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EFFECT OF USING BETAFIN AND/OR BIOPOLYM AS NATURAL ADDITIVES IN PRODUCING NILE TILAPIA FISH IN POLY-CULTURE SEMI-INTENSIVE SYSTEM IN EARTHEN PONDS

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ABSTRACT

In a study on cultivation of Nile tilapia in earthen ponds (each of 1 faddan area) under poly-culture semi-intensive system, betafin was added to diet offered to 1st pond (1 Kg/ton), 2nd pond (2 Kg/ton) and 3rd pond (1 Kg besides biopolym 600 ml/ton). Biopolym was added to diet of 4th pond (600 ml/ton) but sprayed on 5th pond's water (1500 ml then 600 ml each 2 weeks). The 6th pond was a control, without additives. The experimental duration was 6 months. The 3rd pond gave the best results, i.e. the highest average body weight, total and daily body gains, growth rates (relative and specific), total productivity (10.675 ton/faddan), muscular protein %, radius of the muscular bundles, total surface area occupied by the muscular bundles/mm^2 (the least thickness of connective tissues between muscular bundles and thickness of skin and subcutaneous layer), and highest net return (21.970 thousand LE/faddan).

Keywords: Nile tilapia – Betafin – Biopolym – Earthen ponds – Semi intensive system – Performance – Histology – Economics.

INTRODUCTION

There is a shortage (gap) in the Egyptian supply of fish calculated as 25% of the local demand, which is covered via import. So, it is a must to cultivate fish for the over fishing which led to depletion of the wild water-bodies. However, pisciculture is sharing about 47% of the total local fish production. Moreover, 88% of aquaculture production is produced by non-governmental sector (GAFRD, 2003). Nile tilapia fish are the most outspreading wild (FAO, 2002) as well as cultured species (Sadek, 2000 and Shiau, 2002). It was cultivated in ancient Egypt since 2500 years B.C. (Baradach *et al.*, 1972). Aquaculture intensification requires more expensive aquafeed (Suresh, 2000); so, feed additives are often used to stimulate fish performance (Magouz *et al.*, 1996 and Abdelhamid *et al.*, 1997 & 1998).

Betafin (betaine) is a natural by-product (mainly from sugar beet) and an active form of choline, which is very important for different organisms, whether for methylation, metabolism, or to reduce stresses and hence increases the growth (Ombabi, 2002) Whereas, Biopolym (algenate) is also natural product from marine brown algae which is used on the industrial scale for curing waste water, as feed and drinking water additive, anti-bittering agent, stabilizer, carrier and binder (Chango *et al.*, 1993; Travieso *et al.*, 1996 and Joosten *et al.*, 1997). So, the present study aimed to test the effects of using betafine and/or biopolym on tilapia fish performance under semi-intensive production system in earthen ponds.

MATERIALS AND METHODS

The field study was carried out in the Integrated Fish Farm at Al-Manzalah, Ministry of Agriculture during the season of year 2002, for 24 weeks. All facilities (fish, diet and ponds) were kindly offered from this governomental farm, whereas both additives (betafine powder and biopolym liquid) used were kindly gifted from the commercial agents of both producing firms.

Experimental ponds:

Six earthen ponds were used, each of one faddan area (200 x 21 m). The ponds were prepared during 3 weeks by good sun drying, fertilization (50 Kg of each of ammonium nitrate and superphosphate/ faddan), and irrigation (water column of 1.2 – 1.5 m height) from a branch of Bahr Hados drain (salinity: 1.8 – 2.0 ppt, alkalinity: 205 – 425 ppm, and total hardness: 500 – 750 ppm). Each pond was provided by 2 Chinese electric paddle-wheels, each of 2 horses. They functioned for 8 hours daily (from 0 to 8 h). The water flow into the ponds was 5 – 7 L/second. Ten percent of each pond's water were changed daily.

Experimental fishes:

On the 1st of June, over-wintered unsexed *Oreochromis niloticus* fingerlings of an average initial body weight of 22 g and 11 cm length were stocked at a rate of 50 thousand/pond. In addition, 2 and 1 thousand fingerlings of each Mugillidae and common carp per pond were simultaneously stocked at initial body weights of 50 and 30 g/fingerling, respectively. Mid August, 300 fingerlings (average weight of 450 g/fingerling) of catfish/pond were stocked to maintain the carrying capacity of the experimental ponds via predation for any hatchlings.

