EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS AND OXYTETRACYCLINE ON INTESTINAL BACTERIAL LOAD OF NILE TILAPIA FINGERLINGS (OREOCHROMIS NILOTICUS)

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Abstract

A twenty-week experimental feeding period was conducted on Oreochromis niloticus fry to study the effect of different dietary protein levels and Oxytetracycline on the bacterial count inhabiting intestine of Nile tilapia fingerlings (Oreochromis niloticus). Two protein levels (25 and 40%) were used within each protein level 4 oxytetracycline levels (0, 50, 100 and 150 mg/kg diet) were added. One thousand- six hundred fry with average weight of 0.52 g were randomly distributed to 16 aguaria (160 L each), each treatment was performed at two replicates. The addition of the antibiotics to the diet enhanced growth when the diet contained a high level of protein (40%). The predominant species of bacteria inhabiting the anterior, middle and posterior part of the intestinal tract of Oreochroumes niloticus were affected by the protein and oxytetracycline levels. The highest bacterial number was obtained in the posterior part of the alimentary canal of fish. It is suggested that this drug has a sparing effect on the dietary protein normally used for energy, and may possibly be used (under controlled conditions) as growth enhancer. Withdrawal periods of oxytetracycline in a treated fish were 22 days.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most desirable freshwater fish in Egypt and in several countries in the World. The tilaplas (*Oreochromis niloticus*) are warm water cichclids predominantly of African and Middle Eastern origin. Tilapias can survive under a wide variety of environmental conditions, being highly eurythermal, with a temperature ranging between 16°C and 40°C, and a wide range of tolerance to salinity.

Since the discovery of Moor *et al.*(1946) that the addition of antibiotics in subtherapeutical quantities of chicken feed increased growth rate and food conversion, the use of such antibiotics has become widespread in animal husbandry. However, it

has been held for many years that the treated fish failed to show any improved growth.

Snieszko (1957) attributed the failure of antibiotics to enhance growth of salmonids to the held concept that the digestive tract of healthy fish was sterile when empty of food. There has been however, one or two, somewhat equivocal studies that indicated that there might be a growth effect. Ahmed and Matty (1989) have examined a number of antibiotics, both were from the "therapeutic" group and those of the so-called "in-feed" group which are permitted for inclusion at low levels in commercial diets fed to animals over a long period, also, used carp and rainbow trout of initial weight of about 4 grams. The drugs were incorporated into either high protein or low protein diets (40% and 25%) and fed for ten weeks. The addition of verginiamycin at concentration of 40, 80, and 100 mg/kg to the high protein diet of carp resulted in

Two objectives for using the attractants, the first one, using the attractants for fruit flies control (partial bait spray and killing bags), the second one, for detecting and monitoring MFF & PFF adults.

The present investigation is the third one of a series of investigations carried out on mango, citrus, and apricot plantations.

MATERIALS AND METHODS

To evaluate efficacy of some different attractants for adult flies of Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (MFF) and Peach fruit fly (PFF), *Bactrocera zonata* (Saund.), an experiment was carried out on apricot plantation during May - June, 2003 at Sinuris & Ibshaway districts, Fayoum Governorate.

The experiments was carried out in three apricot locations (orchards) which represent the different dynamics of MFF and PFF population.

The experiment was carried out during six weekly inspections (5/5//2003 to 16/6/2003).

The used attractants were:

- Buminal (protein hydrolyzate) as a food attractant in three concentrations, 2.5 %,
 % and 10 %.
- 2. Di-ammonium hydrogen orthophosphate as an aggregating attractant in two concentrations, 2 % and 3 %.

McPhail traps (described by Nicanor *et al.*, 1993) were used on apricot trees and baited weekly with the used attractants.

MATERIALS AND METHODS

1. Experimental fish

Eight hundred fingerlings Nile tilapia (*O. niloticus*) were obtained from Abassa hatchery, Sharkia Governorate. After arrival, all fish were kept for five days in tanks to alleviate stresses to be adapted until they were transferred to the experimental aquarium provided shelter for fish and thus reduced their energy expenditure.

Fish were collected after adaptation to the new environment and were fed on the purified diet containing 30% protein for five days, then, weighed and randomly distributed to the experimental glass aquarium into 8 groups of 50 fish in each aquarium (induplicate). The experiment was conducted in glass aquarium 160 L (80 X 50 X 40 cm) supplied with dechlorinated tap water. The water temperature was maintained at 25 ± 1 °C through the experimental period by an automatic heater. The aquarium was cleaned, and up to two thirds of the water was replaced daily, at the end of each week all the water was replaced. The average weight for fry was 0.52 g.

