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CONTROL OF GYRODACTYLOSIS IN OUTDOOR CULTURE OF MONO-SEX TILAPIA *(OREOCHROMIS NILOTICUS).*

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ABSTRACT

The study was conducted over a 150 days period in twelve earthen ponds to evaluate the ability of benzole25® (anthelmentic drug) to control gyrodactylosis in *Oreochromis niloticus*. The experimental ponds were stocked with 20000 fingerlings / faddan of 20 to 30 g body weight. Fish were fed a balanced diet of 28.50-28.80 % protein at a rate of 3% of the fish biomass as following 1- Basal diet (control group without *benzole25®*). 2- Diet 2 (10 ml *benzole25®*/kg ration). 3- Diet 3 (20 ml *benzole25®*/kg ration) 4- Diet 4 (30 ml *benzole25®*/kg ration). Fish were fed the previously mentioned ration for ten successive days then all groups received non-treated rations as that of the control group. Results indicated that treated groups revealed significant improvement in growth parameters and control of Gyrodactylosis (body weight gain, feed conversion ratio, protein efficiency ratio, and decreased infestation intensity). Doses of 20 ml/kg and 30 ml/kg showed satisfactory results considering; growth performance, feed utilization, general health conditions and economic evaluation. This study suggested that *benzole25®* levels of 20ml/kg and 30ml/kg can be used in the treatment of Nile tilapia.

INTRODUCTION

With the development of aquaculture in the last three decades and further with the increasing number of private and public display aquaria, concentration of hosts and parasites in confined quarters results in enhanced opportunity for infestations and epizootics, (Schmahl, 1998 and Lavilla, 2001). Tilapia culture is becoming an important part of the local fish culture industry in Egypt, (Gafrd, 2007 and Abdelhamid, 2009). But as it continues to intensify, outbreaks of tilapia diseases have been observed to cause considerable financial and economical losses to tilapia growers (Abdelhamid, 2009).

Among farmed fishes, Gyrodactylus (a monogenean trematode which is a parasitic fluke less than one millimeter in length with a direct life cycle not involving any intermediate stages or hosts) are more common parasites of the fins, gills, eyes and body, (Mo, 1994 and Eissa, 2002). They are often sub-clinical and without apparent signs. Under predisposing factors such as poor water quality, high ammonia as a result of high stocking density, remnants of rations, ectoparasites, inadequate handling and stressful conditions, diseases affect fish host (Thoney and Hargis, 2003 and Moraes and Martins, 2004). Appropriate medications are needed to maintain animal health and to manage fish populations. The goal of treatment is to control the progression and spread of the pathogen or parasite within the population and limit morbidity and mortality while maintaining normal growth and development, (Storey, 2005). Chemotherapy is considered as the most effective and flexible weapon against parasitic infestations, (Schmahl, 1998). Various methods have been described for the control of gyrodactylosis, all based on pharmacological bath treatments (Mehlhorn *et al.* 1988, Schmahl *et al.* 1989, Santamarina *et al.* 1991 and Tojo *et al.* 1992).On the other hand oral treatments have a number of advantages, including ease of administration and the fact that there is no need to handle the fish, (Taraschewski *et al.* 1988, Sanmartin *et al.* 1989 and Robinson *et al.,* 2004). Therefore the aim of the present study was to investigate possible oral pharmacological treatments of gyrodactylosis in tilapia rations using Albendazole under farm conditions with doses lower than that recorded by Tojo and Santamarina1, (1998) who considered oral treatment with bendazoles is unlikely to be an economically viable option, since the required dosage (40 ml/kg of feed for 10 days) implies use of large amounts of the drug.

MATERIALS AND METHODS

The present work was conducted in a Fish farm in Kafr El-Sheikh Governorate Elreyad area season 2009 in order to evaluate a commercial drug; benzole25® (suspension of a broad spectrum anthelmintic in which each 1ml contains Albendazole 25mg. Imported by Agricultural Materials Company from Kela company in Belgium, Batch number:13122.10) in cultured tilapia diet on growth parameters,

feed utilization, control of parasitic infestation and economical efficiency.

