

Effect of Feed Storage Time and Storage Temperature on Growth Rate of Tilapia Fry and Efficacy of Sex Reversal

Interim Work Plan, Africa Study 8

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Introduction

Early expansion of tilapia culture was limited because tilapia sexually mature at an early age, which can result in overcrowding in the culture system and in stunted growth. (Hickling, 1963; Hephher and Pruginin, 1981). Methods for culturing exclusively male tilapia, including sex reversal, have been developed to address these problems.

All-male tilapia are produced through sex reversal, that is, by treating fry with a male hormone, such as methyltestosterone or ethynyltestosterone before the primal gonadal cells of females have differentiated into ovarian tissue. Factors that affect sex reversal include: type of hormone, duration of treatment, water quality and temperature, fry stocking density, age and length of fry, quality of feed, and feeding rate. Varadaraj et al. (1994) identified additional factors that should be taken into account: genetics, purity and dosage of hormone, solubility of hormone in solvent, and salinity of the rearing water. The feed manufacturing process can reduce the potency of a steroid. Pure methyltestosterone, a stable, light-sensitive hormone with a melting point between 162 and 167°C, should be stored at room temperature in a sealed, light-proof, amber bottle (Budavari et al., 1989; Sigma Chemical Company, 1994). Varadaraj et al. (1994) also concluded that sub-optimal storage of the hormone and hormone-treated feed can greatly affect their efficacy. Feed should be stored in closed containers of multi-wall construction with plastic liners and kept in a cool, dry place at ambient temperature. Older food should be used first, thus feed containers should be carefully labeled and dated.

Feed stored for longer than 90 days at ambient temperature is subject to the breakdown of oils,

vitamin C, vitamin E, and other vitamins, along with peroxidation of the lipid component (National Research Council, 1981). When fish are given feed deficient in vitamin C, the first vitamin to break down, skin lesions and the disease head and lateral line erosion (HLLE) may result (Jauncey and Ross, 1982). In addition, a mold containing an aflatoxin, which is toxic to fish, may grow in humid environments if storage temperatures rise. Food that has been subject to degradation may inhibit growth and cause vitamin deficiencies, making fish more susceptible to disease (Jauncey and Ross, 1982). Poor storage may also result in off-flavors and odors in the feed, making it less palatable to fish.

Feed availability is often a consideration in sex reversal. Because hormone-treated feed is generally refrigerated, extensive use of the sex-reversal process has been limited to farms with refrigeration facilities. The research described in this report addresses the relative shelf life of hormone-treated feed used for sex reversal at ambient tropical temperature.

Materials and Methods

Research was conducted at the El Carao National Fish Culture Research Center, General Directorate of Fisheries and Aquaculture, Ministry of Natural Resources, Comayagua, Honduras from January 6 to September 6, 1995.

Feed

The basal feed used was Zeigler Mash (55% protein; 13% carbohydrate; 15% fat; 8-10% moisture content; 8-9% ash; < 1% fiber), stored in a -2°C freezer.

Hormone Feed Preparation

A hormone feed containing 60 mg/kg of 17 α -methyltestosterone (MT) was prepared according to the Zeigler Inc. method (personal communication, 1995). A stock solution, stored in a refrigerator at 4°C, was prepared by dissolving 3 g of the MT in 1,000 ml of 95% ethyl alcohol. Sixty ml of the stock solution were then mixed with 630 ml of 90% ethyl alcohol and sprayed on 3 kg of feed in a covered mixer and thoroughly mixed for 20 minutes. Twenty 3-kg batches were pooled together and a 5 cm-layer of feed was then spread on tables in the laboratory at 26°C for 12 hours to allow the solvent to evaporate. The next day the feed was hermetically sealed in plastic zip-lock bags and placed in a freezer at -2°C. The control feed was sprayed with the same alcohol solution as the hormone-prepared diets and remained in the freezer until the day of use. Feeds were taken out of the freezer at their designated times and placed in the refrigerator at 4°C or placed in a sealed cardboard box on a shelf in the laboratory at tropical ambient temperature (28° ± 1.5°C).

Six hormone treated feeds were stored in the following manner:

- 26 days at ambient temperature,
- seven days at ambient temperature,
- zero days at ambient temperature,
- 60 days in the refrigerator and 26 days at ambient temperature,
- 60 days in the refrigerator and seven days at ambient temperature,
- 60 days in the refrigerator and zero days at ambient temperature.

An additional set of fish were fed a non-hormone treated feed that was stored in a dark freezer for 87 days prior to use.

Fry stocking

Oreochromis niloticus fry (Ivory Coast strain; Abdelhamid, 1988), with an initial mean length of 10.4 mm total length, were stocked in hapas. The hapas were suspended from a wooden pier in a 0.1-ha pond with a maximum depth of 1.2 m and a minimum depth of 0.7 m. The hapas measured 1.0 x 1.0 x 0.7 m (length x width x height) and contained 0.5 m³ of water.