2-Diet and feeding regimen

Two groups were fed a high protein (HP) 40% and a low protein (LP) 25%. Four levels of *Oxytetracycline* (0, 50, 100 and 150 mg/kg feed) were used as following.

T1: diet contained 25% protein control diet without *oxytetracycline*.

T2: diet contained 25 % protein + 50mg oxytetracycline /kg from diet.

T3: diet contained 25 % protein + 100mg oxytetracycline /kg from diet.

T4: diet contained 25 % protein 150mg oxytetracycline /kg from diet.

T5: diet contained 40 % protein without Oxytetracycline.

T6: diet contained 40 % protein + 50mg Oxytetracycline/kg diet.

T7: diet contained 40 % protein + 100mg Oxytetracycline/kg diet.

T8: diet contained 40 % protein + 150mg Oxytetracycline/kg diet.

The diets were prepared by mixing in a Hobart mixer and extruding through a mincer.

The extruded strands were cut while wet and the dried pellets screened to remove fines and stored at -20 °C until use. Antibiotic *(Oxytetracycline)* was added to the diets just prior to extrusion. *Oxytetracycline* (OTC) was added at concentrations of 0, 50, 100, and 150 mg/kg feed to both HP (40% protein) and LP (25%) diets.

The experiment lasted for a period of 140 days. The aquaria were cleaned daily without removing the experimental fish. The fish were fed two times daily rate 5% of body weight. Food was offered over a period of 15 minutes in order to ensure complete consumption of food .

Table 1. Means of capture per trap per day "CTD" of MFF in McPhail traps baited with different attractants, in apricot orchards at the three locations during 5/5/2003 to 16/6/2003.

			Mean '	'CTD" of M	FF during i	nspection p	eriods					
Attractants		1 <i>st</i> location		2 <i>nd</i> location		3 rd location			Grand mean			
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Buminal 2.5 %	0.02	0.06	0.08 a	0.01	0.06	0.07_ь	0.16	0.30	0.46 b	0.06 b	0.14 c	0.20 c
Buminal 5 %	0.00	0.02	0.02 a	0.11	0.28	0.39 ab	0.22	0.30	0.52 b	0.11 ab	0.20 <u>bc</u>	0.31 bc
Buminal 10 %	0.01	0.06	0.07 a	0.18	0.54	0.72 a	0.24	0.60	0.84 ab	0.14 ab	0.40ab	0.54abc
di-Ammonium phosphate 2 %	0.00	_0.05	0.05 a	0.23	0.56	0.79 a	0.29	0.61	0.90 ab	0.17 ab	0.41 ab	0.58 ab
di-Ammonium phosphate 3 %	0.02	0.02	0.04 a	0.21	0.64	· 0.85 a	0.51	1.07	1.58 a	0.25 a	0.58 a	0.83 a
Mean	0.01 c	0.04 b	0.05 c	0.15 b	0.41 a	0.56 b	0.28 a	0.58 a	0. <u>8</u> 6 a	0.15	0.34	0.49

	Loc	ations	Attra	actants
Sex	F-value	LSD at 5%	F-value	LSD at 5%
Male	11.83**	0.11	1.74 ns	0.15
Female	16.74**	0.19	4.08**	0.25
Total	15.81**	0.29	3.29*	0.38