1- Fish culture system:

About 240000 fish Nile tilapia (*O. niloticus*) weight 20-30g were obtained from the earthen nursing pond in the same private farm, in Kafr El-Sheikh governorate, Egypt. Fish were randomly distributed among twelve ponds (each pond is about one faddan), the stoking density was about 5 fish/ m^3 (Salah, 2003). The experimental pond supplied with freshwater from drainage canal number 7 in Toulombat 7-Ryad area. The water exchange rate was about 20% of the total pond area/day. The experimental period was about 150 days. Physico-chemical parameters (ammonium, electric conductivity, SD, total disoived solids, salinity, oxygen saturation, dissolved oxygen, PH and water temperature).

in the reservoir ponds were determined insitu with Horiba U-7 water checker according to Abdelhamid(2009).

2- Experimental diets:

The drug (benzole25®) was mixed first in few milliliters of vegetable oil to avoid the rapid dissolution in water. The dry ingredients were grounded through a feed grinder to small particle size (5mm). Such experimental design was nearly close to that of Tojo and Santamarina, (1998). Ingredients were weighed and mixed by a dough mixer for 20 minutes to be homogenous. The estimated amount of oil components (sunflower oil) was gradually added (few drops gradually) and the mixing operation was continued for 20 mimutes. Then 40 ml water per 100g diet were slowly added to the mixture according to Shimeino, *et al* (1993). The diets were pelleted through fodder machine and the pellets were dried under room temperature. The required amount of the diets was prepared every two weeks. Fingerlings were fed daily the commercial diet represented in table (1) at a feeding rate of 3% of fish biomass (six days per week). twice daily (at 8 am and 3 pm).The quantity fed was adjusted at approximately 14-days intervals in response to the increasing body weight (Salem, *et al.* 2009). The diets were designated as diet (1) to (4), in which diet one was considered as a control non treated diet. While, group two, three and four represented the doses of 10, 20 and 30 ml benzole25®/Kg

ration, respectively. Fish were fed the previously mentioned rations for ten successive days then all groups received nontreated rations till the end of the experimental period.

Table (1): Ingredients and composition of the experimental diets.

diet 4	diet 3	diet2	diet 1	Ingredient
10	10	10	10	Fish meal
35.9	35.9	35.9	35.9	Yellow corn
36.6	36.6	36.6	36.6	Soya bean meal
12	12	12	12	Wheat bran
5	5	5	5	Sunflower oil
0.5	0.5	0.5	0.5	Vit. &Min[1].
100	100	100	100	Total
30	20	10	-	*Drug* ml/kg
				Chemical analysis (%).
				Dry matter.
				Crude protein.
				Ether extract.
				Crude Fiber
				Ash
				Nitrogen Free Extract
				Calculated values.
				Gross energy (Kcal/100g)[2]
				Digestible energy (Kcal/100g)[3]
				P/E ratio (mg/Kcal)4

1- Vitamin and mineral mixture (product of HEPOMIX) each 2.5 kg contain: 12.000.000 IU Vit.A; 2.000.000 IU Vit. D3; 10 g Vit. E; 2g Vit. K3; 1g Vit. B1; 5g Vit. B2; 1.5 g Vit. B 6 ; 10g Vit.B12; 30 g Nicotinic acid ; 10 g Pantothenic acid ; 1g Folic acid; 50g Biotin; 250g Choline chloride 50% ; 30g Iron; 10g Copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.

2- (Gross energy) (Kcal/100g), based on 5.6Kcal/g protein, 9.44 Kcal/g lipid, 4.1 Kcal/g carbohydrate, according to (Jobling, 1983).

3- (Digestible energy (Kcal/100g), based on 5.0 kcal/g protein, 9.0 kcal/g lipid, 2.0 kcal/g carbohydrate. according to (Wee and Shu. 1989).

4- (P/E) (protein to energy ratio) = mg crude protein / Kcal of gross energy.

3-Growth parameters:

Average total gain (ATG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR %) was calculated according to the following equations:

a- ATG (g/fish) = [Average final weight (g) – Average initial weight (g)] as reported by (Annet, 1985). b- ADG (g/fish/day) = [ATG (g)/experimental period] c- SGR (%/day) = [Ln final body weight–Ln initial body weight] x 100/experimental period according to Pouomonge and Mbongland (1993). d- FCR= Feed Intake, dry weight

(g)/Live weight gain as reported by De Selva and Anderson (1995). e-PER = Live weight gain (g)/ protein intake (g) as reported by De Selva and Anderson (1995). f-SR = 100[total No. of fish at the end of the experimental /total No. of fish at the start of the experimental.