Fry less than 14 mm and 14 days old were harvested from a 0.05-ha pond, counted by visual

comparison, and stocked into hapas at 4,000/m³. Visual comparison was accomplished by counting 500 fry into 5 cm of water in a 5-gallon white bucket. Fry were then added to a second bucket until the numbers of fry in each bucket appeared to be the same.

Daily ration

Daily feed quantities were weighed the morning of feeding and sealed in clear plastic jars. The jars were placed outdoors in white plastic containers. Fry were fed four times daily for 28 days. The feeding rate was adjusted weekly by measuring 25 fish per hapa to the nearest millimeter and estimating biomass per hapa using the length-weight formula described by Shelton et al. (1978). The weekly feeding rates were 15, 12, 8 and 4% body weight per day during weeks 1, 2, 3, and 4, respectively.

Harvesting

After 28 days of hormone treatment, fry were harvested and weighed to the nearest 0.1 g. One hundred individuals were measured to the nearest millimeter to determine the length-frequency distribution. Five hundred fry were returned to the hapas and grown to a size of at least 4 cm.

After they reached 4 cm, the fingerlings were harvested and stored in 10% formalin. A sample of 100 fish, representing the length-frequency of the population, was sexed following the procedure described by Guerrero and Shelton (1974). Gonads were identified as male, female, or intersex. Intersex gonads were defined as those having ovarian and testicular tissue and were described by the percentage of ovarian tissue present relative to the whole gonad.

Feed Analyses

Feed samples were analyzed for MT degradation and lipid oxidation. The degree of lipid oxidation was found by determining the peroxide value—the amount of iodine liberated from a saturated potassium-iodide solution at room temperature. The lipid is extracted from the feed using the procedures described by Bligh and Dyer (1959) or Folch et al. (1957). The peroxide value is expressed in milliequivalent of peroxide per kilogram (meg/kg) fat (AOAC, 1990). This analysis was done on two samples—the first sample remained frozen from January 30, 1995, to November 22, 1995, and the second sample after stratification and storage for

Table 1. Sex of *Oreochromis niloticus* fry fed for 28-days a diet containing 60 mg MT/kg. The feed was stored for different durations of refrigeration and at ambient air temperature. Data are means of 4 replicates (100 fish/replicate). No significant differences ($P > 0.05$) were observed among treatments for any parameter.

Duration of Storage time (days)		% Males	% Females	% Intersex	Total No. of Fish Sexed
<i>Refrigerator at 4°C</i>	<i>Ambient Temperature</i>				
Control	Control	50	50	0	400
0	0	100	0	0	400
0	7	100	0	0	400
0	28	100	0	0	400
60	0	100	0	0	400
60	7	99	0	1	400
60	28	100	0	1	400

two months in a refrigerator, was kept for 26 days at tropical ambient temperature and returned to the freezer for 180 days (May 25 to November 22, 1995). The analysis was performed by Woodson-Tenent Laboratories, Inc., Memphis, Tennessee.

Methyltestosterone analyses were conducted by CanTest Ltd., Vancouver, B.C., using high performance liquid chromatography (HPLC) with UV detection (Syndel Laboratories Ltd., 1993). Three samples were analyzed:

- 1) a control feed sample that contained no hormone but had been stored in a dark freezer from (January 30 to December 1, 1995);
- 2) a feed sample containing 60 mg MT/kg that had been stored in a dark freezer (January 30 to December 1, 1995); and
- 3) a feed sample containing 60 mg MT/kg stored for two months in the refrigerator followed by 54 days (equivalent to 26 days of storage followed by 28 days of use while sex reversing fry) at tropical ambient temperature, followed by an additional 189 days in the freezer (May 25 to December 1, 1995).

Data collection

A randomized complete block design consisting of seven treatments and four replicates per treatment was used. Maximum and minimum water temperatures and morning dissolved oxygen were recorded daily; Secchi disk visibility was recorded weekly. Data on daily growth, feed conversion, length, and weight were collected weekly and on the day following the final day of treatment.

Survival was determined by counting the fry by weight the day after the last day of treatment. Treatment means for survival, feed conversion, length, weight, and percent males were compared using one-way analysis of variance (ANOVA).

Results and Discussion

Feed storage conditions of MT-treated rations had no effect on efficacy of sex reversal. *O. niloticus* populations receiving non-treated feed averaged 49.5% males. Populations greater than 98% males were produced with MT-treated feeds that had been stored under all of the described storage times (Table 1). Fry received the equivalent of 1.1 to 1.9 μg MT/g fish/d.

