Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

Elixir Aqua. 33 (2011) 2231-2233

ISSN: 2229-712X

Effect of dietary administration of the phytochemical "genistein" (*3,5,7,3,4 pentahydroxyflavone*) on masculine tilapia, *oreochromis niloticus*

A.A. Akinwande[1], A.A. Dada[2*] and F.O. Moody[1]

[1]Dept of Aquaculture, Federal College of Freshwater Fisheries Technology, New-Bussa.

[2]Dept of Fisheries and Aquaculture Technology, Federal university of Technology, Akure, Ondo state.

ARTICLE INFO

Article history:

Received: 19 February 2011;

Received in revised form: 18 March 2011;

Accepted: 27 March 2011;

Keywords

Phytochemical,
Genistein,
Monosex,
Enzymatic aromatase,
Weight gain.

ABSTRACT

In Tilapia aquaculture, it is more desirable to culture all male (monosex) population because they grow bigger and faster. The synthetic steroid hormones such as 17-α-methyl testosterone are commonly used to reverse sex in tilapia. Due to its latent health and environmental hazards, attention is presently being focused on the use of alternatives, such as plant extracts called *phytochemicals*. This study investigated the phytochemical "genistein" as a possible enzymatic aromatase inhibitor that could modulate the sex differential response in the gonad of sexually undifferentiated tilapia. Experimental diet supplemented with genistein (500mg/kg) along with a control diet were evaluated in the feeding of three days old tilapia fry (6mg-8mg) for eight weeks. Result shows that the presence of dietary genistein did not have any significance positive effect on the weight gain, SGR (%) and survival (%), although higher number of males were produced in the genistein fed fish but the phenotypic sex ratio was not significantly different (p>0.05) from the control.

© 2011 Elixir All rights reserved.

Introduction

One of the basic factors of tilapia aquaculture is that male fish grow bigger and faster than the female. To avoid unwanted spawning in a production unit, all male population are preferred and several methods has been successfully used to skew sex-ratios and increase the percentage of males in a population.

The technique of manual sexing is quite wasteful and laborious while hybridization crosses (interspecific) involves maintaining two separate brood lines. Complete sex-reversal is accomplished routinely in aquaculture practices by exposing fish to exogeneous sex steroids during gonadal differentiation. The synthetic steroid 17-α-methyltestoterone (MT), a derivative of testoterone, is frequently used to masculinize tilapia (Green *et al*, 1997; Abucay and Mair, 1997; Gale *et al*, 1999).The latent health effect and environmental hazards make the use of alternative chemicals for sex-reversal in aquaculture to be explored.

A passive alternative approach to the use of steroid hormones may involve the use of phytochemicals such as *isoflavonids*, *flavonoids* and *saponins*. Chemical compounds classified as phytochemicals are natural steroids – like compound derived from soy, tea, fruit and vegetable. These compounds has aromatase inhibition ability that are able to suppress estrogen biosynthesis in cells (Eng *et al*, 2001).

Materials and method

Management of *Oreochromis niloticus* brooders

Earthen nursery pond (7 × 3 × 0.8 m) located within the Federal College of Freshwater Fisheries Technology, New Bussa, Nigeria hatchery complex was used to manage the brooders. The pond was fertilized and mixed zooplankton was inoculated for primary production.

A total of 5 males and 15 females were stocked in the pond and fed with 30% crude protein formulated crushed feed for 2 months. Pond was thoroughly checked daily for tilapia nest and hatchlings of the breeders.

3 days old fry (6mg-8mg) were collected from the nursery pond using a hapa sampling net and fry were gently sorted out from the hapa.

Experimental design

Fry were randomly distributed into six circular indoor breeding tanks with the water level maintained at 40cm and tanks were continuously aerated with Tecax air pump model AP 3000.

An initial density of 100 fish per tank was used with three replicate per treatment (i.e 300 per treatment). Initial mean weight of fry/tank was taken using electronic top – loading balance (mettler E200).

Experimental Diet

Two casein-gelatin based diet were prepared as follows, control diet and genistein 500mg/kg(GEN) based diet, which corresponds to the levels commonly found in seed meal of plant (Bennetean-Pelissero *et al*, 2001). Semi-purified diets were formulated to avoid contamination with natural steroids commonly provided when fish meal products are used (Feist and Schneak, 1990).