Feeding regime:

The fish were adapted for 2 weeks without artificial feeding, thereafter and on the mid June, the basal (control) diet of 25% crude protein and 6 mm diameter was offered for all ponds at a daily rate of 5% of tilapia weight for one month (6 days a week) at 2 meals. This diet consisted of 28% yellow maize, 27% soya meal, 26% rice bran, 16% fish meal, and 3% molasses. The increase of tilapia weight was followed bi-weekly to adjust the daily feed quantity of each pond. The diet was given thereafter at a daily rate of 4% for 1.5 month, 3% for one month, 2% for the following month and lastly 1% for the last 2 weeks.

Experimental treatments:

From mid July the following treatments were given:

1- The 1st pond was given the control diet plus 1 Kg betafin/tone.

2- The 2nd pond was given the control diet plus 2 Kg betafin/ton.
3- The 3rd pond was given the control diet plus 1 Kg betafin and 600 ml biopolym/ton.
4- The 4th pond was given the control diet plus 600 ml biopolym/ton.
5- The 5th pond was sprayed by 1500 ml and thereafter bi-weekly by 600 ml biopolym.
6- The 6th pond was performed as a control without additives.

Measurements:

Water quality criteria (physical, chemical and biological) were tested for all ponds according to APHA (1985) and Boyd (1992). Also growth performance and nutrients utilization of the experimental fish were followed after Jobling (1983) and Abdelhamid (1996 and 2003). Histological examination of the fish muscles was performed too (Drury and Wallington, 1980).

Statistical analysis:

All numerical data collected were statistically analyzed via one-way analysis of variance using SAS (1987) program according to Snedecor and Cochran (1980). When F-test was significant, least significant difference was calculated (Duncan, 1955) to differentiate between means.

RESULTS AND DISCUSSION

1- Water quality criteria:

Using biopolym in feed (diets No. 3 and 4) improved concentrations of dissolved oxygen (DO), phosphate, ammonia and nitrite as well as increased green algae and rotifer counts of ponds' water as shown from Table (1). Betafin improved also plankton populations, since it is a methyl donor (Ombabi, 2002) and naturally found in aquatic organisms (Jasmine *et al.*, 1993; Kolkovskj *et al.*, 1997 and Keller *et al.*, 1999). The other tested parameters were not affected greatly by the experimental treatments. The increased level of DO during day light is due to photosynthesis by algae (Brune *et al.*, 2003). However, the beneficial effects of using biopolym are well confirmed, so it is used for purification and clearing as well as in feeds (Razungles *et al.*, 1992; Bougle, 1995 and Pecht, 1996). The reported values of the tested water criteria are within the suitable ranges for Nile tilapia according to Huet (1972) and Abdelhamid (2003). Similar results wee given too by Gomaah (1997) and Ismail (2001).

2- Fish performance:

Table (2) presents some parameters of growth and feed utilization by the experimental fish. There were significant ($P < 0.05$) differences among the experimental treatments (ponds) in body gain, daily gain, growth rates, and protein productive value (PPV%), in favor of pond No. 3. However there were no significant ($P > 0.05$) differences between both ponds No. 2 and 3 in these parameters. The best final weight and feed conversion were

Table 1: Water quality criteria of the experimental ponds as ranges throughout the experimental period.

