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Effect of orally-administered 17α-methyltestosterone on spermatogenesis in immature milkfish, *Chanos chanos* Forsskal

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Diets containing 17α-methyltestosterone (17α-MT) were fed to milkfish, *Chanos chanos*, both during the spawning season (experiment 1) and after (experiment 2). The daily dosage of 17α-MT per kg of body weight was either 25 mg or 12·5 mg in experiment 1, and either 12·5 mg or 6·25 mg in experiment 2. The fish in both experiments were smaller than the minimum mature size previously reported. After 12 weeks of treatment, the treated fish had GSIs that were four times higher than the control fish in both experiments. Histologically, the testes of the control fish had only spermatogonia, in contrast to spermatozoa in the treated fish. However, no male possessed running milt. So 17α-MT induced spermatogenesis but not spermiation in the immature-sized milkfish.

I. INTRODUCTION

The milkfish, *Chanos chanos* Forsskal, is one of the most extensively cultured species in the world today. It is an important subsistence foodfish throughout South-east Asia. Milkfish farmers currently have to rely on fry of fingerlings collected exclusively from the wild for stocking their ponds. Because of the expansion of culture areas and the inconsistency of these natural supplies, a reliable alternative source of fry is needed. Artificial propagation of this species is, therefore, being pursued.

Two major problems encountered in the effort to spawn milkfish are the unavailability of ripe males and the viscous, non-dispersing nature of the milt (Kuo *et al.*, 1979; Liao & Chen, 1979; Liao *et al.*, 1979; Tseng & Hsiao, 1979; Juario *et al.*, 1980; Lee & Weber, 1983). Juario *et al.* (1980) found Durandron forte 250, a long-acting androgen preparation, to be effective in inducing spermiation and in maintaining newly-caught mature males in good running condition for a maximum of 7 days. However, the technique is only applicable to mature males. An efficient method of inducing maturation and maintaining spermatogenesis and spermiation is still needed.

A recent study of the grey mullet, *Mugil cephalus*, demonstrated that 17α-methyltestosterone (17α-MT), administered orally, can be used to initiate spermatogenesis. In addition, the males of this species can be maintained in good running condition for a full year without adverse effects in sperm quality (Weber & Lee, 1985).

This study examines the response of milkfish testes to different dosages of 17α-MT administered over differing lengths of time. The objective is to test on milkfish the technique developed for inducing spermatogenesis and spermiation in the mullet.

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II. MATERIALS AND METHODS

The study was composed of two experiments, conducted 15 months apart. Both experiments used fish from the same captive broodstock and were conducted in three 54-m^3, cinder block tanks, each 6·7 × 6·5 × 1·2 m deep. Because of the lack of sexual dimorphism in this species, the sex of the individual fish used in both experiments could not be determined at the outset of the experiments. Each fish was marked for identification by a distinct fin-cut on the caudal fin and/or the anal fin. The tanks were supplied with seawater from an underground well (36 ± 2‰ salinity) at a flow rate of approximately 120 gallons h^{-1} (536 l h^{-1}). The average water temperature for both experiments was 26 ± 2° C.

EXPERIMENT 1

Fifty-four 5-year-old milkfish, with an average body weight (body wt) of 1·4 kg (range 1·1–2·0 kg), were distributed among three tanks in May at spawning time. They were fed either Purina Floating Catfish Chow No. 1544 plus codliver oil or Purine Floating Catfish Chow No. 1544 plus codliver oil plus 17α-MT in differing dosages. The high dosage group (HD-1) received 25 mg 17α-MT kg body weight^{-1} day^{-1}. The low dosage group (LD-1) received 12·5 mg 17α-MT kg body wt^{-1} day^{-1}. The control group (C-1) did not receive any 17α-MT. To make up the diets, 17α-MT (Sigma Chemical Company) was mixed with codliver oil which was then combined with Catfish Chow.

Once each week, the fish were removed from the tanks and their identification marks were renewed. At this time the tanks were cleaned to control the algal growth which otherwise would be eaten by the milkfish. Gonadal maturity was assessed by killing three, seven, and eight fish per tank at the end of the 1st, 2nd and 3rd months, respectively.

