts of MT lost by leakage from MT-food in Figure 9, is the amounts of MT detected in the water. The excretion by fish recorded from the water samples from the 7.5 L containers is shown in Figure 10. In both cases, there are no significant differences in the amounts of MT detected by HPLC. The pattern of excretion described in Figure 10, includes the mean values of all treatments per determination since in all cases the amounts of MT detected are very similar.

Objective 2: Evaluation of potential action of phytochemicals on sex differentiation of tilapia

In all experiments, diets containing phytochemicals showed low effect on the sex reversal of the fish, except in the case of MT diet in the all-female experiment at OSU, resulted in significant different values (P < 0.05) of masculinization of 90 to 100%, but males ratios in the remaining diets were only 5 ± 5% males (n=60 per diet). In the all-male tilapia experi-
ments at OSU’s laboratory, there were no significant differences in the sex ratios in the two experiments performed. Values were 98 ± 2% in experiment 1 (n= 48 per diet), and 70 ± 30% in experiment 2 (n=60 per diet).

UJAT experiment 1, with undetermined genotype tilapia, showed similar sex ratios in all groups 55 ± 8% females and 45 ± 8% males (n=50 per diet). In experiment 2, MT diet group exhibited 100% masculinization, and the three remaining groups showed values of males not significantly different at 77 ± 10% (n=60 per diet).

In all cases, growth performance observed in the experiments did not show significant differences (Figures 1, 2, 3 and 4), (Tables 1 and 2). The exception being the second experiment carried out at UJAT (Figure 5), where MT diet group exhibited a significantly (P < 0.05) higher final individual weight gain and SGR (Table 3). Survival values were similar in all experiments carried out at the OSU laboratory (Table 1).

**DISCUSSION**

The phytochemicals evaluated in available literature on fish are mostly related to this activity in vitro using gonadal cells and measuring the coefficients of inhibitions of synthesis of estrogens when flavonoids are present at several concentrations. The possible effect of flavonoids on fish aromatase received attention when sex was reversed in a nutritional study with female sturgeons (Pelissero et al., 1996). However, there was considerable variation in the results obtained after using pure phytoestrogens and their action in vitro in the inhibition of estrogen synthesis (Jeong et al., 1999).

There is not an established mechanism of how flavonoids with steroidal activity are absorbed and metabolized by fish, thus their in vivo effect remains unknown. The major questions here
is to what extent are flavonoids absorbed from the gastrointestinal tract and which factors affect their absorption. Absorption of flavonoids from the diet was long considered to be negligible, as most of the flavonoids, except catechins, are present in plants, bound to sugars as glycosides, and were considered nonabsorbable. Evidently the evaluation of the presence of enzymes that split these predominantly \( \beta \)-glycosidic bonds in fish could be an important step in the evaluation of phytoestrogens, such as flavonoids, to be used as an alternative to steroid synthetic hormones. Studies on the absorption of pure compounds indicate only limited absorption, since up to 60 to 70%
of flavonoid aglycones, such as quercetin, are excreted in urine and feces after administration (Hollman and Katan, 1997). We have not included androstatriene (ATD) among the tested chemical because of its toxicity and the precautionary measures required for its use in the laboratory. The number of reports on ATD published since the first idea surfaced (Kwon et al., 2000; 2001) that provided solid evidence as to the masculinization of tilapia of ATD. In that respect our efforts may have been redundant. The use of this chemical in aquaculture would also not eliminate the need for FDA approval (MT approval has already taken 10 years and is far from completion) and may raise the same public concern as other chemicals in food animals. We did not use gossypol as an agent because of its high toxicity to tilapia (Rinchard et al., 2002) and evidence that it does not affect sex ratio in rainbow trout (Rinchard et al., 2003).

**Conclusion**

We were successful in using the HPLC method for determining excretion/degradation rates of MT following dietary uptake. Phytochemicals have shown potential in affecting sex ratios although its potency was lower compared to MT. Further studies are needed to evaluate other phytochemicals that have known steroidal properties.

**Anticipated Benefits**

The use of phytochemicals as an alternative method to produce monosex populations of tilapia will address human and environmental safety issues. Fish offered to the consumer will not need to be treated with MT and producers may have an alternative method for producing all-male populations of tilapia based on natural products, which do not require FDA approval. The low cost of using phytochemicals or plant extracts should also be an attractive alternative to producers. Moreover, several phytochemicals have proven antioxidant activity and enhance immune resistance in fish.

**Literature Cited**


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