The Evaluation of Synergistic Effect of *Hippophae rhamnoides* and Vitamin E on Growth Performance and Oxidative Stress at *Oreochromis niloticus-Linnaeus, 1758*

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Abstract

The aim of this research is to evaluate the influence of sea buckthorn (*Hippophae rhamnoides*) and vitamin E on growth performance indicators and oxidative stress at Nile tilapia juvenile, reared in a recirculating aquaculture system. The experiment was conducted six weeks, in triplicate. The experimental variants were: V1–control, V2–1% sea buckthorn/kg feed, V3–500mg vitamin E/kg feed and V4–1% sea buckthorn supplemented with 500 mg vitamin E/kg feed. During the experiment was performed an intermediary biometric measurement. Oxidative stress analysis consisted in determination of lipid peroxidation (MDA-malondialdehide) and total antioxidant capacity (TAC) from liver, muscle tissue and gut. Results showed a good evolution of GR, FCR and SGR, during the experiment, in V4–in which feed was supplemented with sea buckthorn and vitamin E. Based on the results obtained in variant V4, in liver and muscle tissue, the oxidative stress was reduced. Regarding MDA and TAC, between experimental variants, were registered significant differences ($p<0.05$) at the level of muscle tissue and gut. In conclusion, the research shows that sea buckthorn (1%/kg feed) in combination with Vitamin E (500mg/kg feed) has a synergistic effect on growth performance indicators and oxidative stress, at *Oreochromis niloticus* juvenile.

Keywords: growth performance, *Oreochromis niloticus*, oxidative stress, sea buckthorn, Vitamin E

1. Introduction

Nowadays, aquaculture represents the fastest food growing industry in the world. In year 2012, the total world aquaculture production was 59.7 million metric tons [1]. From the desire to get as a big fish productions, recently is trying to introduce the different supplements in fish feed to improve growth performance and ensure the welfare status. This aspect is due to food type and its method of administration, because these represent an important factor in fish development and survival at the early stages [2].

In many recent studies it was shown a positive effect of phytobiotics and vitamins administration in fish diets over the health status and growth performance [3-6].

Analysis of oxidative stress may reveal if the fish health is affected or not by a stressors factors.

The term "oxidative stress" was introduced in 1986 by Sies and means all oxidative damage caused by free radicals of oxygen [7]. Practically oxidative stress represents the imbalance between oxidants and antioxidants ratio. Generation of oxidative stress is due, mostly, to the existence of reactive species of some chemical elements. These reactive species presents an unpaired electron, which makes them highly reactive. Thus, were identified reactive oxygen species (ROS), reactive nitrogen species (RNS) and, more recently,

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reactive sulfur species (RSS) with important roles in physiological and pathological processes.
The role of antioxidants is to remove or neutralize reactive oxygen and nitrogen species and peroxidation products of organic molecules [8, 9].
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 [10].
Vitamin E is known for its antioxidant effect, but also because it improves the growth [11-13].
Vitamin C is the main vitamin found in biochemical composition of sea buckthorn fruits, approximately 400 mg/100 g [14].
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 [15].
The scope of this paper is to investigate the influence of sea buckthorn, vitamin E and sea buckthorn in combination with vitamin E on growth performance and oxidative stress at *Oreochromis niloticus* juvenile reared in a recirculating aquaculture system.

2. Materials and methods

The research was carried out in the pilot recirculating system from the Department of Aquaculture, Environmental Science and Cadastre, of "Dunarea de Jos" University of Galati. The experiment lasted from 25 October to 7 December, 2012.
The recirculating system design includes the following components: 12 rearing units, with a volume of 64L each, 12 external filter (Tetratec EX 400) for water quality conditioning units, 12 automatic heaters with thermostat, used to obtain the optimal temperature for tilapia growth, 12 aeration stones and a pump for compressed air type RESUN Quiet LP 100. The important physicochemical parameters of technological water (oxygen, concentration of nitrites, nitrates, ammonia, pH and temperature) were maintained between normal limits (Table 1). Temperature and dissolved oxygen was daily measured with Hannah HI 98186 oximeter, and $N\text{-}NO_2$, $N\text{-}NO_3$, $N\text{-}NH_4$, were measured, two times per week, at Spectroquant Nova 400 with Merck kits. pH was measured with WTW inoLab series (Terminal 740).