EXPERIMENT 2

Forty-eight 6-year-old milkfish with an average body wt of 1·7 kg (1·2–2·6 kg) were used for the study begun in October, after the spawning season. The diets consisted of Purina Trout Chow No. 6 plus two different levels of 17α-MT. The high dosage group (HD-2) received 12·5 mg 17α-MT kg body wt^{-1} day^{-1}, while the low dosage group (LD-2) received 6·25 mg 17α-MT kg body wt^{-1} day^{-1}. The control group (C-2) did not receive any 17α-MT. The experimental diet was prepared by dissolving the 17α-MT in 95% ethanol which was then sprayed on the Trout Chow; the alcohol was allowed to evaporate off overnight at room temperature. The diet for the control group was sprayed with alcohol alone.

The fish were checked monthly for maturity by applying gentle abdominal pressure and looking for the release of milt. At the end of the 12th week of the experiment, eight fish from each tank were killed for histological examination.

The special diets for both experiments were prepared weekly and kept refrigerated. They were given in the morning, plain Catfish Chow or Trout Chow being given in the afternoon, *ad libitum*.

In both experiments, body weights and lengths of the killed fish were recorded, gonads were removed, weighed, fixed in Bouin's solution and prepared for histological examination, and sections stained with hematoxylin and eosin. The gonadosomatic index (GSI) was calculated as:

$$GSI = \frac{\text{Gonad wt}}{\text{Body wt}} \times 100$$

Comparisons were made between the data obtained from each treatment by one-way analysis of variance and *t*-test (Sokal & Rohlf, 1969).

III. RESULTS

At the start of both experiments, milt could not be detected in any of the fish, either by applying abdominal pressure or by aspiration with a cannula.

TABLE I. Monthly variation of mean gonadosomatic index of male milkfish administered 17α-methyltestosterone orally in experiment 1; *n* in parentheses

Dosage of 17α-MT (mg kg body wt^{-1} day^{-1})	Weeks from start of experiment (Mean GSI ± S.E.)			
	4	8	12	Total
25 (HD-1)	0·0455 (2)	0·0613 (1)	0·0514 ± 0·0289 (4)	0·0543 ± 0·0210 (7)
12·5 (LD-1)	0·0587 (1)	0·0599 ± 0·0113 (5)	0·0738 ± 0·0270 (3)	0·0644 ± 0·0172 (9)
0 (C-1)	0·0112 (2)	0·0130 ± 0·0033 (5)	0·0143 ± 0·0054 (3)	0·0130 ± 0·0036 (10)

TABLE II. Mean gonadosomatic indices for male milkfish receiving 17α-methyltestosterone for 12 weeks in experiment 2

Dosage of 17α-MT (mg kg body wt^{-1} day^{-1})	Mean GSI ± S.E.	*n*
12·5 (HD-2)	0·0722 ± 0·0176	7
6·25 (LD-2)	0·0991 ± 0·0273	3
0 (C-2)	0·0193 ± 0·0104	4

EXPERIMENT 1

Of the 54 fish used, 26 were male. Milt was not produced by any fish throughout the experiment. However, the GSI of the treated males was approximately four times greater than that of the control males (Table I). This held true throughout the experiment. There was no marked monthly change in GSI for males within the three groups, so the data for each group were pooled for statistical comparisons. The average GSIs for the HD-1 group and the LD-1 group were significantly greater ($P < 0·05$) than that of the control group (Table I). All the fish in this experiment possessed very small gonads in comparison with maturing fish.

The results of the histological examination revealed that male fish in the control group possessed testes at a very early stage of spermatogenesis throughout the experiment. The fish from LD-1 group had spermatogonia with very few spermatocytes in both week 4 and week 8; at week 12, spermatogonia through to spermatozoa were found. In the HD-1 group, fish had testes at stages of spermatogonia to spermatozoa in week 8 and had less developed testes in the 12th week. The histological stage of the testes of the HD-1 group in week 4 was the same as that found in the LD-1 group.

