THE INFLUENCE OF SOME PHYTOBIOTICS ON GROWTH PERFORMANCE AT *OREOCHROMIS NILOTICUS* REARED IN AN INTENSIVE RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The aim of this research is to evaluate the influence of several phytobiotics on growth performance indicators at Oreochromis niloticus species, reared in an intensive recirculating aquaculture system. During six weeks of experiment, a total number of 180 fish, with an initial average biomass of 125.41 ± 34.33g, were divided randomized in four rearing units. The experimental variants were: V1-control, V2-1% thyme (Thymus vulgaris) / kg feed, V3-1% fenugreek (Trigonela foenum graecum) / kg feed, V4-1% neem (Azadirachta indica) / kg feed. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein. Fish were fed four times per day with a daily ration of 3.4% of fish body weight. During the experiment, the physico-chemical parameters of technological water were situated in normal range for optimal growth. Technological parameters analysed had revealed the following aspects at the end of the experiment: the best value of SGR was recorded in V2 – 1.81 %/day and the lowest was encountered in V1 and V4 case – 1.65 %/day; regarding FCR, the best value was found at V2 - 1.26 g feed/g biomass gain and the lowest was recorded at V1 and V4 – 1.44 g feed/g biomass gain. In conclusion,the research shows that the thyme, in 1% concentration per kg diet, has the best effect on growth performance of Oreochromis niloticus.

Key words: phytobiotics, growth performance, *Oreochromis niloticus*, recirculating aquaculture system

INTRODUCTION

The recent expansion of intensive aquaculture practices has led to the appearance of several concerns like those regarding biological material welfare or growth performance, if we refer especially to recirculating aquaculture systems. To avoid these concerns, different types of natural stimuli, such as probiotics, prebiotics or phytobiotics, were introduced into fish diets.

Because the effects of medicinal plants on human health are well known, as they are used for hundreds of years, their use among fish diets was tested while reducing various chemicals, usually used in aquaculture.

It had turned to this because phytobiotics have a multitude of properties, such as immune stimulators, antioxidants, antivirals, antibacterial, antifungal, anti-stress, appetite stimulators and not at least, promoters of growth [16, 4].

Phytobiotics are natural compounds which, being incorporated into diets, lead to a optimization of animal productivity [4]. They are also less expensive than other therapeutic methods used in aquaculture.

However, the use of medicinal and aromatic plants into fish diets is still limited, this being accomplished only at experimental scale.

Increasingly number of recent studies presents the positive aspects of phytobiotics administration, in diets, at different fish species [6, 2, 10, 1]. It is necessary a comparison between different phytobiotics used, to determine the nutritional potential of each one and also their ability to be used in commercial diets, at industrial level [20].

The aim of this paper is to investigate the influence of thyme, fenugreek and neem's on *Oreochromis niloticus* growth performance, under intensive recirculating aquaculture system conditions.

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MATERIAL AND METHOD

The material base of this study consisted in pilot recirculating system from the Department of Aquaculture, Environmental Science and Cadastre, from "Dunarea de Jos" University of Galati. The experiment lasted six weeks, from 20.06.2012 to 1.08.2012.

The recirculating system design includes the following components: 4 rearing units, with a volume of $1m^3$ each, and a series of water quality conditioning units (drum filter, sand filter, activated carbon filter, trickling filter, UV lamp and oxygenation units) [5].

A total number of 180 Nile tilapia, with an initial average weight of 125.41 ± 34.33g, were randomly distributed in 4 rearing units. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein. The feed biochemical composition is shown in Table 1.

Table 1 The biochemical composition of SOPROFISH 38/7 pelleted feed

Composition	Quantity
Protein %	38
Water %	10
Fat %	7
Ash %	10
Cellulose	4
Total Ca	1,6
Total P	1,2
Total Na	0,2
Vitamin A (IU/kg)	15000
Vitamin D (IU/kg)	2500
Vitamin E (mg/kg)	90
Vitamin C (mg/kg)	200
Lysine %	2,3
Methionine+Cysteine %	1,2
Ingredients: fish meal, soybean protein content, corn, wheat.	