Storage time did not have an adverse effect on growth, survival, or feed conversion ratio (FCR) ($p > 0.05$; Table 2). Feed conversion ratios ranged from 0.85 to 0.87 across treatments. FCRs in other sex reversal studies have ranged from 1.0 to 1.3 (Phelps and Cerezo, 1993). Low FCRs in this study may be attributed to the lack of available, natural foods for the fry. Schroeder (1983) concluded that 50 to 70% of the growth of *O. aureus* hybrids came from natural food organisms. Storage durations apparently did not degrade nutrient quality sufficiently to affect food consumption; any deficiencies in feed quality were possibly supplemented by natural foods. At the end of the 28-day treatment period fish averaged 0.9 g. In other studies, average fry weights at the end of 21-28 days of treatment in hapas suspended in outdoor tanks and ponds ranged from 0.25 to 0.72 g (Buddle, 1984; Popma, 1987; Guerrero and Guerrero, 1988).

Table 2. Initial and final body weights, lengths, survival, and feed conversion ratios (FCR) of *Oreochromis niloticus* fry reared in 0.5-m³ hapas. A diet with 17 α -methyltestosterone and stored for different numbers of days in a refrigerator (refrig.) and at tropical ambient temperature (AT) (0 d-0 d, 0 d-7 d, 0 d-26 d, 60 d-0 d, 60 d-7 d, 60 d-26 d- or a control diet (non-treated) was fed to the fry for 28 days. Data are presented as means of 4 replicates.

	Refrigerate AT						
	Control	0 d - 0 d	0 d - 7 d	0 d - 26 d	60 d - 0 d	60 d - 7 d	2 d - 26 d
INITIAL*							
Weight (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Length (mm)	10.4	10.4	10.4	10.4	10.4	10.4	10.4
FINAL							
Weight (g)	0.9	1.0	0.9	0.9	0.8	0.9	0.9
Length (mm)	36.0	37.0	36.1	36.3	35.0	36.0	35.9
% Survival	55.3	59.5	54.1	57.0	61.0	63.0	58.0
FCR	0.9	0.8	0.9	0.8	0.8	0.8	0.8

* No significant (P > 0.05, ANOVA) differences were observed among treatments for any parameter.

Methyltestosterone concentration was relatively stable. Initial hormone concentration in the feed was 60.4 mg MT/kg of feed; hormone concentration, after two months of refrigerator storage plus 26 days on the shelf was 54.8 mg MT/kg of feed. Factors that influence the degradation of MT include temperature and light sensitivity; however, these factors did not affect MT-feed concentrations in this study.

Initial peroxide value was 13 meq peroxide/kg feed for the feed stored in the freezer; feed stored for two months in the refrigerator plus 26 days at tropical ambient temperature was 20 meq/kg feed. Feeds with peroxide values between 3 and 30 meq/kg/feed are classified as having some oxidation but are not rancid.

The National Research Council (1981) reported that feed stored in an area with high moisture and/or high temperatures will cause peroxidation of the lipid and degradation of vitamins. Results indicate that storage times of up to two months in the refrigerator followed by 26 days at tropical ambient temperature do not cause adverse effects on the efficacy of *O. niloticus* fry sex reversal in an outdoor environment. Varadaraj (1994) found that 100% male tilapia were produced when given feed (stored in a light-proof desiccator) mixed with MT which had been stored for 11 days at room temperature in a light-proof desiccator. He found that only 54% males were produced when fry were

given feed prepared with MT which had been stored at room temperature and had been exposed to light and air, despite past-preparation storage of the MT-treated feed at 4°C in a light-proof dessicator. Only 55% males were produced when fry were given MT-treated feed, which had been stored at room temperature, exposed to light and air, and prepared with MT stored at room temperature in a light-proof dessicator.

Conclusions

MT storage times of up to 60 days in the refrigerator followed by 26 days at tropical ambient temperature did not affect growth, survival, feed conversion efficiency, or sex reversibility in *O. niloticus* fry fed a hormone-containing diet of 60 mg MT/kg for 28 days. Greater than 99% males were produced after fry were fed with feeds stored under six different regimes. After 60 days in the refrigerator followed by 26 days at tropical ambient temperature, feeds were not rancid (final peroxide value of 20 meq/kg feed), and MT levels were reduced from 60.4 mg MT/kg feed to 55.0 mg MT/kg feed.

Literature Cited

Abdelhamid, A.A., 1988. Genetic homogeneity of seven populations of *Tilapia nilotica* in Africa, Central America, and Southeast Asia. M.S.

