THE SYNERGISTIC INFLUENCE OF *THYMUS VULGARIS* AND VITAMIN E ON GROWTH PERFORMANCE AND OXIDATIVE STRESS AT *OREOCHROMIS NILOTICUS* SPECIES

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Abstract

The aim of this research was to evaluate the influence of thyme and vitamin E on growth performance indicators, biochemical composition of meat and oxidative stress at Oreochromis niloticus species, reared in a recirculating aquaculture system. The experiment was conducted in duplicate, for one month. The experimental variants were: V1 – 1% thyme (Thymus vulgaris)/kg feed and V2 – 1% thyme/kg feed supplemented with 500 mg vitamin E/kg feed. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein. At the end of the experiment, the results showed that in V2 variantwere recorded the best values of SGR (0.65 %/day) and FCR (1.42 g feed/g biomass gain). Regarding the biochemical composition of meat, significant differences were recorded only in case of fat content ($p=0.03$; $p<0.05$). In terms of oxidative stress, was observed a decrease in lipid peroxidation (MDA-malondialdehyde)in V2 variant,significant differences ($p<0.05$; $p=0.01$) were obtained only in case of bloodplasma. But, the increase of total antioxidant capacity (TAC)was registeredin V1 variant at the level of bloodplasma, muscle tissue, liver and gut. In conclusion, the research shows that the administration of thyme (1%/kg feed) in combination with vitamin E (500mg/kg feed) has a synergistic effect on growth performance indicators and lipid peroxidation, at Nile tilapia.

Key words: thyme, vitamin E, growth performance, *Oreochromis niloticus*, aquaculture system

INTRODUCTION

With the recent fast growth in aquaculture, governance of this sector has become increasingly important and has made remarkable progress, because the fish and fishery products represent a valuable source of nutrients of fundamental importance for diversified and healthy diets. For developing aquaculture certification standards is required the essential minimum criteria, among wich food safety, animal health and welfare, environmental integrity and socio-economic aspects, for ensuring a development and implementation of credible aquaculture certification schemes [7].

Studies reveal that between fish nutrition and their health maintaining are strongly connected. For this reason, food improving and an effectively diet adopting is an important step in ensuring the welfare of the fish.

Recent research shows the beneficial effect of the introduction of phytobiotics in fish diet both the fish welfare, but also on growth performance. Moreover, vitamin E is known for its anti-oxidant effect, but also because it improves the growth.

It's know that natural antioxidants can protect the biologically important cellular components from oxidative processes (free radicals) caused by reactive oxygen species (ROS) [16]. Determination of oxidative stress is made, among others, by analyzing lipid peroxidation (MDA) and total antioxidant capacity (TAC).

Regarding this aspect has shown that some herbal plant, due to phenolic compounds, have shown a positive relationship between antioxidant activity and vitamin C, vitamin E, and beta-carotene content [19].

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Because Lee et al. (2005) and Rota et al. (2008) have demonstrated that thyme has strong antimicrobial and antioxidant activity due to its very high contents of thymol, p-cymene, carvacrol, eugenol and 4-allylphenol, besides vitamin E we chose a thyme (*Thymus vulgaris*) as a phytobiotic, for this experiment [10, 15].

Thymus vulgaris L. (thyme) is an aromatic plant belonging to the *Lamiaceae* family, used for medicinal (because shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus *Thymus*) [18] and spice purposes almost everywhere in the world [12].

Adwan et al., (2006) have revealed that the many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites [1].

The aim of this paper is to investigate the influence of thyme and thyme with vitamin E on growth performance, biochemical composition of meat and oxidative stress at *Oreochromis niloticus* species reared in a semi-intensive aquaculture system.

MATERIALS AND METHODS

The research was conducted at the research laboratory of Aquaculture, Environmental Science and Cadastre from "Dunarea de Jos" University, Galati. The experiment lasted from 30.07.2012 to 29.08.2012. The recirculating aquaculture system includes four rearing units, with a volume of 0,5 m^3 each. For each growth units corresponded two external filters - Tetratec Ex 400 - for water recirculation, while the water aeration was performed using a compressor for aeration and aeration stones.

A total number of 100 Nile tilapia, with an initial average weight of 328.36 ± 37.68g, were randomly distributed in 4 rearing units. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Antache et al. (2013) [3].

The experimental variants were performed in duplicate and were organized as follows: V1 – 1% thyme (*Thymus vulgaris*)/kg feed, V2 – 1% thyme + 500mg vitamin E / kg feed.

Fish were fed three times per day with a daily ration of 1% from fish body weight.

The most important physicochemical parameters of technological water (oxygen, pH, temperature, the concentration of nitrites, nitrates, and ammonia,) were maintained between normal limits.

