Vet. Med. J., Giza, 39, No. 3, 763-774 (1991)

STUDIES ON NILE TILAPIA (OREOCHROMIS NILOTICUS) MALE PRODUCTION AND THEIR SUSCEPTIBILITY TO BACTERIAL AND FUNGAL INFECTIONS

BY

A.F. BADRAN AND M.A.K. DANASOURY*

Dept. of Avian and Aquatic Medicine, Fac. of Vet. Med., Suez Canal University.

* Dept. of Animal and Fish Production, Fac. of Agriculture, Suez Canal University.

(Received:25 . 2 . 1991)

INTRODUCTION

Monosex culture of tilapia has long been recognised as one of the most satisfactory solution to over - population (Brown and Van Someren, 1953). Many workers (Hickling 1967, Pruginin 1967, Shell 1967 and Bardach et al., 1972) have observed that male tilapia grow at a faster rate than females in culture, and Fryer and Iles (1972) confirmed this to be true of certain wild fish. Male tilapia can be obtained by: (1) sorting of tilapia fingerlings according to sexual characters and this method is wasteful in terms of the female fish are discarded, as well as being laborious and time consuming, (2) sex modification by irradiation, chemical castration and sex reversal. The best method for sex reversal is feeding of methyltestosterone or ethyltestosterone during the first weeks of life (Guerrero 1976)

Epidermis and mucous layer of fish provide the first line of defence against infection by potential environmental pathogens and are involved in the basic

processes of locomotion, osmoregulation and mechanical protection. Changes in the structure of the fish epidermis (reducing the numbers of goblet cells and consequently mucous secreation) are associated with the age, sex and state of maturity (Pottinger and Pickering, 1985). These changes disturb the equilibrum between the forces favouring parasitic invasion and the forces resisting colonization. Therefore, the incidence of skin parasites as *S. diclina* type 1 (Richards and Pickering, 1978 and Willoughby, 1978) and Protozoan flagellate Ichthyobodo (Robertson, 1979) is greater in sexually mature fish compared with immature fish sampled at the same time from the same body of water. Moreover, sexually mature males are more readily infected by a variety of ectoparasites and fungal pathogens than are mature females or immature fish of either sex (White 1975, Richards and Pickering 1978 and Pickering and Christie 1980).

This work was conducted to explain the relation between Nile tilapia males produced by hormonal treatment and their susceptibility to skin parasite infections (one bacterial, *F. columnaris*, and one fungal, *S. diclina*,).

MATERIAL AND METHODS

(1) All male production:-

Four treatment feeds, 0.0, 15.0, 30.0 and 45.0 ug of 17 ∝ methyltestosterone (Sigma LTD Germany)/g diet were prepared as the method described by Guerrero (1975). The pulverised treated diets were stored in airtight plastic bags and refrigerated until feeding. Fish purchased from Abbassa station 11 hrs after hatching were reared in aerated glass aquaria (300 L capacity) where the water temperature was 24±1 C. The fish were devided randomly into 4 groups, each

containing 400 fish, after absorption of the yolk sac. The fish was fed the treated diets ad libitum 3 times daily at 9.00 am, 1 pm and 5 pm. The feeding periods were 14,21 and 42 days for 45, 30 and 15 ug of 17 ∝ methyltestosterone/g diet respectively, after that the hormone diet was omitted and the fish received the diet of control group. The fish were sexed by dissection after 120 days from hatching. The experiment was repeated again and the obtained fish were exposed to artificial infections.

(2) Artificial infections:-

The infections were performed with strictly skin parasitic organisms, one bacterial *(F. columnaris)* and one fungal (*S. diclina*) , at 30, 60, 90 and 120 days of fish age. The organisms, *F. columnaris* and *S. diclina* were isolated from diseased Nile tilapia (*O. niloticus*) by Badran and Eissa (1991) and Badran et al. (1991) respectively . For each infection 100 fish from each group were used, 50 fish for *F.columnaris* and 50 fish for *S. diclina* infections. The fish were placed in glass aquaria each supplied with 118 liters of dechlorinated tap water and the water temperature was maintained at 20 ± 1 C throughout the period of challenge. The mortalities of each challenge were recorded daily throughout 30 days and were used for *F. columnaris* and *S. diclina* reisolation.