4 – Proximate analysis:

Dry matter, crude protein, ether extract, crude fiber and ash contents of the feed ingredients and whole body of fish at the beginning and at the end of the experiment were performed according to A.O.A.C. (1990).

5- Parasitic Examination

All fishes were grossly examined for the detection of any external abnormalities on the body surface (skin, fins, gills, eyes and mouth).

Scrapings of body surface was taken from each fish and examined on a glass slide with cover slip for parasitic infection according to Woo (1995). Identification by light microscopy (40x) was done according to, Tojo and Santamarina (1998) and Eissa (2002).

6- Infestation intensity

Infestation intensity was recorded on a 5-point scale, after examination of a sample area of 24 X32 mm, as follows: 'minimal', only 1individual of *Gyrodactylus* sp. detected in the sample, 'low', from 1-10, 'moderate'; 10 to 50, 'high'; >50 and 'zero' (-); *Gyrodactylus* sp. not detected in the sample, (Tojo and Santamarina, 1998).

7 - Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F- test was significant, least significant difference was calculated according to Duncan (1955).

RESULTS AND DISCUSSION

1 Chemical composition of the experimental diets:

Chemical composition of different diets are presented in Table (1). There were no differences observed among diets in DM, CP, EE, CF and ash. The CP content was between 28.55 to 28.80% on DM basis. Such level was within the range suggested by Jauncy and Ross (1982) and NRC (1993). The calculated energy was similar in the tested diets, where the GE values ranged from (435.47 to 453.66

Kcal/100g); it was higher than that suggested by NRC (1993) for tilapia. However, it was nearly similar to that used by Hassanen *et al.* (1995), Abd-El-Maksoud *et al.* (1998) and Salem *et al.* (2009).

2 Quality parameters of rearing water:

Table (2): water analysis

NH4	E.C.	SD	TDS	Sal.	Sat.	DO	PH	Temp.
1.4	3.1	13.1	2.4	2.0	65.1	6.2	9.1	28

Results of Table (2) shows that All tested water quality criteria were suitable for rearing *O. niloticus* fingerlings as cited by Abdel-hakim *et al.* (2002) and Abdelhamid (2009).

3 Growth performance and survival rate:

At the end of experimental period, both groups; diet 3 and diet 4 revealed significant increase in the parameters of Growth performance and survival rate. There were significant ($P \leq 0.05$) differences among various groups of fish as group 3 and diet 4 seem better than 1 and 2. These results are demonstrated in Table (2). Feed and Protein Utilization showed that both group 3 and 4 were the best ($P \leq 0.05$) treatment in comparison with 1 and 2. While, there were no significant differences between group 1 and 2 in all studied parameters. Such results were closely near to that reported by Storey (2005). However, significant differences ($p > 0.05$) were observed among fish groups offered feed with doses (20 or 30ml/kg) of benzole25®.

The economic parameters of the tested rations are presented in Table (3). The calculation depends on the average prices of ration ingredients at year (2009) which was around: (vit. And min. mixture 9000LE/ton, Fish meal 9000LE/ton, yellow corn 1500LE/ton, soybean meal 2000LE/ton, wheat bran 1250LE/ton, and Oil 6500LE/ton and benzole25® 45 LE/liter. The diet 1 recorded the lowest price being 2560.2 LE/ton but, rations of group 3 and 4 showed decreased cost/kg gain (7.23-6.91 LE) compared with diet 1 and 2 that gave the higher cost/kg (8.13 and 8.24LE). This was in a disagreement with the findings of Tojo and Santamarina1, (1998) who considered treatment with bendazoles is an uneconomical option.