RESULTS

- 1-The growth response of the experimental fish over 140 days feeding period is shown in Table 1. Averages of body weight at experimental start were found to be 50.02, 46.55, 49.4, 49.4, 50.9, 51.7, 50.6 and 49.1g for T1, T2, T3, T4, T5, T6, T7 and T8, respectively. The difference among the groups was insignificant, indicating the homogeny of the experimental groups at the start of the experiment. Ten days after the experiment started, the group of fish on T7 had a significant (p<0.05) higher body weight than the rest of experimental groups. Twenty days after the experiment, started, the group of fish on T6 and T7 had a significant (p<0.05) higher body weight than the rest of experimental groups. Fifty and sixty days after the experiment started the group of fish on T7 had a significant (p<0.05) higher body weight than the rest of experimental groups. From 100 to 140 days after the experiment started the group of fish on T8 (40% protein and 150mg oxytetracycline) had a significant (p<0.05) higher body weight than the rest of experimental groups.
- 2-The bacterial species inhabiting the intestinal tract of *O. Niloticus* and its total bacterial count before beginning of the experiment are presented in Table 2. It was found that predominant species of bacteria inhabiting the anterior part of the intestine were *Aeromonas hydrophila*, *Esherichia coli and Proteus sp. Flavobacterium columinare* and *Staphylococcus saprophyticus*. The total bacterial count of the intestinal content of fish was 52000/g. The predominant species of bacteria inhabiting the middle part of the intestine were *Aeromonas hydrophila*, *Esherichia coli Proteus sp.* The total bacterial count of the intestinal content of fish was 57000/g. Also, the predominant species of bacteria inhabiting in the posterior part of the intestine were *Aeromonas hydrophila*, *Esherichia coli* and *Staphylococcus saprophyticus*. The total bacterial count of the intestinal content of fish was 60000/g.
- 3-The bacterial species inhabiting the anterior, middle and posterior part of the intestinal tract of *Oreochroumes niloticus* fed two protein levels (25 and 40%) and different *oxytetracycline* levels (0, 50, 100 and 150 mg/kg diet) were presented in Table 3.
- 4- The total bacterial count of the intestinal content of fish was recorded in Table 4.
- 5-Results of oxytetracycline residues in Table 5 showed that the oxytetracycline residues in gills, kidneys, liver and muscle were sufficient to inhibit the growth of

tested organism for variable periods after stopping the antibiotic treatment. Annion.phosph. 3 % and the other four attractants, while there were insignificant differences among the three attractants, Buminal 2.5, Buminal 5 % and Buminal 10 %.

• The 3*rd* location: Represent relatively mid population for PFF. Table 2 showed that the mean "CTD" was 1.63, 2.09, 2.81, 8.43 and 6.15 flies for Buminal 2.5 %, Buminal 5 %, Buminal 10 %, di-Ammon.phosph. 2 % and di-Ammon.phosph. 3 %, respectively.

The statistical analysis showed significant differences between di-Ammon.phosph. 2 % and the three attractants, Buminal 2.5 %, Buminal 5 % and Buminal 10 %, while there were insignificant differences among Buminal 2.5 %, Buminal 5 % and Buminal 10 %, also there were insignificant differences in between Buminal 10 % and di-Ammon.phosph. 3 %, and also between di-Ammon.phosph. 2 % and di-Ammon.phosph. 3 %.

A nutritional role for this flora has been suggested by Trust (1979), but the evidence is not overwhelming.

In the present study, the bacterial number did not seem to affect Nile tilapia even when its number increased (Table 4). Also, it did not appear to play any obvious part in the antibiotic stimulation of growth of Nile tilapia, for, except on one occasion, when there was no increase or decrease in bacterial counts even through growth was enhanced. However, a change in the metabolism of the bacterial flora after antibiotic treatment has been suggested as a possible way in which host growth becomes improved. The present study revealed that the protein and *oxytetracycline* levels affect in the predominant species of bacteria inhabiting anterior, middle and posterior part of the intestine. It was also shown that the anterior and middle part of the intestine were more rich in the bacterial species than posterior one which agreed with Enany (1979).

The present investigation revealed that the bacterial count of *Oxytetracycline* medicated foods (25% protein) in *Oreochromis niloticus* fish was increased comparatively with the non–treated, first and second treatments (50 and 100mg/kg diet), while, in the third treatments (150mg/kg diet) was decreased.

On the other hand, it was shown that the bacterial count of *Oxytetracycline* medicated foods (40% protein) in *Oreochromis niloticus* fish was increased in all the three treatments (Table 4) (50, 100 and 150mg/kg) compared with the non – treated group. This may be attributed to the high protein level (40%) when it encouraged the rapid reproduction of intestinal bacteria even with high level of *Oxytetracycline*. This may support the findings met with Depaola *et al.* (1995).

Several countries have regulation concerning withdrawal periods for days administered to fish to diminish the risk of unwanted effect on fish, environment and consumers. *Oxytetracycline* is one of the most important widely used antibiotic in medicated feeds, it has a broad spectrum antibacterial action both in vivo and in vitro.

The present study revealed that the oral systemic treatment with Oxytetracycline in O. niloticus led to detectable residues for different periods in muscles and different organs of or both diseased and healthy ones. Similar results were obtained by Snieszko (1957), Eissa et al. (1998). They reported that Oxytetracycline residues were the highest in liver and the lowest in muscle of fish. The observed high concentration of oxytetracycline in liver could be attributed to the

hepatic route of *oxytetracycline* excretion (Cravedi *et al.*, 1987) or to the fact that the liver is the target organ in drug metabolism (Haddad and Winchester, 1990).