2.1. Bacterial infection:

The growth of *F. columnaris* was carried out in 2 transfers of cytophage broth, 100 ml and 1 L each in sequence, at 28 C for 24 hrs each (Kuo et al., 1981). At the end of the second transfer , the bacterial culture was diluted to 1/5 in 0.5% saline to provide a cell suspention of 0.5-1.2 x 10^8 colony forming unit (CFU)/ml which used for infection by

immersion method. Before immersion in the cell suspension for 10 minutes, the fish were pre-immersed in 1.0% Nacl for 2 minutes.

2.2. Fungal infection:-

The fish of each group were exposed to *S. diclina* infection by indirect contact with 5 clinically diseased Nile tilapia fingerlings which were infected by direct contact with scale removal (Singal et al., 1987) and then added to each aquarium.

RESULTS

(1) All male production:-

The results of sex ratio are represented in **Table (1)** and they revealed that, the male percent were 68, 100 and 56 in groups fed 45, 30 and 15 ug of 17 α methyltestosterone/g diet for 14, 21 and 42 days respectively while the male percent of the control group that was fed untreated diet was 52%.

(2) Artificial infections:-

The results of artificial infections with *F. columnaris* and *S. diclina* are demonestrated in **Table, (1)** and revealed that, the mortalities were directly proportional with the male percent in each group especially at 90 and 120 days of fish age. The mortalities in the group of entire males (100%) at 120 days of fish age were 40 and 42% while in the control group were 30 and 28% respectively.

DISCUSSION

The results of the present study revealed that, sex ratio corresponded to the hormone (17α methyltestosterone) concentration and duration of hormone

Table (1). Results of Nile tilapia (O. niloticus) male production and their susceptability to bacterial and fungal infections .

Fish group	* Hormone level	Duration of feeding	Male %	Pathogen of artificial infection	+ Total fish	Fish age (days) at the artificial infections							
						30		60		90		120	
						Dead fish	Mortal. %	Dead fish	Mortal. %	Dead fish	Mortal. %	Dead fish	Mortal %
1	45 ug/g	14 days	68	F. columnaris	50	17	34	17	34	18	36	18	36
				S. diclina	50	16	32	16	32	17	34	17	34
2	30 ug/g	21 days	100	F. columnaris	50	17	34	18	36	20	40	20	40
				S. diclina	50	17	34	19	38	21	42	21	42
3	15 ug/g	42 days	56	F. columnaris	50	16	32	16	32	15	30	15	30
				S. diclina	50	15	30	15	30	15	30	14	28
4	Control	———	52	F. columnaris	50	16	32	15	30	15	30	15	30
				S. diclina	50	15	30	15	30	14	28	14	28

* 17∝ methyltestosterone / g of fish diet.

\+ Total fish (50 fish) for each artificial infection.

Mortal. = Martality .

feeding. Since, male percentage was 68, 100 and 56% in the groups fed 45, 30 and 15 ug hormone/g diet during 14,21 and 42 days of the first life respectively. The male percentage in the control group was 52%. Although, the treated fish in different groups received the same quantity of hormone throughout treatment periods, the production of entire males was obtained in group fed 30 ug hormone/g diet for 21 days. This result indicated that the success of all male production depends upon the correspondence of gonadal differention stage and specific hormone dose with its feeding duration. These results explain those reported by Guerrero (1976) and Macintosh et al. (1985).

Mucous layer covering the external body surface of fish provide the first line of defence against infection by prevention of colonisation of bacteria , fungi and parasites and thus acts as a chemical defence barrier (Jakowska, 1963). It is secreated by specialised goblet cells present in the epidermal layer (Harris et al., 1973 and Pickering 1974). The depletion of mucous - secreating goblet cells goblet cells accompanied with age, sex and state of maturity (Pickering, 1977) or with sex modification by feeding of hormonal treated diets (Pottinger and Pickering 1985) favour the parasitic invasion and colonisation through the skin. This phenomenon is supported by the results obtained from the present study where the susceptibility to artifical infections were directly proportional with the male percentage in each group. Since, the mortalities of *F. columnaris* and *S. diclina* infections at 120 days of fish age were 40 and 42% in the group of 100% males while they were 30 and 28% in the control group (52% males). The results coincided with those reported by White (1975), Richards and