Parameters	Experimental ponds No.					
	1	2	3	4	5	6
Temperature, °C at 5 a.m.	18.0 – 31.1	17.8 –31.0	17.9 –31.1	17.8 –31.2	17.9 –31.1	17.9 –32.0
at 10 a.m.	22.0 –32.5	19.5 –32.8	19.5 –32.6	19.4 –30.5	19.5 –32.5	19.6 –33.1
Secchi, cm	13.0 –19.5	12.0 –21.0	11.0 –20.0	13.5 –22.0	10.5 –24.0	14.0 –20.0
pH at 5 a.m.	7.2 – 8.1	7.2 – 8.1	7.3 – 8.1	7.2 – 8.1	7.2 – 8.1	7.2 – 8.1
at 10 a.m.	7.4 – 8.7	7.5 – 8.4	7.6 – 8.7	7.5 – 8.7	7.6 – 8.7	7.4 – 8.6
O_2, ppm at 5 a.m.	0.4 – 2.5	0.4 – 2.2	0.4 – 2.2	0.6 – 2.5	0.3 – 2.5	0.3 – 2.3
at 10 a.m.	1.5 – 8.6	3.0 – 9.8	3.5 -10.7	3.1 -12.1	2.4 – 9.0	3.0 –10.2
Alkalinity, ppm	205 – 400	265 – 350	265 – 425	220 – 390	250 – 395	235 – 415
Hardness, ppm	550 -650	500 – 650	500 -650	550 -700	550 -650	550 – 750
PO_4, ppm	0.12 –0.77	0.00-0.98	0.00 - 0.87	0.12 – 1.05	0.15 – 0.83	0.35 – 0.58
NH_4, ppm	0.31 –3.92	0.29-2.84	0.30 – 2.77	0.30 – 2.90	0.29 – 3.54	0.28 – 3.44
NH_3, ppm	0.02 –0.12	0.01-0.14	0.02 –0.15	0.01 - 0.15	0.02 – 0.13	0.01 -0.20
NO_2, ppm	0.01 -0.05	0.00 -0.04	0.00 - 0.04	0.00 -0.04	0.00 -0.05	0.00 -0.05
Phytoplankton,100/L Green algae	62 – 157	15 – 363	48 – 540	32 – 594	43 – 340	32 – 222
Blue-green algae	0 –115	0 –123	0 –124	0 –95	0 –37	3 –87
Euglena	42 – 198	70 – 203	5 – 160	12 – 265	7 – 283	3 – 219
Diatom	7–54	0–23	0–18	0–15	0–72	1–57
Zooplankton, U/L						
Rotefera	117 –2060	58 – 1281	138 –4801	58 – 5024	413 –3572	18 – 3040
Ciladosera	1–24	1–22	4–39	1 – 215	1 – 814	3 – 117
Copipoda	0–6	0–8	1–16	0 – 812	0 – 168	2 – 127
Estracoda	0–7	0–17	0 – 582	0–5	0 – 108	0–54
Naboli	0–2	0–12	1–32	0–2	0–14	0–12

Table 2: Means of tilapia fish performance and nutrients utilization criteria.

Parameters	Experimental ponds No.					
	1	2	3	4	5	6
Final weight, g	214	241	243	223	226	203
Body gain, g	192.0^{b}	219.5^{a}	220.7^{a}	200.5^{ab}	204.2^{ab}	180.5^{b}
Daily gain, g	1.16^{b}	1.33^{a}	1.34^{a}	1.22^{ab}	1.24^{ab}	1.09^{b}
Relative growth rate, %	875^{bc}	1020^{a}	992^{a}	891^{b}	940^{ab}	811^{c}
Specific growth rat, %/d	0.599^{bc}	0.636^{a}	0.629^{a}	0.603^{b}	0.616^{ab}	0.511^{c}
Feed conversion	2.71	2.27	2.22	2.39	2.29	2.66
Protein efficiency ratio	1.49	1.55	1.57	1.48	1.51	1.43
Protein productive value, %	23.7^{bc}	26.2^{ab}	28.4^{a}	22.4^{c}	24.3^{bc}	22.6^{c}
Energy utilization, %	14.7	15.5	15.4	13.9	14.6	14.2

a - c: Means in the same row followed by similar letter do not significantly ($P > 0.05$) differ.

realized in fish of pond No. 3. This means that betafin (with or without biopolym) is essential for feed intake, digestion, absorption, metabolism, feed and nutrients utilization and growth of fish (Przybyl *et al.*, 1999; Kasper *et al.*, 2002; Magouz, 2002; Ombabi, 2002 and Felix and Sudharsan, 2004). However, biopolym had sparing effect for betafin as shown by using 600 ml biopolym + 1 Kg betafin/ton feed for pond No. 3 was similar to using 2 Kg betafin/ton feed for pond No. 2. Moreover, algenate is used as a binder in aquafeed (Morales *et al.*, 1993), so preventes losses of nutrients and feed additives (Duis *et al.*, 1995), therefore encourages feed and nutrients utilization leading to better growth (Anzai *et al.*, 1995 and Nishide *et al.*, 1995). Algenate increases also weight and length of stomach (Siri *et al.*, 1992 and Nishide *et al.*, 1995) so increases feed digestion and absorption and consequently growth.