Fish were fed the experimental diets at 10% body weight for eight weeks and adjusted fortnightly based on weight increase. After eight weeks of feeding with experimental diet, fish were fed with commercially prepared diet (30% C.P) for six weeks until they attained a minimum of 7cm in length.

Growth Performance/Sex Ratio Evaluation

Performance was evaluated in terms of Specific growth rate (%/day), weight gain (g) and survival (%). Sexes were dissection of abdominal cavity to facilitate gonad removal.

Tele:
E-mail addresses: dadaayokanmi@yahoo.com

© 2011 Elixir All rights reserved

Statistical Analysis

Data on growth performance and survival were subjected to One-way analysis of variance (ANOVA) at 95% probability level. The chi-square analysis was used to determine the significant difference in the sex ratio.

Results and Discussion

Composition of experimental diet for first feeding of *O. niloticus* expressed as percentage of dry matter per 100g is shown in Table 1. All ingredients were Sigma product obtained from a standard Laboratory in Lagos, Nigeria. Data on growth performance of the fish is shown in Table 2 while Table 3 shown the sex ratio of *O. niloticus* fed the two experimental diets. Result shows that the sex ratio was not affected by inclusion of genistein in the diet.

There was no significant difference in the growth performance of *O. niloticus* using the chi square (p>0.05) (Table 2). Levy *et al*. (1995) also reported that following genistein treatment of rats during early pregnancy, the number of males was higher than females among the progenies although the sex ratios were not different from that in control. The GEN group showed a lower final mean weight (0.7 ± 0.05g) but the difference was not statistically significant (p>0.05). Observed overall mean survival rate in experimental fish was close to 66% in both treatment. Genistein based diet had high number of males (18) but not significantly different (p>0.05) from the control. However tamoxifen, another aromatase inhibitor, has been reported to have masculinizing effect (100 mg/kg of food) in a hybrid tilapia (Hines and watts 1995)

Genistein was used for this study due to its relative abundance in nature.The content in soybeans product which are also used as ingredients in production of aquaculture feed ,is up to 900mg per kg (Liggin *et al*, 2002).

This phytochemical have received special attention given its ability to interfere with endogenous estrogen biosynthesis by aromatase inhibition. Studies in Japanese *medaka* (O*ryzias latipes*) have showed that quercentin administration can cause endocrine disruption in female (Weber *et al*, 2002). Genistein (*3,5,7,3,4 pentahydroxyflavone*) induced increased level of endogeneous testoterone as indication of aromatase inhibition in rainbow trout.

This study indicates that dietary administration of genistein did not have a significant effect (p>0.05) even though higher number of male was recorded. The number of male tilapia compared to female in genistein supplemented feed in this study is higher but not significant. Based on the results and foregoing, it could be concluded that genistein has aromatase inhibition but has no significant impact in increasing male ratio in this study.

There are limited numbers of studies on the biological effect of phytochemical on sex-reversal in aquatic species. Flavonids (gossypol) present in cotton seed cake has been postulated to affect sex ratio in favour of male when rainbow trout (*Oncorhynchus mykiss*) brood stocks were fed diet containing cotton seed meal (Richard *et al*, 2003). Some flavonoids, such as chrysin, are natural aromatase inhibitors (Chen *et al.,* 1997) and may be used to boost low levels of testosterone in ageing male rats. Gastric incubation of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in rat has also been proved to have anabolizing and virilizing effects (Moundipa *et al.,* 1999).

An attempt to evaluate the effect of dietary administration of genistein in *O. niloticus* in this study did not show any significant impact on sex ratio. It is suggested that in further studies, dietary inclusion levels should be increased, probably the sex ratio may be dosage dependent.

References

1.Abucay JS, and Mair GC (1997) Hormonal sex reversal of tilapia: Implication of hormone treatment application in closed water systems .*Aquaculture Res.* 28 841-845.