At the end of the experiment, fish were weighed, measured and were calculated the growth performance indicators (biomass growth (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER)), were determined the biochemical composition of meat and welfare status of the Nile tilapia. The welfare status was achieved by determining oxidative stress, analyzing the lipid peroxidation (determined by the concentration of malondialdehyde; MDA nmol/ml) and total antioxidant capacity (TAC mM Trolox).

The determination of biochemical composition from Nile tilapia meat was performed from fresh meat (on muscle tissue samples). The samples were then weighed and minced in a tissue grinder, to ensure homogeneous samples for the analysis. When collecting the samples, the uniformity of exemplars, was taken into account, to eliminate the errors which consist in weight differences between exemplars.

The proximate composition of diets was carried out using the Association of Analytical Chemists methods [2].

For the analysis of *Oreochromis niloticus* meat biochemical composition was carried out to determine the body percentage of moisture, protein content, fat content, ash and dry matter.

Proteins were determined with Gerhardt type equipment by using Kjeldahl method, fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment, dry matter was determined by heating at temperature of 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C, in a Nabertherm furnace.

To determine the oxidative stress, the MDA and TAC were analyzed

spectrophotometrically from tissue muscle, liver, gut and plasma.

The determination of malondialdehyde was performed in accordance with Draper and Hadley method [4], where one molecule of MDA reacts with two molecules of thiobarbituric acid (TBA), giving a pink color that is measured at an optical density of 532 nm.

Total antioxidant capacity (TAC mMTrolox) was measured at an optical density of 734 nm, using the ABTS - (2,2-azinobis 3-ethylbenzothiazoline-6sulphonic acid) in accordance with the method described by Re Roberta [14]. The inhibition degree was calculated with the following formula:

% inhibition = [1 - (Abs P / Abs B)] x100;

where:

AbsP - sample absorbance,

AbsB - blank absorbance.

The calibration curve of % inhibition dependingonmM Trolox are presented in figure 1.

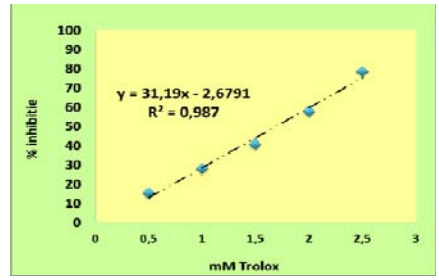

Fig. 1 The calibration curve of % inhibition depending on mM Trolox

The data were statistically analyzed using descriptive statistics and T test.Programs used were Microsoft Excell 2010 and SPSS Statistics 17.0.

RESULTS AND DISCUSSIONS

Growth performance

Technological performance indicators, obtained at the end of the experiment, are shown in Table 1. During the experiment, mortalities were not recorded.

Table 1 Technological performance indicators obtained at the end of the experiment (mean/variant)

Growth performance indicators	V1 (thyme)	V2 (thyme+vitamin E)
Initial numbers of fish	25	25
Final numbers of fish	25	25
Survival (%)	100	100
Days of rearing	31	31
Initial stocking density (g)	8210	8208
Initial stocking density(kg/m3)	**20.53**	**20.52**
Final stocking density (g)	9924	10044
Final stocking density (kg/m3)	**24.81**	**25.11**
Biomass gain (g)	1714	1836
Biomass gain (kg/m3)	5.71	6.11
Initial mean individual weight (g/ex)	328.40	328.32
Final mean fish weight (g/ex)	396.96	401.76
Specific growth rate SGR (%/day)	**0.61**	**0.65**
Feed conversion ratio FCR (g/g)	**1.52**	**1.42**
Protein efficiency ratio PER (g/g)	**1.73**	**1.85**

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

The average individual weight, at the end of the experiment, was higher in variant V2 – 401.76 g/fish, followed by variant V1 – 396.96 g/fish. Regarding the specific growth rate, the best value was recorded in V2 – 0.65 %/day, followed by V1 – 0.61%/day.

The best FCR was found in V2 – 1.42 g feed/g biomass gain, followed by V1 – 1.52 g feed/g biomass gain.

Protein efficiency ratio (PER) has a high value in V2 variant (1.85 g biomass increase/g feed).

Zaki et al., (2012) showed that dietary supplementation with 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 [21]. At *Dicentrarchus labrax* species (european seabass) the best values of growth indicators (SGR, FCR, PER) were obtained in case of thyme variant (1% thyme/kg feed) [20].

However, our results show the occurrence of a synergistic effect of thyme combination with vitamin E on technological performance indicators at *Oreochromis niloticus* species.

Biochemical composition of meat

The biochemical composition of meat varies considerably depending on several factors like: fish age, size, sex, environmental conditions (temperature, dissolved oxygen, pH, salinity, etc) [8], and appears to be largely influenced by feed composition [6]. In figure 2 are presented the values of moisture, protein, fat and ash content.