- Thesis. Auburn Univ., AL 53 pp., Philipp. Dev. VII (14):20-25.
- Association of Official Analytical Chemists (AOAC), 1990. Peroxide value of oils and fats (965.33). In: K. Helrich (Editor), Official Methods of Analysis, 15th Edition. AOAC Inc. Inc., Arlington, VA, pp. 956-957.
- Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37:911-917.
- Budavari, S., M.J. O'Neil, A. Smith, and P.E. Heckelman (Editors), 1989. Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 11th Edition. Merck and Co., Rahway, New Jersey, 1463 pp.
- Buddle, C.R., 1984. Androgen-induced sex-inversion of *Oreochromis* (Trewavas) hybrid fry stocked into cages standing in an earthen pond. *Aquaculture*, 40:233-239.
- Feldman, D.J. Gagon, R. Hofmann, and J. Simpson, 1988. Statview SE + Graphics (software). Abacus Concepts, Inc., Berkeley, California.
- Folch J., M. Lees, and G.H. Sloane Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226:497-509.
- Guerrero, R.D., and L.A. Guerrero, 1988. Feasibility of commercial production of sex-reversed Nile tilapia fingerlings in the Philippines. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 183-186.
- Guerrero, R.D. and W.L. Shelton, 1974. An acetocarmine squash method for sexing juvenile fishes. *Prog. Fish-Cult.*, 36:56.
- Hepher and Pruginin, 1981. Commercial Fish Farming. John Wiley and Sons, New York, 291 pp.
- Hickling, C.F., 1963. The cultivation of tilapia. *Scientific American*, 208(5):143-153.
- Jauncey, K. and B. Ross, 1982. A Guide to Tilapia Feeds and Feedings. Institute of Aquaculture, University of Stirling, Scotland, 111 pp.
- National Research Council, 1981. Nutrient Requirements of Coldwater Fishes. National Academy of Sciences, Washington, D.C., 63 pp.
- Nakamura, M. and M. Iwahashi, 1982. Studies on the practical masculinization in *Tilapia nilotica* by the oral administration of androgen. *Bull. Jpn. Soc. Sci. Fish.*, 48:763-769.
- Nakamura, M. and Takahashi, H., 1973. Gonadal sex differentiation in *Tilapia mossambica* with special regard to the time of estrogen treatment effectiveness in inducing feminization of genetic fishes. *Bull. Fac. Fish., Hokkaido University*, 24:1-13.
- Pandian, T.J., and K. Varadaraj, 1987. Techniques to regulate sex ratio and breeding in tilapia. *Current Science (Bangalore)*, 56:337-343.
- Pandian, T.J., and K. Varadaraj, 1988. Techniques for Producing all-male and all-triploid *Oreochromis mossambicus*. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai, and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 243-249.
- Phelps, R.P. and G. Cerezo, 1992. The effect of confinement in hapas on sex reversal and growth of *Oreochromis niloticus*. *Journal of Applied Aquaculture*, 4:73-81.
- Popma, T.J., 1987. Freshwater fishculture development project-ESPOL, Guayaquil, Ecuador. Final Technical Report. Auburn University, Alabama, 75 pp.
- Schroeder, G.L., 1983. The role of natural foods in tilapia growth: A study based on stable isotope analysis. Proceedings of the International Symposium on Tilapia in Aquaculture, 8-13 May 1983, Nazareth, Israel, pp. 313-322.
- Shelton, W.L., K.D. Hopkins, and G.L. Jensen, 1978. Use of hormones to produce monosex tilapia for aquaculture. In: R.O. Smitherman, W.L. Shelton and J.H. Grover (Editors), Symposium on the Culture of Exotic Fishes. Fish Culture Section, American Fisheries Society, pp. 10-13.

- Shelton, W.L., R.D. Guerrero, and J. Lopez-Macias, 1981. Factors affecting androgen sex reversal of *Tilapia aureus*. *Aquaculture*, 25:59-65.
- Sigma Chemical Company, 1994. Material Safety Data Sheet. St. Louis, MO, USA, 2 pp.
- Soliman, A.K., R.J. Roberts, and K. Jauncey, 1983. The pathological effects of feeding rancid lipid in diets for *Oreochromis niloticus*. In: L. Fishelson and Z. Yaron (Editors), *International Symposium on Tilapia in Aquaculture*, Nazareth, Israel. Tel Aviv University, Tel Aviv, Israel, pp. 193-199.
- Syndel Laboratories Ltd., 1993. High-performance liquid chromatography (HPLC) with UV detection. Syndel Laboratories Ltd., Vancouver, B.C., 2 pp.
- Varadaraj, K., S.S. Kumari, and T.J. Pandian, 1994. Comparison of conditions for hormonal sex reversal of Mozambique tilapias. *Prog. Fish. Cult.*, 56:81-90.
- Vera Cruz, E.M. and G.C. Mair, 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus*. *Aquaculture*, 122:237-248.
- Zeigler, Inc., personal communication, October 31, 1995.