3- Fish production characteristics:
Although the initial biomass of fish in all the experimental ponds was the same, pond No. 3 (followed by pond No. 2) produced the heaviest final biomass and hence also total gain of fish. Whereas tilapia yield was better in pond No. 2 and % tilapia No. 1 was better in pond No. 5. However, pond No. 3 reflected the significantly ($P < 0.05$) highest dressing and boneless meat percentages comparing with the control (pond No. 6) as shown from Table (3). However, betafin is known with its stimulating effect on fish growth (meat production) as given by Castro *et al.* (1998), Papatryphon (2000) and Magouz (2002). Also, algenate improves growth via increasing nutrients utilization (Gubar and Tarasov, 1994; Anzai *et al.*, 1995 and Nishide *et al.*, 1995).

Table(3): Total production and tilapia production and its **characteristics.**

Items	Experimental ponds No.					
	1	2	3	4	5	6
Initial biomass, Kg	1870	1870	1870	1870	1870	1870
Final biomass, Kg	10539	12545	12812	12018	12438	10783
% from the best pond	82.3	97.9	100.0	93.8	97.1	84.2
Total gain, Kg	8669	10675	10942	10148	10568	8913
% Tilapia from total crop	85.0	86.5	83.1	85.9	81.2	76.9
% Tilapia No. 1 from total crop	42.9	43.0	38.8	40.0	44.3	31.3
% Tilapia No. 1 from Tilapia crop	50.4	49.7	46.7	46.6	54.6	40.7
Dressing, %	60.7^{c}	64.5^{a}	63.0^{ab}	62.4^{b}	62.1^{bc}	61.6^{bc}
Boneless meat, %	38.7^{c}	41.5^{a}	41.1^{ab}	39.6^{abc}	39.8^{abc}	39.3^{bc}

a - b: Means in the same row followed by similar letter do not significantly ($P > 0.05$) differ.

4- Composition and structure of the fish dorsal muscles:

The highest protein percent, thickness of the large connective tissue, and intensity of muscular bundles/mm^2, from one side, and from the other side the lowest fat percent, thickness of small connective tissue, thickness of skin and subcutaneous layer and smallest/largest diameter ratio of muscular bundles were realized in dorsal muscles of tilapia fish fed on the diet containing 1 Kg betafin and 600 ml biopolym/ton in pond No. 3 (Table 4 and Figs. 1 - 14).

The superiority of fish from pond No. 3 was true through the high growth performance, feed and nutrients utilization, and characteristics of the fish production. So, it not surprising to obtain better results herein concerning chemical composition and histological structure of dorsal muscles from fish of pond No. 3. Since, Virtanen *et al.* (1989) found that betafin increased dry matter content of the freshwater fish muscles. Additionally, Hruby *et al.* (2001) reported that betaine reduces fat content but increases protein content of fish carcass. The better histological structure due to betafin supplementation was also given by Ombabi (2002), since betafin increases protein synthesis in the muscular cells, hence leads to muscular growth. Moreover, alginate also increases dry matter (via increased water excretion) and reduces fat contents (via lowering digestion of dietary fat) as mentioned by Spyridakis *et al.* (1989); Anzai *et al.* (1995) and Nishide *et al.* (1995). The negative correlation between fish muscular fat and protein contents was confirmed also before (Abdelhamid *et al.*, 1995, 1998 and 1999).

Table (4): Chemical and histological composition of the dorsal muscles of tilapia fish from different experimental ponds.

Parameters	Experimental ponds No.					
	1	2	3	4	5	6
Dry matter, %	24.0[b]	25.8[a]	24.2[b]	23.7[b]	25.0[ab]	24.3[b]
Protein, %	80.5[a]	72.7[b]	82.7[a]	73.3[b]	78.7[a]	78.3[a]
Fat, %	15.5[b]	23.1[a]	12.7[b]	22.1[a]	16.3[b]	16.7[b]
Ash, %	4.00[c]	4.11[c]	4.54[bc]	4.64[abc]	4.95[ab]	5.19[a]
Thickness of the connective tissues septa, µm						
Small	4.00	4.50	2.50	5.00	3.00	3.00
Medium	30.0[ab]	31.0[ab]	30.5[ab]	21.5[c]	26.5[b]	35.0[a]
Large	65.5[a]	69.5[a]	70.0[a]	43.5[b]	47.0[b]	65.0[a]
Thickness of skin, µm	61.0[ab]	64.0[a]	44.0[d]	49.5[cd]	65.5[a]	54.5[bc]
Thickness of subcutaneous, layer, µm	69.0[c]	52.5[d]	46.0[e]	74.0[b]	64.5[c]	84.5[a]
Diameter of subcutaneous fat granules, µm	73.5[a]	34.5[b]	42.5[b]	48.5[b]	35.5[b]	72.5[a]
Diameter of muscular bundles, µm						
Smallest diameter	22.0	23.0	20.5	22.5	20.5	22.5
Largest diameter	34.5[b]	35.5[b]	46.0[a]	46.5[a]	38.0[b]	35.0[b]
Mean diameter	28.3[b]	29.3[b]	33.3[a]	34.5[a]	29.3[b]	28.8[b]
Smallest/largest ratio	0.64	0.64	0.45	0.48	0.54	0.64
Intensity of muscular bundles/mm^2	1080[a]	1144[a]	1009[a]	981[a]	999[a]	790[b]
Muscular bundles area, mm^2	0.68[d]	0.77[c]	0.88[b]	0.92[a]	0.67[d]	0.51[e]
% of muscular bundles area/mm^2	67.7	76.9	87.6	91.7	67.2	51.3
% of connective tissue/mm^2	32.3	23.1	12.4	8.3	32.8	48.7