In terms of biochemical composition of Nile tilapia meat, a significant difference ($p<0.05$) was observed in the fat content ($p=0.03$), between the experimental variants. The values of fat content were: 1.161±0.02% in V1 and 1.334±0.09% in V2.

Regarding to ash content, Olagunju et al. (2012) affirm that an ash content between 1.17 and 1.79 % of fish, revealed that they are a good source of minerals such as calcium, potassium, zinc, iron and magnesium [13].

In case of dry matter, the values obtained were 22.69±0.69 %in V1 and 22.47±0.63 % in V2.

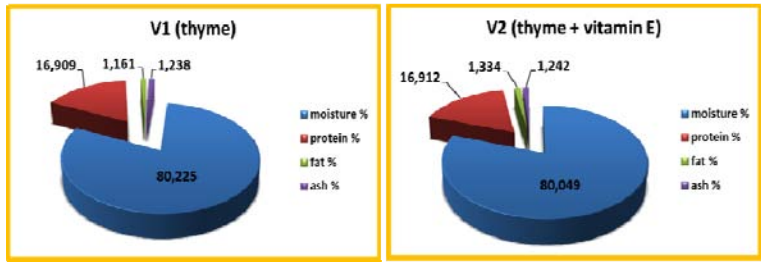

Fig. 2 The biochemical composition of *Oreochromis niloticus* meat fed with thyme and thyme + vitamin E.

Welfare status

Duncan (2005) said that the welfare status is correlated with biological functioning including physiological stress responses, which can be determined by various analysis. In this experiment we evaluated the fish welfare by analyzing oxidative stress (by determining the concentration of malondialdehyde and the total antioxidant capacity) [5].

Table 2 The analysis of malondialdehyde from different tissues, at Oreochromis niloticus species

MDA (nmol/ml)				
Experimental variant		**V1 (thyme)**	**V2 (thyme+vitamin E)**	**p value***
plasma	M±ES	10.01±1.44	7.37±1.09	0.01
muscle tissue	M±ES	9.19±1.27	6.57±0.44	0.15
liver	M±ES	7.21±0.37	6.93±0.79	0.40
gut	M±ES	9.50±0.70	8.14±0.56	0.13

***Note:** p value is 0.05. For signifiant differences p<0.05*

Determination of lipid peroxidation plays an important role because it is responsible for plasma membranes damage [11]. Results on malondialdehyde (MDA) dynamics are presented in Table 2.

Comparing the experimental variants, in V2 variant can be observed a decrease of MDA concentration, in tissue, liver and gut; and a decrease with a significant difference ($p<0.05$) in plasma level ($p=0.01$).

These results lead to the following affirmation: the combination between thyme and vitaminE lead to decrease the lipid peroxidation, respectively decrease the oxidative stress.

Total antioxidant capacity is a relatively new test and the advantage of this test is that it measures the antioxidant capacity of all antioxidants, from a biological sample [9].

Table 3 The analysis of total antioxidant capacity of different tissues at *Oreochromis niloticus* species

TAC (mM Trolox)				
Experimental variant		**V1 (thyme)**	**V2 (thyme+vitamin E)**	**p value***
plasma	M±ES	23.79±0.61	22.53±2.25	0.16
muscle tissue	M±ES	15.01±0.09	11.12±3.05	0.21
liver	M±ES	22.71±0.37	18.19±1.65	0.11
gut	M±ES	9.51±0.05	5.63±2.01	0.15

***Note:** p value is 0.05. For signifiant differences p<0.05*

The analysis of total antioxidant capacity of different tissues is presented in Table 3.

Although in V2 variant was registered a decrease in a total antioxidant capacity, but were not significantly difference between the variants of statistical point of view.

However, is observed that dietary supplementation with a concentration of 1% thyme led to an increase a TAC (mM Trolox) with 68.91% in gut, 34.98% in tissue muscle, 24.84% in liver and with 5.59% in plasma, in comparation with a dietary supplementation with a combination between thyme and vitamin E.

Some studies show that between the malondialdehyde and total antioxidant capacity there is a correlation. So, while the malondialdehyde concentration decrease, the concentration of total antioxidant capacity increase and vice versa.

But, Suresh et al. (2010) showed that there can be a compensatory mechanisms to overcome the lipid peroxidation (increased MDA levels) by increasing the antioxidants in vivo which can maintain the normal oxidant:antioxidant ratio (without oxidative stress) [17].

CONCLUSIONS

In conclusion, this experiment reveals that the thyme combination with vitamin E shows a synergistic effect leading to an improvement in growth performance and to a reduction of lipid peroxidation in tissue, liver, gut and especially in plasma level in which were significant differences ($p<0.05$).

It has been found that dietary supplementation with a concentration of 1% thyme led to an increase a total antioxidant capacity (mM Trolox) with 68.91% in gut, 34.98% in tissue, 24.84% in liver and with 5.59% in plasma, in comparation with a dietary supplementation with a combination between thyme and vitaminE.