The present study revealed that, withdrawal periods of oxytetracycline in both healthy or treated fish were 22 days. Similar results were obtained by Eissa *et al.* 1998. In addition, the present investigation suggested that the absence of such antibiotics may be due to the lower doses given to fish (50, 100 and 150 mg/kg) which are considered prophylactic and therapeutic doses.

It could be concluded from the present study that drugs used for treatment of fish must be stopped for at last 21 days before fish are used for human consumption to allow the traces of the active drug and its metabolites to be eliminated from the body of the fish. The widespread use of antimicrobial agents for healthy fish to reduce bacterial disease in aquaculture must be stopped. (Shu-Peng *et al.*, 2000). Concerning antibiotics, they are used to enhance growth and *Oxytetracycline* is the preferable as it is a drug approved by the US Food and Drug Administration (FDA) to control certain diseases in salmonids, catfish and other fish (Stehly *et al.*, 1999) who reported that triplicate samples of rainbow trout fillet tissue fortified with OTC at 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 ppm and fillet tissue with incurred OTC at approximately 0.75, 1.5, and 3.75, ppm were analyzed by high-performance liquid chromatography (HPLC) and the microbial inhibition assay. The results indicated that the 2 methods are essentially identical in the tested range, with mean coefficients of variation of 1.5% for the HPLC method and 3.94% for the microbial inhibition assay.

In Egypt, it was recorded that the withdrawal period of *Oxytetracycline* as after 18 days before the *Oreochromis niloticus* fish are marketed for human consumption. Also, the veterinary regulation does not permit harvesting fish within 21 days after cease of treatment.

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Table 1. Average body weight (g) Changes of fingerlings Nile tilapia.

Protein level		25%	6			40%				
Days	T1	T2	Т3	T4	T5	Т6	77	T8		
OTC mg/kg	0	50	100	150	0	50	100	150		
0	50.02±1.7	49.55±3.6	48.4±1.9	49.4±1.7	50.9±2.1	51.7±2.7	50.6±1.9	49.1±4.2		
10	61.3±1.6d	59.99±1.8d ^e	57.8±0.9°	62.1±3.1 ^d	64.5±3.6°	67.8±4.4 ^b	70.1±4.9ª	58.4±1.9de		
20	73.49±4.2 ^c	69.74±3.7d	66.7±2.1fe	75.2±5.7 ^b	76.6±5.7 ^b	81±4.9ª	81.5±9.5°	74.6±7.9 ^b		
30	86.25±4.9 ^d	82.99±4.7e	78.6±7.6 ^f	90.1±4.2°	92.5±6.2°	99±4.8ª	95.7±6.4 ^b	92.3±8.3°		
40	95.4±1.6 ^f	98.9±2.1 ^e	94.5±3.7 ^f	101.4±1.2d	105.8±3.5°	113.3±7.ª	113.8±5.9ª	109.3±4.2 ^b		
50	103.1±2.59	108.1±1.5 ^f	108.2±3.4 ^f	111±2 ^e	116.8±2.8d	125.7±6.5ª	129.7±3.9°	120±3.5°		
60	111.4±3.99	116.8±5.7 ^f	121.2±6.5e	121.1±4.e	125.9±4.9 ^d	135.3±6.1 ^b	141.4±5.9ª	130.8±9.3°		
70	125.1±10 ^f	130.9±10e	132.6±12e	135.2±8. ^d	139.7±10 ^c	150.7±8.8ª	150.3 <u>±6.</u> 7⁵	148,6±6 ^b		
80	148.7±13 ^f	153.2±15e	148.1±17 ^f	156.1±15 ^d	161.6±17°	174.6±17ª	167.7±21°	174.5±11a		
90	169.5±8.3e	176.4±11 ^d	173±8.9°	185.8±13 ^b	186.1±14b	199.7±15°	200.8±5.6 ^b	202.7±1.9ª		
100	184.7±4.8 ^f	198.2±3.2e	199.3±5.4e	209.9±2.9d	213.1±2.5°	227.6±1.9b	226.8±10ª	232.8±3.1°		
110	212.11±5f	224.5±3°	225.3±4.4 ^e	237.2±5.7 ^d	242.4±5.2°	257.3±17 ^b	255.4±8.6 ^b	263.5±14ª		
120	239.4±10 ^h	248.8±7 ⁹	252.8±5.9 ^f	256.6±14°	273.7±10 ^d	288.5±18 ^b	285.6±3b	302.7±5.7°		
130	267.1±49	276.3±8 ^f	276.2±9.8f	285.5±13 ^e	306.6±21 ^d	321.6±20 ^b	316.7±20°	336.4±15ª		
140	289.9±28 ^h	296.4±30 ⁹	302.9±18 ^f	311,4±19 ^e	332.5±19 ^d	348.5±7.2°	351.2±12b	376.1±3.8ª		