a - e: Means in the same row followed by similar letter do not significantly ($P > 0.05$) differ.

Table 5: Total costs and net return from different experimental ponds in Egyptian pounds (L.E).

Items	Experimental ponds No.					
	1	2	3	4	5	6
Total inputs	43082	44361	44601	44301	44013	43156
Total outputs	52762	63573	66571	62799	64752	53235
Net return	9680	19212	21970	18498	20739	10079

Fig. (1): Cross-section in dorsal muscles of control fish showing the large space between muscles (x 40, E & H stains).

Fig. (2): Cross-section in dorsal muscles of fish in the 3rd treatment showing the large space between muscles (x 40, E & H stains).

Fig. (3): Cross-section in skin and subcutaneous layer of control fish (x 100, E & H stains).

Fig. (4): Cross-section in skin and subcutaneous layer of fish in the 2nd treatment (x 100, E & H stains).

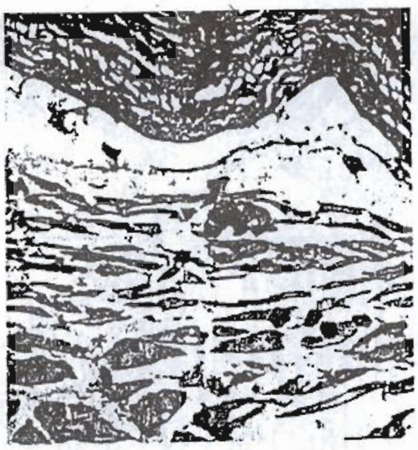

Fig. (5): Cross-section in skin and subcutaneous layer of fish in the 3rd treatment (x 100, E & H stains).

Fig. (6): Cross-section in skin and subcutaneous layer of fish in the 5th treatment (x 100, E & H stains).

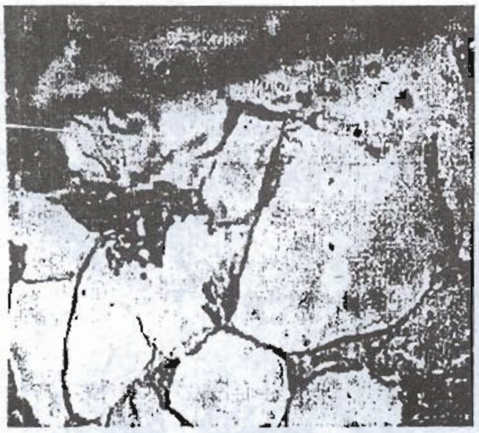

Fig. (7): Cross-section showing fat droplets in subcutaneous layer of fish in the 1st treatment (x 400, E & H stains).

Fig. (8): Cross-section showing fat droplets in subcutaneous layer of fish in the 2nd treatment (x 400, E & H stains).

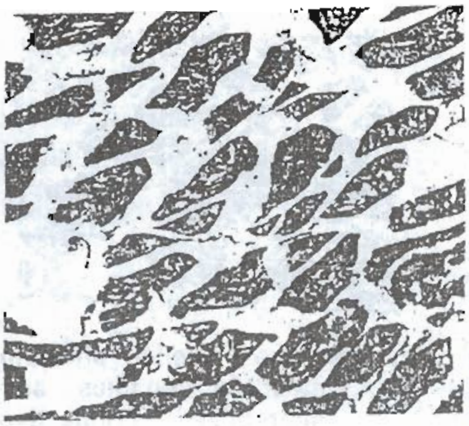

Fig. (9): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of control fish. (x 100, E & H stains).