Regarding of biochemical composition of Nile tilapia meat between the experimental variants a significant difference was observed only in the fat content. The highest value were recorded in V2.

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REFERENCES

[1] Adwan G., Abu-Shanab B., Adwan K.,Abu-Shanab F., 2006: Antibacterial Effects of Nutraceutical Plants Growing in Palestine on *Pseudomonas aeruginosa*, Turk J. Biol., nr. 30, p. 239-242.

[2] AOAC (Association of Official Analytical Chemists), 17th Edition, A.O.A.C., Washington DC, 2000, nr. 21, p. 447.

[3] Antache A., Cristea V., Dediu L., Grecu I., Docan A., Vasilean I., Mocanu (Crețu) M., Petrea Șt. M., 2013: The influence of some phytobiotics on growth performance at *Oreochromis niloticus* reared in an intensive recirculating aquaculture system. Lucrări Științifice, Seria Zootehnie, Iași, Vol. 60(18), p. 204-208.

[4] Draper H.H., Hadley M., 1990: Malondialdehyde determination as index of lipid peroxidation. Methods.Enzymol.nr. 186, p. 421 – 431.

[5] Duncan I.J.H., 2005: Science-based assessment of animal welfare: farm animals. Rev Sci Tech Off Int Epiz.,nr. 24, p. 483–492. http://www.fao.org/docrep/016/i2727e/i2727e.pdf

[6] El-Zaeem S. Y., Mohamed M. M. A., Mohamed E.S. S., Abd El-Kader W. N., 2012: Flesh quality differentiation of wild and cultured Nile tilapia (*Oreochromis niloticus*) populations. African Journal of Biotechnology Vol. 11(17), p. 4086-4089.

[7] FAO Fisheries and Aquaculture Department, Rome 2012. The state of world fisheries and aquaculture 2012.http://www.fao.org/docrep/016/i2727e/i2727e.pdf

[8] Hernández-Sánchez F., Aguilera-Morales M. E. 2012: Nutritional richness and importance of the consumption of tilapia in the Papaloapan Region, REDVET, Vol.13, nr.6. http://www.veterinaria.org/revistas/redvet/n060612/061205.pdf

[9] Kusano C., Ferrari B., 2008: Total antioxidant capacity: a biomarker in biomedical and nutritional studies. Journall of Cell and Molecular Biology 7(1), p. 1-15.

[10] Lee K.J., Dabrowski K., Sandoval M., Miller M.J.S., 2005: Activity-guided fractionation of phytochemicals of maca meal, their antioxidant activities and effects on growth, feed utilization and survival in rainbow trout (*Oncorhynchus mykiss*) juveniles. Aquaculture, 244(1-4),293-301.

[11] Lupoae M., Cristea V., Coprean D., Mocanu M., Patriche T., Bocioc E., 2011.Biochemical determinations and oxidative stress evaluation on *O. mykiss* grown in recirculating system. Lucrări Științifice - vol. 55, Seria Zootehnie, p. 306 – 310.

[12] Morales R., 2002: Medicinal and Aromatic Plants – Industrial Profiles, vol. 24-Thyme, E. Stahl-Biskup and F. Saez, eds., Taylor&Francis, p. 16.

[13] Olagunju A., Muhammad A., Mada S. B., Mohammed A., Mohammed H. A., Mahmoud K. T., 2012: Nutrient Composition of *Tilapia zilli*, *Hemisynodontis membranacea*, *Clupea harengus* and *Scomber scombrus* Consumed in Zaria. World J. Life Sci. and Medical Research, (2), p.16.

[14] Re Roberta P. N., Proteggente A., Pannala A., Min Yang, Rice-Evans C., 1999: Antioxidant Activity Applyng an Improved ABTS Radical Cation Decolorization Assay Free Radical. Biology & Medicine, Vol. 26, nr. 9/10, p. 1231–1237.

[15] Rota M.C., Herrera A., Martinez R.M., Sotomayor J.A., Jordan M.J., 2008: Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control, 19(7): 681-687.

[16] Su L., Yin J.-J., Charles D., ZhouK., Moore J., Yu L., 2007: Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food Chemistry, nr. 100, p. 990-997.

[17] Suresh D. R., Kumaran S., Hamsaveena A. V., 2010: Age related changes in malondialdehyde: total antioxidant capacity ratio – a novel marker of oxidative stress. International Journal of Pharma and Bio Sciences V1(2).

[18] Thompson J., Chalcha J., Michet A., Linhart Y., Ehlers B., 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. J. Chem. Ecol., 29(4):859-80.

[19] Velasco V., Williams P., 2011: Review: Improving meat quality through natural antioxidants. Chilean Journal of Agricultural Research 71(2) April-June, p. 313-322.

[20] 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.

[21] 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.