EXPERIMENT 2

Of the 24 fish sampled after 12 weeks, 14 were male. As in experiment 1, the average GSI (Table II) for the males in both treatment groups was approximately

four times that in the control group. The difference was significant ($F > 0{\cdot}005$). Histological examination of the testes revealed that the males in the control group possessed only spermatogonia in the testes, whereas both treatment groups had males with more advanced stages of maturation. All but two of the males in the HD-2 group possessed spermatids and spermatozoa. The development of testes in the males of LD-2 group was less than that observed in the HD-2 group; only one of the three fish had testes with spermatids and spermatozoa.

The GSI of females are not included in the Tables, but are available on request.

IV. DISCUSSION

Two major constraints to conducting reproductive studies on milkfish are limitations on the number of available fish and the lack of dimorphism in this species. Hence, numbers of males in the different groups in these two experiments were rather low.

Liao & Chen (in press) have found that males older than 5 years and females older than 6 years showed gonadal maturation in captivity. However, Tampi (1957) determined that the minimum age for maturation in wild milkfish was between 4 and 5 years, with a body wt of 11 kg. The smallest mature male recorded in Hawaii weighed 2·5 kg (Oceanic Institute, 1977). The testes of a tank-reared, 2·3-kg, 5-year-old milkfish at Tungkang Marine Lab. in Taiwan contained sperm at various stages (Liao & Chang, 1976). Sexually mature milkfish less than 2 kg body wt were found only in the hypersaline ponds of Christmas Island (Crear, 1980). Therefore, the 5-year-old fish in experiment 1 and the 6-year-old fish in experiment 2 had reached the minimum age at which milkfish are known to mature. However, their average size of 1·4 kg in experiment 1 and 1·7 kg in experiment 2 may still have been too small for the males to have responded to the treated diets.

GSIs ranging from 0·09 to 0·76 have been classified as maturing male milkfish (Tan, 1982). In this study, GSIs of treated groups were significantly higher than the control groups, but LD-2 was the only group with a mean GSI greater than 0·09 (Tables I, II). GSIs in the treatment groups showed negative correlations to the amount of 17α-MT given (Fig. 1). However, the fish fed 12·5 mg 17α-MT kg body wt^{-1} day^{-1} (LD-1 and HD-2) had testes that histologically were more advanced than other treatment groups by week 12. In addition, the males in HD-2 group had more developed testes than males in the LD-1 group, even though the dosages of 17α-MT were the same. The LD-1 group was expected to show a better response than the HD-2 group, as experiment 1 was carried out during the spawning season: one explanation is that fish used in experiment 2 were a year older and a little larger, and thus more responsive to the treatment.

The males in the HD-1 group had higher GSIs and less developed testes at week 12 than at week 8. The reason for this observation could not be determined from this study, although it does imply that long-term administration of 17α-MT at high dosages may cause a reverse effect on the maturation of fish of immature size. Hirose & Hibiya (1968) reported the inhibition of spermatogenesis through a feedback mechanism while feeding rainbow trout with chlorotestosterone, and Yamazaki (1976) and Billard *et al.* (1982) reported degeneration of spermatogonia in other fish treated with methyltestosterone. However, Weber & Lee (1985) did

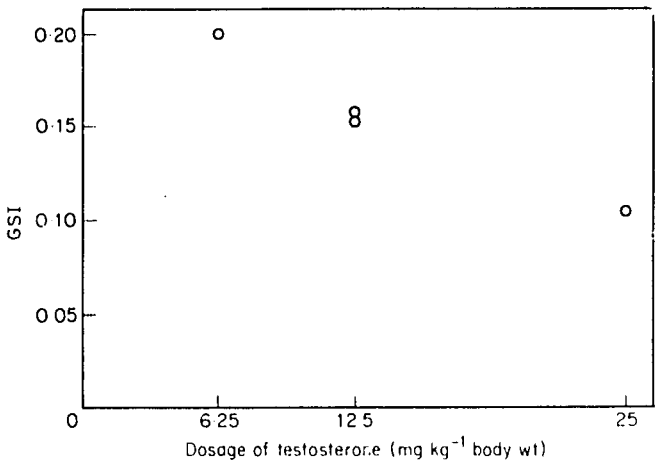

Fig. 1. Correlation between gonadosomatic index and dosage of 17α-methyltestosterone in the daily diet of immature male milkfish in experiments 1 and 2.

not encounter any negative feedback in adult mullet after prolonged treatment. The possibility of negative feedback of 17α-MT in milkfish should be investigated.