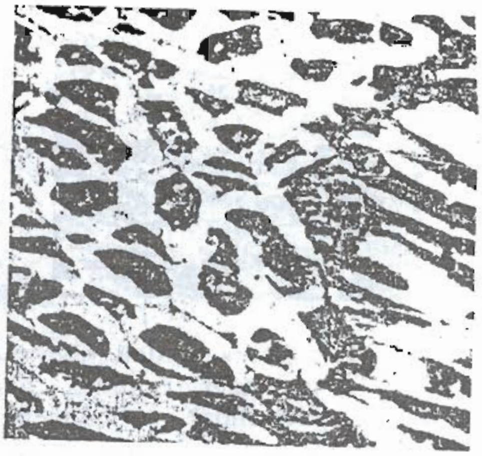

Fig. (10): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of fish in the 1st treatment (x 100, E & H stains).

Fig. (11): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of fish in the 2nd treatment (x 100, E & H stains).

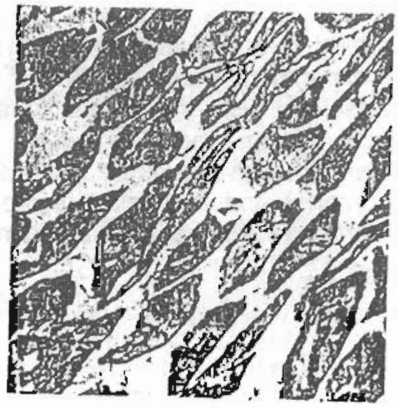

Fig. (12): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of fish in the 3rd treatment (x 100, E & H stains).

Fig. (13): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of fish in the 4th treatment (x 100, E & H stains).

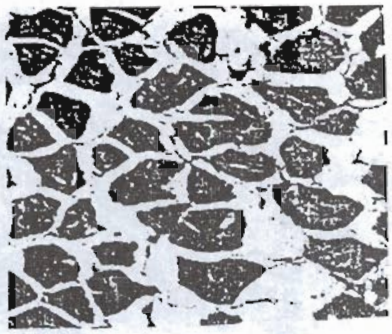

Fig. (14): Cross-section showing muscular bundles and interstitial connective tissue of the dorsal muscles of fish in the 5th treatment (x 100, E & H stains).

CONCLUSION

Betafin, itself, was not useful for tilapia culture in earthen ponds (Abdelhamid and Ibrahim, 2003); thus, its beneficial effects in the present study may be due to its synergetic effect with biopolym at the used concentrations with the diet (3rd treatment, i.e. 3rd pond) which led to the best results of fish growth, quality, and return. Therefore, it is recommended to use this treatment in feeding tilapia fish cultured in earthen ponds under semi-intensive production system to maximize fish production (to save imports) and to partially solve the fish production shortage gap than the demand for human consumption.

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تأثير استخدام البيتافين و/أو البيوبوليم كإضافات طبيعية فى إنتاج أسماك البلطى النيلى بنظام متعدد الأنواع شبه مكثف فى أحواض ترابية

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فى دراسة على البلطى النيلى المستزرع فى أحواض ترابية (كل منها سعة فدان) بنظام تعدد الأنواع شبه المكثف، أضيف البتافين فى عليقة الحوض الأول (١ كجم/طن)، والثانى (٢ كجم/ طن)، والحوض الثالث (١ كجم/طن مع البيوبوليم ٦٠٠ مل/طن)، وأضيف البيوبوليم لعليقة الحوض الرابع (٦٠٠ مل/طن) وتم رشه على ماء الحوض الخامس (١٥٠٠ مل ثم ٦٠٠ مل كل أسبوعين)، وترك الحوض السادس بدون إضافات كمقارنة، واستمرت التجربة ٦ أشهر. أظهرت معاملة الحوض الثالث أنها أفضل المعاملات، إذ أدت إلى أعلى متوسط وزن جسم، زيادة كلية فى الوزن، متوسط الزيادة اليومية فى الوزن، معدلى النمو النسبى والنوعى، إنتاجية كلية (٦٧٥ر١٠ طن/فدان)، % بروتين عضلات، قطر الحزم العضلية، مساحة كلية للحزم العضلية/مم[٢] (وأقل سمك للأنسجة الضامة بين الحزم العضلية وأقل سمك للجلد وطبقة تحت الجلد)، وأقصى عائد صافى (٩٧٠ر٢١ ألف جنية/فدان).