The feeding frequency was 4 times per day, at an interval of 3 hours (9:00, 12:00, 15:00, 18:00) and was done manually. Daily ration was 3.4% of total body weight.

During the experiment, fish feed was supplemented with a concentration of 1% phyitobiotic / kg of feed. The phytobiotics used in the experiment were: thyme (*Thymus vulgaris*), fenugreek (*Trigonela foenum graecum*) and neem (*Azadirachta indica*). Thus, the following experimental variants were established: V1 - control variant, V2 - 1% thyme; V3 - 1% fenugreek, V4 - 1% neem.

The introduction of phytobiotics into fish feed was made as follows:

- the mixture of phytobiotic with gelatin - 2% concentration,
- uniform spray the mixture over the surface, correlated with continuous shaking,
- the final mixture is dried at 25 °C, for 2 hours.

The main physico-chemical water quality parameters were monitored daily: oxygen (mg/l) with TriOxmatic 700IQ WATT (W) sensor, temperature (°C) with sensor TrioxiTherm WATT, pH with WAT Sensolyt 700 IQ (SW) sensor and the concentration of $N\text{-}NO_2$ (mg/l), $N\text{-}NO_3$ (mg/l) and $N\text{-}NH_4$ (mg/l) was also monitored twice a week using Spectroquant photometer, Nova 400.

At the end of the experiment, fish were weighed and measured and the following growth performance indicators were calculated: biomass growth (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and retained protein (RP). The formulas that were used are shown below:

- Weight gain (WG) = Final weight (Wt) – Initial weight (W0) (g);
- Food conversion ratio (FCR) = Total feed (F) / Total weight gain (W) (g/g);
- Specific growth rate (SGR) = 100 x (ln Wt – ln W0) / t (% BW/day);
- Protein efficiency ratio (PER) = Total weight gain (W) / amount of protein fed;
- Retained protein (RP) = Total feed (F) x Crude protein (PB%) / 100.

The final data were statistically analyzed using descriptive statistics in Microsoft Excel. Results are expressed as mean ± standard error.

RESULTS AND DISCUSSIONS

Growth performance is influenced in a large part by water quality, both physically and chemically. During the experiment, water temperature ranged between 25.2 - 27°C, with a mean of 26.22 ± 0.60°C. The values were maintained in an optimal range for growth of Nile tilapia. Many authors claim that optimal range for growth, at this species, is between 25-30°C [7,12,13,14], although for normal growth, the temperature

must be somewhere between 20 and 35°C. Above 25°C, the growth rate is even higher at tilapia [15].

Oxygen and pH mean values are shown in Figure 1, where V1 is control variant, V2 – thyme variant, V3 – fenugreek variant, V4 – neem variant, FB - biological filter and FM - mechanical filter. Among experimental variants, oxygen values ranged between 6.66 mg/l and 9.16 mg/l, with an average value of 8.54 ± 0.91mg/l. These values are higher than those reported by Ridha et al in 2006, where the range was between 6.1-7.2 mg/l [14].

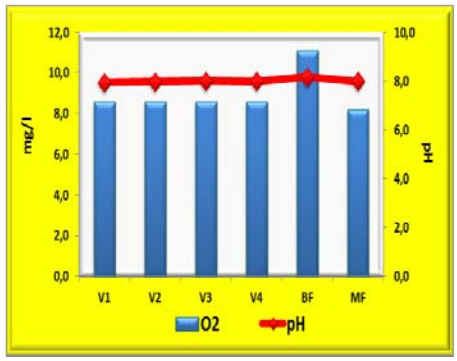

Fig. 1 Mean values of O_2 and pH concentrations

Although on very short period of time tilapia can survive even at an oxygen value of 0.3 mg/l, for normal growth, increase metabolism and disease resistance, O_2 concentration should be above 2 mg/l [13.12]. Also, for optimal growth of *O. niloticus* species, pH should be maintained between 6 and 9 [8.13].