Table 1. Physicochemical parameters of technological water obtained during the experiment

Parameters/Variants	V1	V2	V3	V4
T (°C)	25.65±0.99	25.80±1.16	25.99±1.03	26.53±0.80
DO (mg/L)	7.02±0.68	6.52±0.89	6.81±0.58	6.79±0.58
pH	7.45±0.14	7.51±0.07	7.48±0.07	7.51±0.15
$N\text{-}NO_2$ (mg/L)	0.61±0.38	0.53±0.46	0.91±0.46	0.61±0.49
$N\text{-}NO_3$ (mg/L)	163.65±79.96	143.43±41.05	176.55±73.37	153.30±52.53
$N\text{-}NH_4$ (mg/L)	0.05±0.04	0.30±0.15	0.20±0.11	0.28±0.17

The experiment was performed in triplicate with a total number of 684 Nile tilapia juveniles, with an initial average weight of 1.81±0.01 g/fish. The fish were randomly distributed in 12 rearing units. Fish were fed with NUTRA PRO ”0” pelleted feed, with 54% crude protein. The feed biochemical composition is presented in Table 2. Fish were fed five times per day with a daily ration of 10% of fish body weight until 16.11.2012 and 5% until 7.12.2012.
The experimental variants were: V1–control, V2–1% sea buckthorn (*Hippophae rhamnoides*)/kg feed, V3–500mg vitamin E/kg feed and V4–1% sea buckthorn mixed with 500mg vitamin E/kg feed. The introduction of phytobiotic and vitamin E in fish feed was presented in paper of Plăcintă et al., 2012 [16].
At the end of the experiment, fish were weighed, measured and the following growth performance indicators were calculated: biomass growth (BG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER).
To quantify the oxidative stress was determined:

- lipid peroxidation (malondialdehyde-MDA nmol/ml) performed in accordance with Draper and Hadley (1990) method, at an optical density of 532 nm [17];
- total antioxidant capacity (TAC mM Trolox) using the ABTS-(2,2-azinobis 3-ethylbenzothiazoline-6sulphonic acid) in accordance with the method described by Re et al. (1999), at an optical density of 734 nm [18].

These analyzes were measured using the spectrophotometer SPECORD 210 Analytikjena.

MDA and TAC were determined from muscle tissue, liver and gut. Prior to sampling, fish were anesthetized with 2-phenoxyethanol.

Table 2. The biochemical composition of NUTRA PRO "0" pelleted feed

Composition	Quantity
Crude Protein %	54
Crude Fat %	18
Crude Fibre %	1
Crude Ash %	8.5
Na %	0.9
Ca %	2
P %	1.5
Vitamin A UI/kg	7500
Vitamin D3 UI/kg	1500
I (Potassium iodide) mg/kg	3
Zn (Zinc sulphate monohydrate) mg/kg	135
Mn (Manganous sulphate monohydrate) mg/kg	23
Fe (Ferrous sulphate heptahydrate) mg/kg	60
Cu (Cupric sulfate pentahydrate) mg/kg	8

Ingredients: fish meal, wheat gluten, fish oil, wheat, gluten corn,"marine zooplankton" food, peas protein, soy beans concentrate

The data were statistically analyzed using SPSS Statistics (17.0) program. Under this program was applied descriptive statistics, Post Hoc analysis for multiple comparisons (more precisely Duncan test), Levene test for homogeneity of variance, for as parametric test was used One-Way ANOVA test and as non-parametric test was used Kruskal-Wallis test (this is a correspondent of ANOVA one-way test when applying parametric test). The boxplots were performed in SPSS Statistics (17.0) and the graphs which represents total length-body weight regression have been made in Microsoft Excel (Office 2010).

3. Results and discussion

Growth performance

Technological performance indicators, obtained at the end of the experiment, are shown in Table 3. At the end of experiment the survival rate was 98.25% in V1, 98.83% in V2, 98.83% in V3 and 99.42% in V4.

From data analysis, a higher biomass growth can be observed in the variant V4, in which were administrated sea buckthorn in combination with vitamin E (1236.27±22.02g).

Table 3. The synthetic table regarding the technological performance indicators for Nile tilapia

Growth performance indicators	V1	V2	V3	V4
Initial numbers of fish	57±0.00	57±0.00	57±0.00	57±0.00
Final numbers of fish	56.00±0.00	56.30±0.82	56.30±0.41	56.70±0.41
Days of rearing	42	42	42	42
Initial stocking density (kg/m^3)	1.64±0.002	1.62±0.006	1.62±0.003	1.62±0.002
Final stocking density (kg/m^3)	18.09±0.41	17.40±0.31	18.78±0.61	19.32±0.34
Biomass gain (kg/m^3)	16.45±0.41	15.78±0.30	17.16±0.61	17.70±0.35
Initial mean individual weight (g/ex)	1.837±0.003	1.820±0.007	1.815±0.003	1.816±0.002
Final mean fish weight (g/ex)	20.67±0.47	19.78±0.60	21.34±0.69	21.82±0.44
Daily growth rate (g/day)	25.07±0.63	24.05±0.46	26.15±0.94	26.97±0.53
Specific growth rate SGR (%/day)	5.72±0.06	5.65±0.04	5.84±0.08	5.90±0.04
Feed conversion ratio FCR (g/g)	0.569±0.009	0.577±0.012	0.541±0.014	0.543±0.006
Protein efficiency ratio PER (g/g)	3.26±0.05	3.21±0.07	3.42±0.09	3.41±0.04

Note: The results are presented as mean±standard deviation per variant

Regarding the individual average weight between growth units of the same variants were not registered significant differences, Duncan test grouping the results in only a subset of data. Thus, in accordance with ANOVA test p values were

0.56, 0.37, 0.21 and 0.64 for the variant V1, V2, V3, respectively V4.