Pickering (1978) and Willoughby (1978) who found that the incidence of saprolegniosis was greater in sexually mature male brown trout and salmonid fish than in mature females or immature fish of both sex. Yet, Pickering and Christe (1980) also reported that the incidence of infestation by the ectoparasites, Ichthyophthirius, Trichodina, Scyphidia and Saprolegnia, was significantly greater in sexually mature males brown trout compared with mature females. Moreover, Puckridge et al. (1989) reported that,the adults of bony bream fish, rather than juveniles , are affected with mycotic dermatitis as a result of hormonal changes associated with sexual maturation which may increase the susceptibility of fish to disease. On the other hand, the results disagree with those reported by Watson and Dick (1980) who found that metazoan parasites of pick exhibited definite patterns of abundance with host age and season and this resulted from changes in host diet and behaviour, while no difference in parasite abundance existed between the host sexes. This difference was attributed to the route of Metqzoan infection to fish which was either by ingestion of infected host or through gills according to metazoan species. Also, several investigation have failed to demonstrate any differences in ectoparasitic (Smith 1969 and 1972, Hanek and Fernando 1978 a & b) or endoparasitic infection (Lawrence 1970 and Evans (1978) which could be related to the sex of the host.

From the present study, it is concluded that:-

1. The entire males of Nile tilapia *(O. niloticus)* was obtaine by feeding of 30 ug 17 ∝ methyltestosterone/g of fish diet during the first 21 days of life.

2. Intensive culture of entire males of Nile tilapia should be under Veterinary observation and mangment

to menimise the environmental stress factors as the males were readily to contract the diseases especially that caused by skin parasitic organisms.

SUMMARY

Nile tilapia *(O. niloticus)* male production was established by feeding of 17 ∝ methyltestosterone at various levels for different durations. Four months from hatching, the fish were sexed by dissection. The fish fed hormonal treated and untreated diets were exposed monthly, from hatching till 120 days of age, to artificial infection with *Flexibacter columnaris* and *Saprolegnia diclina*.

All male production (100% male) was obtained from the group fed diet containing 30 ug 17 ∝ methyltestosterone/g diet for 21 days, while the male percentage in groups fed diets containing 45 and 15 ug of hormone/g diet for 14 and 42 days were 68 and 56 respectively. The male percent in the control group was 52%.

The mortality ratio of artificial infection with *F. columnaris* and *S.diclina* in groups fed the hormonal treated and untreated diets were directly proportional with the male percent in each group. Since, the mortalities of *F. columnaris* and *S. diclina* infection in the group of all male production at 120 days of age were 40 and 42% respectively, followed by the mortalities in the gorup of 68% male which were 36 and 43% respectiely. The mortality ratio in the control gorup (52% male) were 30 and 28% for the same organisms respectively and at the same time.

REFERENCES

1. Badran A.F. and Eissa I.A.M. (1991): Studies on bacterial diseases among cultured fresh water fish (Oreochromis niloticus) in relation to the incidence of bacterial pathogens at Ismailia Governerate. Vet. Med. J. Giza. (Under publication).

2. Badran A.F., Ezzat M. and El-Tarabili M. (1991): Investigation on saprolegniosis among Nile tilapia (Oreochromis niloticus) with special reference to its contrcl. Zagazig Vet. J. 19, (1), 26-40.

3. Bardach J.E.; Ryther J.H. and McLarney W.O. (1972): Aquaculture: The farming and husbandry of freshwater and marine organisms. Wiley-Interscience; New York, London. p. 868.

4. Brown J.M. and Van Someren V.D. (1953): New fish culture methods for Tilapia in East Africa. Nature, Lond., 172, (4373): 330-332.

5. Evans N.A. (1978): The occurrence and life history of Asymphylodora kubanicum (Platyhelminthes: Digenea: Monorchidae) in the Worcester-Birmingham Canal, with special reference to the feeding habits of the definitive host, Rutilus rutilus., J. Zool., Lond., 184, 143-153.

6. Fryer G. and Iles T.D. (1972): The cichlid fishes of the great lakes of Africa: Their biology and evolution. Oliver and Boyd; Edinburgh. p. 541.

7. Guerrero R.D. (1975): Sex reversal of tilapia. FAO Aquaculture Bull., 7, (3-4): 7.

8 . Guerrero R.D. (1976): Sex reversal of tilapia. FAO Aquaculture Bull., 8, (1): 5-6.