Fish in the HD-1 group lost their appetite after 8 weeks on the experimental diet. This change could decrease the amount of 17α-MT in the fish and result in a lesser degree of gonadal development, so the appetite of the fish was improved by changing the way in which the treated feed were prepared between experiments. The fish in experiment 2, using a different feed preparation, were treated for 9 consecutive months and did not produce milt, showing that those fish could not respond to the treatment of 17α-MT. Preliminary results of induction of maturation of milkfish by employing similar methods have produced positive results in larger-sized milkfish in the Philippines (Santiago, pers. comm.) and Taiwan (I. C. Liao, pers. comm.), which strongly suggests that further experimentation on larger milkfish is important.

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References

Billard, R., Fostier, A., Weil, C. & Breton, B. (1982). Endocrine control of spermatogenesis in teleost fish. *Can. Fish. Aquat. Sci.* **39,** 65–79.

Crear, D. (1980). Observations on the reproductive state of milkfish populations (*Chanos chanos*) from hypersaline ponds on Christmas Island (Pacific Ocean). *Proc. World Maricult. Soc.* **11,** 548–556.

Hirose, K. & Hibiya, T. (1968). Physiological studies on growth-promoting effects of protein-anabolic steroids on fish. II. Effects of 4-chlorotestosterone acetate on rainbow trout. *Bull. Jap. Soc. Sci. Fish.* **34,** 473–481.

Juario, J. V., Quinitio, G. F., Banno, J. E. & Natividad, M. (1980). Effects of exogenous hormone injections on milt consistency in newly caught, wild milkfish. *Philipp. J. Biol.* **9(2-3),** 321–326.

Kuo, C.-M. & Weber, G. M. (1983). Preliminary studies on the maturation of milkfish *Chanos chanos* in an environmentally controlled system. Paper presented at Second Int. Milkfish Aquacult. Conf., Iloilo, Philippines, 4–8 October, 1983.

Liao, I. C. & Chang, Y. S. (1976). A preliminary report on the gonadal development of adult milkfish, *Chanos chanos*, reared in tanks. In *Proc. Int. Milkfish Workshop Conf.*, Tigbauan, Iloilo, Philippines, 19–22 May 1976, pp. 121–132.

Liao, I. C. & Chen, T. I. (1979). Report on the induced maturation and ovulation of milkfish (*Chanos chanos*) reared in tanks. *Proc. World Maricult. Soc.* **10**, 317–331.

Liao, I. C. & Chen, T. I. (in press). Report on the gonadal development of milkfish, *Chanos chanos*, reared in tanks. *Aquaculture*.

Liao, I. C., Juario, J. V., Kumagai, S., Nakajima, H., Natividad, M. & Buri, P. (1979). On the induced spawning and larval rearing of milkfish, *Chanos chanos*, reared in tanks. *Aquaculture* **18**, 75–93.

Oceanic Institute. (1977). Annual report on *Research in Artificial Propagation of Milkfish*. 32 pp.

Sokal, R. R. & Rohlf, F. J. (1969). *Biometry*. San Francisco: W. S. Freeman and Co. 776 pp.

Tampi, P. R. S. (1957). Some observations on the reproduction of the milkfish *Chanos chanos* (Forsskal). *Proc. Indian Acad. Sci., Bangalore*. **46B(4)**, 256–273.

Tan, J. D. (1982). A histological study of the hypophysial-gonadal system during sexual maturation and spawning in the milkfish *Chanos chanos* Forsskal. M.S. thesis submitted to Univ. of Philippines. 41 pp.

Tseng, L. C. & Hsiao, S. M. (1979). First successful case of artificial propagation of pond-reared milkfish. *China Fish.* **320**, 9–10 (in Chinese).

Weber, G. M. & Lee, C.-S. (1985). Effects of 17α-methyltestosterone on spermatogenesis and spermiation in the grey mullet, *Mugil cephalus L. J. Fish Biol.* **26**, 77–84.

Yamazaki, F. (1976). Applications for hormones in fish culture. *J. Fish. Res. Bd Can.* **33**, 948–958.