Table (3): Performance data of Nile tilapia *O.niloticus* fed on the experimental fish:

Diet No. parameters	Diet 1 *O.niloticus*	Diet 2 *O.niloticus*	Diet 3 *O.niloticus*	Diet 4 *O.niloticus*
Av. Initial body weight (g).	10.6a	10.5a	10.8a	10.4a
Av. Final body weight (g).	140d	145c	170b	185a
Av. Gain in wt (g).	129.4d	134.5c	159.2b	174.6a
Av. daily gain (g/day).	0.86d	0.89c	1.06b	1.16a
S.G.R. %	1.72b	1.75b	1.83a	1.92a
F.C.R.	3.17a	3.12a	2.66b	2.47b
P.E.R.	1.09b	1.11b	1.31a	1.42a
Survival rate %	90b	85b	100a	100a
Cost(LE) of one ton diet	2560.2	2640.2	2720.2	2800.2
Feed cost/kg gain (LE)*	8.13	8.24	7.23	6.91

a,b,c, and d means in the same column bearing different letters differ significantly at 0.05 level.
Diet 1 =Ration1 (without benzole25®), Diet 2= (Ration2 +10 mg/kg benzole25®), Diet 3= (Ration3+20 mg/kg benzole25®), Diet 4= (Ration4 +30 mg/kg benzole25®).
*Feed cost/kg gain (LE) =feed intake x cost (LE) of one ton feed/1000xtotal gain.

4- Body composition:

The results of carcass composition of Nile tilapia showed no significance ($p>0.05$) in dry matter and crude protein among fish treatments. Meanwhile, the percentage of ether extract and ash differed significantly among fish groups which may be attributed to the presence of Albendazole.

Table (4): Means ± standard error of proximate analysis (% on the dry matter basis) of experimental fish fed on graded levels of benzole25®.

Ash	EE	CP	DM	Treatment
19.77±0.88b 19.45±0.07b 20.12±1.68a 19.20±0.23b	23.38±0.95b 24.90±0.27a 23.35±0.02b 23.97±0.02b	58.00±0.83 a 58.50±0.30 a 58.90±1.20 a 58.97±0.25 a	26.38±0.18 a 25.89±0.16 a 25.61±0.20 a 25.23±0.11 a	1- Diet 1 2- Diet 2 3- Diet 3 4- Diet 4

A and b means in the same column bearing the same letter do not differ significantly at 0.05 level. Diet 1 =Ration1 (without benzole25®), Diet 2= (Ration2 +10 mg/kg benzole25®), Diet 3= (Ration3+20 mg/kg benzole25®), Diet 4= (Ration4 +30 mg/kg benzole25®y).

5-Parasitic Examination

Morphology of Gyrodactylidae (Viviparous, eyes absent, parent worm contains a distinct well differentiated embryo, Vitellaria not distinct and one pair of anchors firmly attached by two bars) closely met that recorded by Woo (1995), Tojo and Santamarina (1998) and Eissa (2002).

6-Infestation

Regarding the number of worms per field, significant decrease in the treated groups was seen when compared to the control non treated group, table (5). The number of worms in all groups were less than those recorded by Tojo and Santamarina1, (1998). This my be due to the use of different sources of water for fish culture, as our source of water is somewhat higher in salinity as salt can be used to treat external protozoas and monogenetic trematodes (Plumb, 1992). More over, used as prophylaxis against external parasites of freshwater fishes (Baticados and Paclibare, 1992, Swann and Fitzgerald, 1993).

Table (5): Infestation intensity

Days/ Infestation	Diet 1	Diet 2	Diet 3	Diet 4
1	12-14	12-14	12-14	12-14
2	12-14	10-11	9-10	9-10
3	12-15	8-6	6-7	5-6
4	13-15	6-7	2-3	2-4
5	12-14	4-5	1-2	2-3
6	14-15	2-3	0-1	0-1
7	12-14	1-2	0-1	0
8	13-15	0-1	0-1	0
9	13-14	0-1	0	0
10	15-16	0	0	0

Diet 1 =Ration1 (without benzole25®), Diet 2= (Ration2 +10 mg/kg benzole25®), Diet 3= (Ration3+20 mg/kg benzole25®), Diet 4= (Ration4 +30 mg/kg benzole25®y).

7-Pathology

Fish heavily infected with Gyrodactylus appear pale, due to excessive mucus secretion and epithelial proliferation. In heavily infected skin zones there is skin erosions, blackening, desquamation of the skin epithelium, focal haemorrhagic lesions and loss of scales. On the other hand, feeding mostly on the eye's surface damages tissues and causes great irritation to the fish and exophthalmia ending with complete eye erosion and sunken eyes, figure (1).