9 . Hanek G. and Fernando C.H. (1978 a): The role of season, habitat, host age, and sex on gill parasites of Lepomis gibbosus (L.). Can. J. Zool., 56, 1247-1250.

10. Hanek G. and Fernando C.H. (1978 b): The rolw of season, habitat, host age and sex on gill parasite of Ambloplites rupestris (Raf.). Can. J. Zool., 56, 1251-1253.

11. Harris J.E., Watson A. and Hunt S. (1973): Histochemical analysis of mucous cells in the epidermis of brown trout, Salmo trutta L., J. Fish Biol., 5, 345-351.

12. Hickling C.F. (1967): Fish hybridization. FAO Fish. Rep., 44, (4): 1-11 (IV/R-1).

13. Jakowska S. (1963): Mucus secretion in fish-a note. Ann. N.Y. Acad. Sci., 106, 458-462.

14. Kuo S.C., Chung H.Y. and Kou G.H. (1981): Studies on artificial infection of the Gliding bacteria in cultured fishes. Fish Pathol. 15 (3/4) 309-314.

15. Lawrence, J.L. (1970): Effects of season, host age, and sex on endohelminths of Catostomus commersoni. J. Parasit., 56, 567-571.

16. Macintosh D.J., Varghese T.J. and Satyanarayana G.P. (1985): Hormonal sex reversal of wild - spawned tilapia in India. J. Fish Biol. 26, 87-94.

17. Pickering A.D. (1974): The distribution of mucous cells in the epidermis of the brown trout, Salmo trutta L., and the char, Salvelinus alpinus L.,. J. Fish Biol., 6, 111-118.

18. Pickering A.D. (1977): Seasonal changes in the epidermis of the brown trout, Salmo trutta L.,. J. Fish Biol., 10 , 561-566.

19. Pickering A.D. and Christie P. (1980): Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, Salmo trutta L.,. J. Fish Biol. 16, 669-683.

20. Pottinger T.G. and Pickering A.D. (1985): The effect of 11-ketotestosterone and testosterone on the skin structure of brown trout, Salmo trutta L.,. General and Comparative Endocrinology 59, 335-342.

21. Pruginin Y. (1976): Culture of carp and Tilapia hybrids in Uganda. FAO Fish Rep., 44 (4): 223-229.

22. Puckridge J.T., Walker K.F., Daley C. and Beakes G.W. (1989): Mycotic dermatitis in a freshwater gizzard shadm the bony bream, Nematalosa erebi (Gunther), in the River Murray, South Australia. J. of Fish Dis., 12, 205-221.

23. Richards R.H. and Pickering A.D. (1978): Frequency and distribution patterns of Saprolegnia infection in wild and hatchery-reared brown trout, Salmo trutta L., and char, Salvelinus aplinus L.,. J. Fish Dis. 1, 69-82.

24. Robertson D. (1979): Host parasite interaction between Ichtyobodo necator (Hennegy 1883) and farmed salmonids. J. Fish Dis. 2, 481-493.

25. Shell F.W. (1967): Mono-sex culture of male Tilapia nilotica (Linnaeus) in ponds stocked at three rates. FAO Fish. Rep., 44, (4): 253-258 (V/E-5).

26. Singhal R.N., Jeet S. and Davies R.W. (1987): Experimental transmission of Saprolegnia and Achlya to fish. Aquaculture, 64, 1-7.

27. Smith J.W. (1969): The distribution of one monoger an and two copepod parasites of whiting , Merlangius merlangus L., caugh in British waters. Nytt Mag. Zool., 17, 57-63.

28. Smith J.W. (1972): The occurrence of Diclidophora esmarkii (Nilsson, 1855), in the northern North Sea and to the north and west of Scotland. J. Cons. perm. int. Explor. Mer 34, 256-261.

29. Watson R.A. and Dixk T.A. (1980): Metazoan parasites of pike, Esox lucius Linnaeus, from Southern Indian Lake, Manitoba, Canada. J. Fish Biol., 17, 255-261.

30. White D.A. (1975): Ecology of an annual Saprolegnia sp. (Phycomycete) outbreak in wild brown trout. Verh. int. Verein. theor. angew. Limnol., 19, 2456-2460.

31. Willoughby L.G. (1978): Saprolegnias of salmonid fish in Windermere: a critical analysis. J.Fish Dis., 1, 51-67.