Nitrite ranged from 0.15 ± 0.06 mg/l to 0.16 ± 0.06 mg/l among experimental variants, higher values being recorded only at mechanical filter outlet. Yanbo and others, in 2006, reported that the lethal dose, LC50, of nitrite, at *O. niloticus* species, is 28.18 mg/l [18]. However, Ridha reported at tilapia values of $N\text{-}NO_2$ concentrations between 0.01 and 0.46 mg/l [14]. Regarding nitrate, during the experimental period, the highest average value was recorded in neem variant. The concentration of ammonium ($N\text{-}NH_4$) varied among the four experimental variants, fact that can be seen in Figure 2.

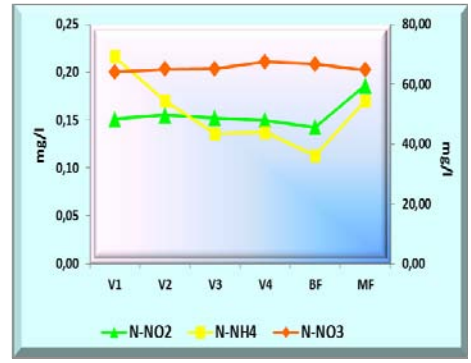

Fig. 2 Mean values of various nitrogen compounds.

Technological performance indicators, obtained at the end of the experiment, are shown in Table 2. During the experiment, mortalities were not recorded.

From data analysis, a higher biomass growth can be observed at the variant where thyme was administrated.

The average individual weight, at the end of the experiment, was higher at V2 - 268.33 g/fish, followed by V3 - 253.89 g/fish, V4 - 251.56 g/fish and V1 - 251 g/fish.

Regarding the specific growth rate, the best value was recorded in V2 - 1.81%/day, followed by V3 - 1.68%/day (Figure 3).

The best FCR was found in V2 - 1.26 g feed/g biomass gain, followed by V3, V4 and V1 (Figure 3).

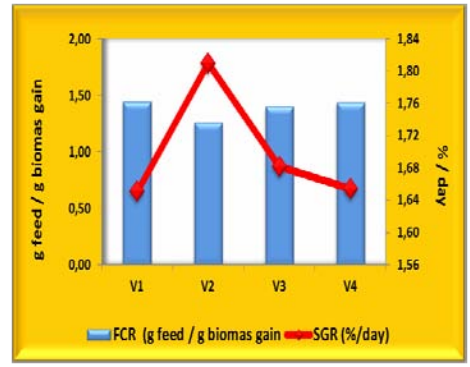

Fig. 3 SGR and FCR values after the administration of phytobiotics.

Mostafa et all., in 2009, supplemented the diet of Nile tilapia juvenile with fenugreek seed

powder at a concentration of 0, 0.5, 1 and 1.5%/kg diet, for 12 weeks, fact that showed an improvement of growth performance at 1% fenugreek/kg diet variant [11].

At *Dicentrarchus labrax* species (european seabass), after the administration of thyme, rosemary and fenugreek diets, in a concentration of 1% / kg feed, the best values of growth indicators (SGR, FCR, PER) were met in case of thyme variant[19].

Protein efficiency ratio (PER) has a high value at thyme variant (30.13 g biomass increase/g feed), followed by fenugreek variant (27.08 g biomass increase / g feed distributed). This can be seen in Figure 4.

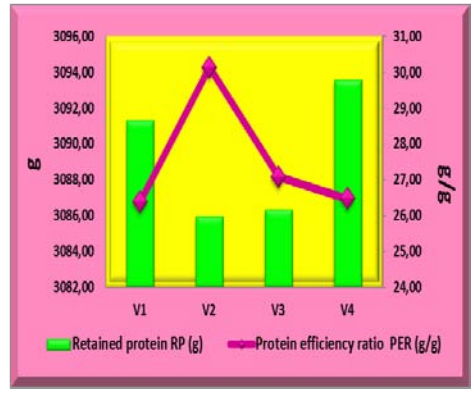

Fig. 4 PER and RP values after the administration of phytobiotics

Table 2 Technological performance indicators, obtained at the end of the experiment