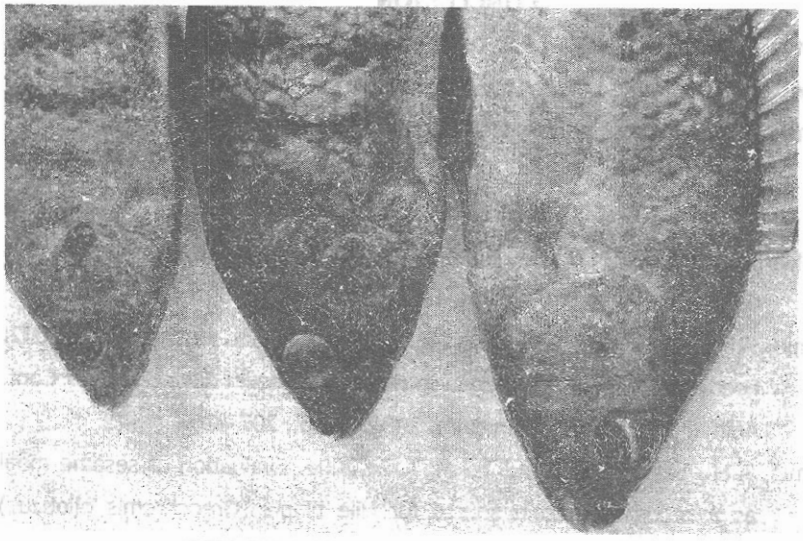

figure (1):Showing various degrees of eye opacity and skin blackening.

Such clinical signs were also reported by Kabata, (1985) and Thoney and Hargis, (2003). On the other side our results were some what faraway from the findings of Moraes and Martins, (2004) who stated that signs are often sub-clinical and without apparent signs and under predisposing factors such as poor water quality, high ammonia, high stocking density and poor feeding, ectoparasites, inadequate handling and other stressful conditions; diseases affect fish host. This difference may be attributed to different species of fish and/or the culture system.

8- Dosage

Results of table (3 and 5) showed that the antiparasitic effect of benzole25®

at a dose of 20-30ml/Kg ration is both effective and economic. Such findings are somewhat in a disagreement with the recommendations of Tojo and Santamarina1, (1998) who mentioned that oral treatment with bendazoles is unlikely to be an economically viable option, since the required dosage (40 g per kg of feed for 10 d) implies using large amounts of the drug. This difference may be attributed to the route of administering the drug.

CONCLUSION

From the pervious results, it could be concluded that treating with benzole25® with dose of 20-30ml/Kg ration for Nile tilapia showed anti-parasitic effect, improvement of growth parameters, feed utilization, health status and economics.

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التحكم في مرض الجيروداكتيلوزس للبلطي النيلي(وحيد الجنس ذكور) في المزارع

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وحدة بحوث الثروة السمكية بسخا، المعمل المركزى لبحوث الثروة السمكية ، العباسة- شرقية- مصر.

المخلص العربي

أجريت هذه الدراسة فى خلال ١٥٠ يوم فى ١٢ حوض ترابي لتقييم قدرة مادة الالبنزول ٢٥ (قاتل للديدان لمقاومة مرض الجيرودكتلوسس فى اسماك البلطي النيلي وحيد الجنس وكانت كثافة الأسماك فى الأحواض ٢٠٠٠٠ إصبعية لكل فدان بمتوسط وزن من ٢٠-٣٠ جرام لكل سمكة. تم تغذية الأسماك على علائق متزنة فى القيمة الغذائية (٢٨% بروتين) بمعدل ٣% من الوزن الحي بالحوض على النحو التالي المجموعة الأولى (المجموعة القياسية بدون إضافة للدواء) المجموعة الثانية إضافة (١٠ملى/كيلوجرام علف) المجموعة الثالثة (٢٠ملى/كيلوجرام علف) والمجموعة الرابعة (٣٠ملى/كيلوجرام علف). وتم تغذية الأسماك بالعلائق السابق لمدة ١٠ أيام متتالية ثم التغذية على علائق بدون دواء لكل المجاميع باقي مدة التجربة. وأظهرت النتائج أن استخدام الجرعة من ٢٠-٣٠ ملي/كيلوجرام كانت المثلى من حيث معدلات النمو والحالة الصحية والاقتصادية وباقة القياسات الأخرى . ومن هذه الدراسة نقترح استخدام مادة الالبنزول ٢٥ كعلاج بجرعة ٢٠-٣٠ ملي/كيلوجرام فى مزارع اسماك البلطي النيلي.