2.Bennetau-Plissero C, Breton B, Bennetau B, Le Menn F and Kaushick SJ (2002) Effect of genistein enriched diet on the sex steroid endrocinology and the reproductive efficiency of the rainbow trout *Oncorhynchus mykiss*. veterinaire *Reveu de medecine* 155, 513-516

3.Chen S, Kao YC and Laughton CA (1997) Binding characteristics of aromatase inhibitors and phytoestrogens to human aromatase. *J. Steroid Biochem. Molec. Biol., 61:107115*..

4.Eng ET, William D, Mandava U, Kirma U, Tekmal RR and Chen S (2001) Suppression of aromatase (estrogen synthetase) by red wine phytochemicals. *Breast Cancer Research and Treatment* 67, 133-146.

5.Feist G and Schreck CB (1990) Hormonal content of commercial fish diets and of young coho salmon (Oncorhynchus kisutch) fed these diets .*Aquaculture* 86, 63-75.

6. Gale GL, Fitzpatrick MS, Lucero M,Contreras-Sanchez WM and Schreck CB (1999) Masculinization of nile tilapia (*Oreochromis niloticus*) by immersion in androgens. *Aquaculture* 178, 349-357.

7. Green BW, Veverica KL and Fitzpatrick MS (1997) Fry and Fingerlings production in: Egna H and Boyd C (Ed), Dynamics of pond aquaculture. *CRC Press, Boca Raton*, FL, pp. 215-243.

8. Hines G.A and Watts S (1995) Nonsteroidal chemical sex manipulation of tilapia. *J. World Aquacul. Soc*., 26:98102.

9. Levy JR, Faber KA, Ayyash L and Hughes CL Jr (1995) The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. *Proc. Soc. Exp. Biol. Med.*, 208:6066.

10. Moundipa FP, Kamtchouing P, Koueta N, Tantchou J, Foyang NPR and Mbiapo FT(1999) Effects of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in mature rat testis function. *J. Ethnopharm*., 65:133139.

11. Liggins J, Mulligan A, Runswick S and Bingham SA (2002) Daidzein and genistein content of cereal. *European journal of Clinical Nutrition*. 56, 961-966.

12. Richard J, Lee KJ, Czensy S, Ciereszko A and Dabrowski K (2003) Effect of feeding cottonseed meal containing diets of broodstock rainbow trout and their impact on growth of their progenies. *Aquaculture* 227, 77-87.

13. Weber LP, kiparissis Y HW, Ang GS, Niimi AJ, Janz DM and Metcalfe CD (2002) Increased cellular apoptosis after chronic aqueous exposure to non-lyphenol and quercetin in adult medaka (*Oryzias latipes*). Comparative Biochemistry and Physiology Part C – *Toxicology and Pharmacology*. 131c 51-59.

Table 1. Composition of experimental diets.

[1]Fish protein hydrolyzate from National Institute for Freshwater Fisheries Research, New bussa, Nigeria.

[2]Oils and vitamin/premix from Albarka feed mill Ilorin, Nigeria .

	Experimental Diets	
Ingredient	**Control**	**Genistein**
Casein (vitamin free)	36.00	36.00
Gelatin	6.00	6.00
Dextrin	32.60	32.60
Fish protein hydrolyzate[1]	5.00	5.00
Cod liver oil[2]	3.50	3.50
Soy bean oil[2]	3.50	3.50
Vitamin/Mineral premix[2]	0.8	0.8
Carboxymethyl cellulose	2.00	2.00
L – Methione	0.6	0.6
L – Lysine	0.6	0.6
Choline chloride	1.00	1.00
Phospitan C[4]	0.02	0.02
Genistein	0.00	0.05
Cellulose	1.18	1.18

Table 2. Summary of Weight gain (WG), Specific growth rate (SGR) and Survival of *O. niloticus* in each dietary treatment.

Treatment	Weight Gain (g)	SGR (%/day)	Survival (%)
CON	0.8 ± 0.1^a	8.3 ± 1.1^a	65 ± 8.2^a
GEN	0.7 ± 0.05^a	8.2 ± 0.08^a	66 ± 1.4^a

Mean values in the vertical row with similar alphabets are not significantly different (P>0.05)

Table 3. Summary of male/female ratio of *O. niloticus* fed the two experimental diets

Treatment	Total no. of fish examined	No. of male	No. of female
CON	70	15	55
GEN	70	18	52