Growth performance indicators	**V1**	**V2**	**V3**	**V4**
Initial numbers of fish	45	45	45	45
Final numbers of fish	45	45	45	45
Survival (%)	100	100	100	100
Days of rearing	42	42	42	42
Initial stocking density (g)	5647	5636	5638	5651
Initial stocking density(kg/m3)	**14,12**	**14,09**	**14,10**	**14,13**
Final stocking density (g)	11295	12075	11425	11320
Final stocking density (kg/m3)	**28,24**	**30,19**	**28,56**	**28,30**
Biomass gain (g)	5648	6439	5787	5669
Biomass gain (kg/m3)	14,12	16,09	14,47	14,17
Initial mean individual weight (g/ex)	125,49	125,24	125,29	125,58
Final mean fish weight (g/ex)	251,00	268,33	253,89	251,56
Specific growth rate SGR (%/day)	**1,65**	**1,81**	**1,68**	**1,65**
Feed conversion ratio FCR (g/g)	**1,44**	**1,26**	**1,40**	**1,44**
Protein efficiency ratio PER (g/g)	**26,38**	**30,13**	**27,08**	**26,46**
Retained protein RP (g)	3091,30	3085,98	3086,36	3093,58

Note: Experimental variants: V1–control, V2–thyme, V3–fenugreek, V4–neem

Dietary supplementation with different phytobiotics, at many species of fish, in different development stages, is increasingly studied.

For example, dietary supplementation, at Nile tilapia, with a mixture of plants extracts, with digestive properties, natural emulsifying agents and co-factors of digestion, generated a higher feed conversion ratio and protein efficiency [3].

Zaki et all, in 2012, tested several phytobiotics, in two concentrations, 1% and 2%, at *Oreochromis niloticus* species, with an average initial weight of 0.82 ± 0.3 g /fish. The results showed that dietary supplementation with fenugreek, eucalyptus, pepper, chamomile and thyme, in a concentration of 1%, had a positive effect on growth performance parameters, feed conversion ratio, nutrient utilization, protein efficiency and also on physiological parameters [20].

Khalafalla, in 2009, reported that administration of diets, at Nile tilapia (2.5 g / fish), with a concentration of 1% marjoram leaves, caraway seeds, chamomile flowers and fenugreek powder seeds, generated a higher effect on growth performance than did 0.5% concentration [9].

Unlike the thyme and fenugreek, neem dietary supplementation had little effect on Nile tilapia growth performance, reported to control variant. This was seen at *Tilapia zillii* species, which was fed for 60 days with diets supplemented with *Azadirachta indica* [17].

CONCLUSIONS

The results of this research show that thyme, added in a concentration of 1% / kg feed, has a beneficial effect over the productivity of *Oreochromis niloticus* species, reared in a recirculating aquaculture system. In our case, also fenugreek helped to increase growth performance, but less successful than thyme.

In this experiment, the concentration of 1% neem / kg feed did not generated a significantly higher growth performance, reported to control variant.

As a final conclusion, we can affirm that some phytobiotics, included in aquatic organisms diets, may lead to an increase of productivity and efficiency of feed utilization, at tilapia species.

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REFERENCES

[1] Abdelhamid H.M.B., 2010: Physiology and nutritional studies on improving growth of Nile tilapia (O. niloticus) fry using some medicinal plants as a feed additives. MSc.Thesis, University of Kafr El-Sheikh, Egypt.

[2] Al-Absawy A.N.M., 2010: Nutritional requirements for Nile tilapia, O. niloticus, cultured in El-Max research station with special references to their growth and feeding habits.M.Sc. Thesis, Faculty of Science Al-Azhar University. Cairo, Egypt.

[3] Ceulemans S., Robles R., Coutteau P., 2009: Innovative Feed Additives Improve Feed Utilization In Nile Tilapia, Global Aquaculture Advocate November/December.

[4] Cristea V., Alina Antache, Iulia Grecu, Angela Docan, Lorena Dediu, Mirela (Creţu) Mocanu, 2012: The use of phytobiotics in aquaculture. Lucrări Ştiinţifice, Seria Zootehnie, Iaşi. Vol. 57 (17).