Where: OTC = Oxytetracycline PrL = protein level. AvBW= average body weight

Values in the same row having the same superscript letter were not significantlent

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Table 1. Average body weight (g) Changes of fingerlings Nile tilapia.

Protein level	<u></u>	25%) 		40%				
Days	Т1	T2	T3	T4	T 5	T6	T 7	Т8	
OTC mg/kg	0	50	100	150	0	50	100	150	
0	50.02±1.7	49.55±3.6	48.4±1.9	49.4±1.7	50.9±2.1	51.7±2.7	50.6±1.9	49.1±4.2	
10	61.3±1.6 ^d	59.99±1.8de	57.8±0.9e	62.1±3.1 ^d	64.5±3.6°	67.8±4,4 ^b	70.1±4.9°	58.4±1.9de	
20	73.49±4.2°	69.74±3.7 ^d	66.7±2.1f°	75.2±5.7 ^b	76.6±5.7 ^b	81±4.9³	81.5±9.5°	74.6±7.9 ^b	
30	86.25±4.9°	82.99±4.7°	78.6±7.6	90.1±4.2°	92.5±6.2°	99±4.8°	95.7±6.4 ^b	92.3±8.3°	
40	95.4±1.6 ^f	98.9±2.1e	94.5±3.7 ^f	101.4±1.2d	105.8±3.5°	113.3±7.ª	113.8±5.9ª	109.3±4.2 ^b	
50_	103.1±2.5°	108.1±1.5	108.2±3.4 ^f	111±2°	116.8±2.8d	125.7±6.5³	129.7±3.9ª	120±3.5°	
60_	111.4±3.9°	116.8±5.7 ^f	121.2±6.5e	121.1±4.e	125.9±4.9 ^d	135.3±6.1 ^b	141.4±5.9a	130.8±9.3°	
70	125.1±10 ^f	130.9±10°	132.6±12e	135.2±8.d	139.7±10°	150.7±8.8°	150.3±6.7 ^b	148.6±6 ^b	
80	148.7±13 ^f	153.2±15e	148.1±17 ^f	156.1±15 ^d	161.6±17°	174.6±17°	167.7±21 ^a	174.5±11 ^a	
90	169.5±8.3°	176.4±11 ^d	173±8.9°	185.8±13 ^b	186.1±14b	199.7±15°	200.8±5.6 ^b	202.7±1.9 ^a	
_ 100	184.7±4.8f	198.2±3.2e	199.3±5.4e	209.9±2.9d	213.1±2.5°	227.6±1.9b	226.8±10 ^a	232.8±3.1 ^a	
110	212.11±5 ^f	224.5±3 ^e	225.3±4.4e	237.2±5.7 ^d	242.4±5.2°	257.3±17⁵	255.4±8.6 ^b	263.5±14ª	
120	239.4±10 ^h	248.8±79	252.8±5.9 ^f	256.6±14 ^e	273.7±10 ^d	288.5±18 ^b	285.6±3 ^b	302.7±5.7°	
130	267.1±4°	276.3±8 ^f	276.2±9.8 ^f	_285.5±13 ^e	306.6±21 ^d	321.6±20 ^b	316.7±20°	336.4±15ª	
140	289.9±28 ^h	296.4±309	302.9±18 ^r	311.4±19 ^e	332.5±19⁴	348.5±7.2°	351.2±12 ^b	376.1±3.8°	

Where: OTC = Oxytetracycline PrL = protein level. AvBW= average body weight

Values in the same row having the same superscript letter were not significantlent

Table 2. The bacterial species inhabiting the intestinal tract of *Oreochroumes noliticus* before beginning of the experiment and its total bacterial count.

Intestinal part	Microorganism	T.B.C/g
Anterior part	Aeromonas hydrophila Esherichia coli Proteus sp. Flavobacterium columinare Staphylococcus saprophyticus	52000
Middle part	Aeromonas hydrophila Esherichia coli Proteus sp.	57000
Posterior part	Aeromonas hydrophila Esherichia coli Staphylococcus saprophyticus	60000

Where: T.B.C = Total bacterial count.