[5] Cristea V., Grecu I., Ceapa C., 2002: Ingineria sistemelor recirculante de acvacultură, Editura Didactică şi Pedagogică R. A. Bucuresti.

[6] El-Dakar A.Y., Hassanien G.D., Gad S.S., Sakri S.E., 2008: Use of Dried Basil Leaves as a Feeding Attractant for Hybrid Tilapia, O. niloticus x O. auraus, Fingerlings. Mediterranean Aquaculture Journal 1(1): p. 35- 44.

[7] El-Sayed A.F.M., 2006: Tilapia culture, Alexandria University, Egypt.

[8] Guerrero R. D., 1997: A Guide to Tilapia Farming. Aquatic Biosystems. Bay Laguna, Philippines.

[9] Khalafalla M.E., 2009: Utilization of Some Medical Plants as Feed Additives for Nile Tilapia, *Oreochromis niloticus*, feeds. Mediterranean Aquaculture Journal. 2(2), p. 10-19.

[10] Khalil F.F., Farrag F.H., Mehrim A.I., 2009: Using Marjorana hortensis against contamination of mono-sex Nile Tilapia, O.niloticus diets by lead oxide. Abbassa international journal for Aquaculture. ISSN 1687-7683, special issue for Global Fisheries Research Conference, Cairo international Convention Center, 24- 26 October 2009,pp 407-428.

[11] Mostafa A.A.Z.M., Ahmad M.H., Mousallamy A, Samir A., 2009: Effect of using dried fenugreek seeds as natural feed additives on growth performance, feed utilization, whole-body composition and entropathogenic Aeromonas hydrophila-challenge of monosex Nile Tilapia O. niloticus (L) fingerlings. Aust. J. Basic Appl. Sci., 3(2): 1234-1245.

[12] Peterman Mark Alan, 2011: Evaluation of Production Characteristics of Four Strains of Nile Tilapia Oreochromis niloticus and a Red Variety Under Two Sets of Intensive Culture Conditions. A thesis submitted to the Graduate Faculty of Auburn University, Alabama.

[13] Popma T. and Masser M., 1999: Tilapia Life History and Biology. Southern Regional Aquaculture Center (SRAC). Publication Number 283.

[14] Ridha T.M., 2006: Comparative study of growth performance of three strains of Nile tilapia, Oreochromis niloticus L. At two stoking densities, Aquacul. res., 37: p 172–179.

[15] Savin C., Cristea V., Patriche N., Marilena Talpeş, Hoha G., Magdalena Tenciu, Elpida Paltenea, Elena Mocanu, Păsărin B., 2012: Preliminary data regarding tilpia growth performances in a recirculating aquaculture system, Lucrări Ştiinţifice, Seria Zootehnie, Iaşi. Vol. 57 (17).

[16] Thavasimuthu Citarasu, 2010: Herbal biomedicines: a new opportunity for aquaculture industry, J. Aquacult. Int. 18, p. 403-414.

[17] Temitope Jegede, Oyedapo Fagbenro, 2008: Dietary neem (azadirachta indica) leaf meal as reproduction inhibitor in redbelly tilapia, Tilapia zillii, 8th International Symposium on Tilapia in Aquaculture, p. 365-373.

[18] Yanbo, W., Wenju, Z. and Zirong X., 2006: Acute toxicity of nitrite on tilapia, Oreochromis niloticus, at different external chloride concentrations. Fish Physiology and Biochemistry 1, 49-54.

[19] Yılmaz S., Ergün S., Çelik E. Ş., 2012 : Effects of herbal supplements on growth performance of sea bass (Dicentrarchus labrax): Change in body composition and some blood parameters, J. BioSci. Biotech. 1(3), p. 217-222.

[20] Zaki M.A., Labib E.M., Nour A.M., Tonsy H.D., Mahmoud S.H., 2012: Effect of some medicinal plants diet on mono sex Nile tilapia (Oreochromis niloticus), growth performance, feed utilization and physiological parameters. Asia-Pacific Chemical, Biological & Environmental Engineering Society, p. 220-227.