Table 3. The bacterial species inhabiting the intestinal tract of *Oerchroumes nolitica* fed with 25 and 40 % protein diet and different *Oxytetracycline* levels (0, 50, 100 and 150 mg/kg diet)

Intestinal	Microorganism		25%	0			40)%	
parts		T1	12	T3	T4_	T5	T6_	17	T8
	Aeromonas hydrophila Esherichia coli	+	+	-		+	+		=
Anterior part	Proteus sp.	+	[-	-	-	+	-	_	-
)	Flavobacterium columinare	+	/ +	+	-	+	} +	+	} -
ļ	Staphylococcus saprophyticus	+	<u> </u>	+		+	L +	<u> + </u>	
	Aeromonas hydrophila	+	+	-	-	+	+	[-
Middle part	Esherichía coli	+	+	-	-	+	│ +	-	-
ĺ	Proteus sp.	+			Ĺ	+	<u> - </u>	<u> </u>	<u>-</u>
Posterior part	Aeromonas hydrophila Esherichia coli Staphylococcus saprophyticus	+++++	+ + +	- - +	- - +	+ + +	+ + +	- - +	-

Table 4. Bacterial counts of the intestinal content of fish fed with 25 % and 40% protein in different concentrations of *Oxytetracycline*:

Protein percent %		25	%		40%			
OTC level mg/kg	0	50	100	150	0	50	100	150
Diets	T1	T2	T3	T4	T5	Т6	T7	Т8
Total bacterial Count/g	83000	70800	59500	52000	86000	75900	69000	59000

OTC = Oxytetracycline.

Table 5. Results of oxytetracycline residues in O. niloticus fish.

Treatment	Samples	Days after treatment						
		2	7	12	17	22	28	
Control	Gills	0/1	0/1	0/1	0/1	0/1	0/1	
	Kidneys	0/1	0/1	0/1	0/1	0/1	0/1	
	Liver	0/1	0/1	0/1	0/1	0/1	0/1	
	Muscle	0/1	0/1	0/1	0/1	0/1	0/1	
Oxytetracycline	Gills	3/4	3/4	2/4	1/4	0/4	0/4	
	Kidneys	4/4	3/4	3/4	1/4	0/4	0/4	
	Liver	4/4	4/4	3/4	2/4	1/4	0/4	
	Muscle	4/4	3/4	2/4	0/4	0/4	0/4	

The results are expressed as the number of samples with positive inhibition in relation to the number examined.

تأثير إضافة مستويات مختلفة من البروتين والاكسىتتراسيكلين على الحمل البكتيري في أمعاء اصبعيات البلطى النيلي

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تم دراسة تأثير إضافة المضاد الحيوي (الاكسى تتراسيكلين) في علائق اصبعيات سمك البلطي النيلى كمنشطات للنمو من خلال استخدام مستويين من البروتين داخل كل مستوى أربع مستويات من الاكسى تتراسيكلين (٠٠،٠٠، ١٠٠، ١٥٠ ملليجر ام/كيلوجر ام عليقة) على الحمل البكتيري في أمعاء أصبعيات البلطي النيلي.

تم استخدام ١٦٠٠ سمكة متوسط وزن ٢٥٠٠جرام قسمت على ٨ معاملات (٢ مكررة داخل كل معاملة) وتم استخدام أحواض زجاجية سعة ١٦٠ لتراً بمعدل تخزين ٥٠ سمكة/حوض (٢) واستمرت هذه التجربة ١٤٠ يوما. إضافة المضاد الحيوي (الاكسىتتراسيكلين) في العلائق ساعد على النمو عندما تحتوى العلبقة على مستوى عالى من البروتين (٤٠%). إضافة الاكسى نتراسيكلين و البروتين أثر تأثيرا مباشرا على أنواع البكتريا التي تقطن الجزء الأمامي، الجزء الأوسط وكذلك الجزء الأخير من أمعاء اصبعيات البلطي النيلى. وكان أكثر عدد للبكتريا في القناة الهضمية في الجزء الأخير منها. ونستخلص من هذا العمل إن إضافة هذا الدواء يعمل على تنشيط النمو بنسبة بسيطة ويمكن أن يستعمل في علائق الأسماك (تحت الرقابة والسيطرة). فترة إفراز الدواء من الأسماك المعاملة تصل إلى ٢٢ يوماً.