But between the four experimental variants have met significant differences ($p<0.05$; $p=0.024$) in the individual average weight.

Ducan test divided in two subsets of data the average of the obtained values from the experimental variants. The first subset includes average weight from V2 and V1 variants and the second subset includes the average weight from V1, V3 and V4 variants. This shows that the individual average weight from V2 variant was significantly lower ($p<0.05$; $p=0.024$) compared with V3 and V4 variants. Post-hoc testing was performed after Levene pre-testing which confirmed that values presents homogeneous variances ($p>0.05$; $p=0.205$).

In Figure 1 are graphically presented in the form of box plots, the values of individual average weight and total length obtained in the experimental variants at the end of the research.

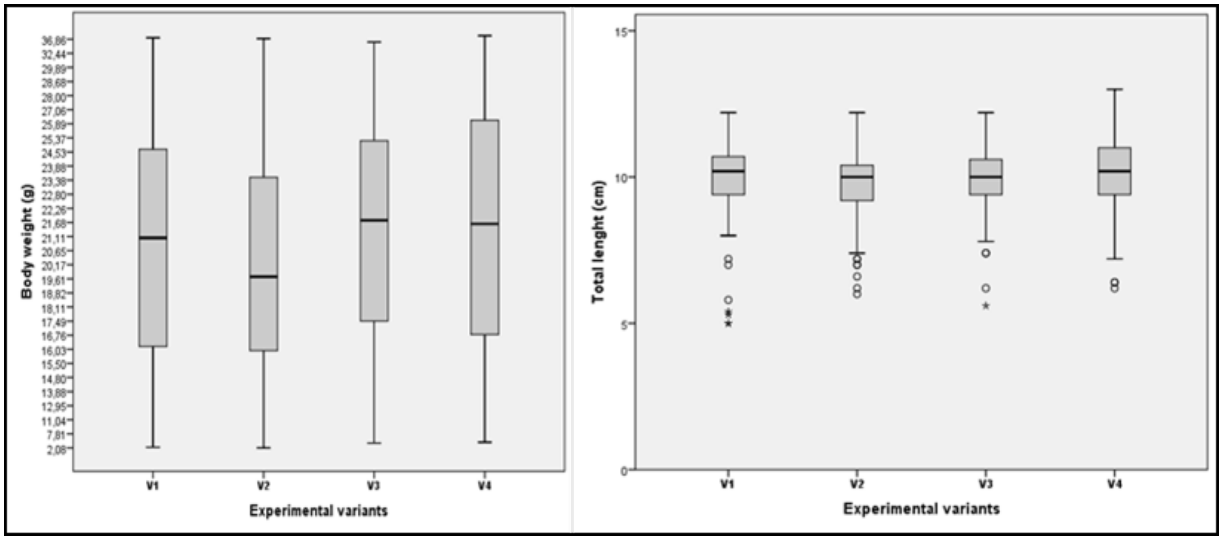

Figure 1. Variation of final body weight and total length in experimental variants-boxplot (median, minimum, maximum and quartiles)

In Figure 2 are presented the power regression of the individual weight and total length of the experimental variants, we can notice slight differences regarding the allometric factor, respectively the condition of the exemplars from the experimental variants.

The allometric coefficient "b" of the regression revealed a better condition of fish from variant V2 (b=3.0418), followed by V1 (b=3.0217), V4 (b=2.9362) and V3 (b=2.8389).

In terms of growth performance, the most suggestive technological indicators are SGR and FCR. From table 3 it can be seen that the best value of SGR was recorded in V4 and was 5.90±0.04 %/day, and the lowest was 5.65±0.04 %/day, obtained in V2. SGR value from V4 is significantly different ($p<0.05$; $p=0.021$) than those obtained in V2 and V1. The best values of FCR was registered in variant in which was administered vitamin E (V3) and in which was administered sea buckthorn in combination with vitamin E (V4), these being significantly different compared to value obtained in V2 ($p<0.05$; $p=0.048$). Protein efficiency coefficient has increased with 4.91% and 4.60% in V3, respectively V4, and decreased with 1.53% in V2 compared to V1. The changes were not statistically significant ($p>0.05$; $p=0.05$).

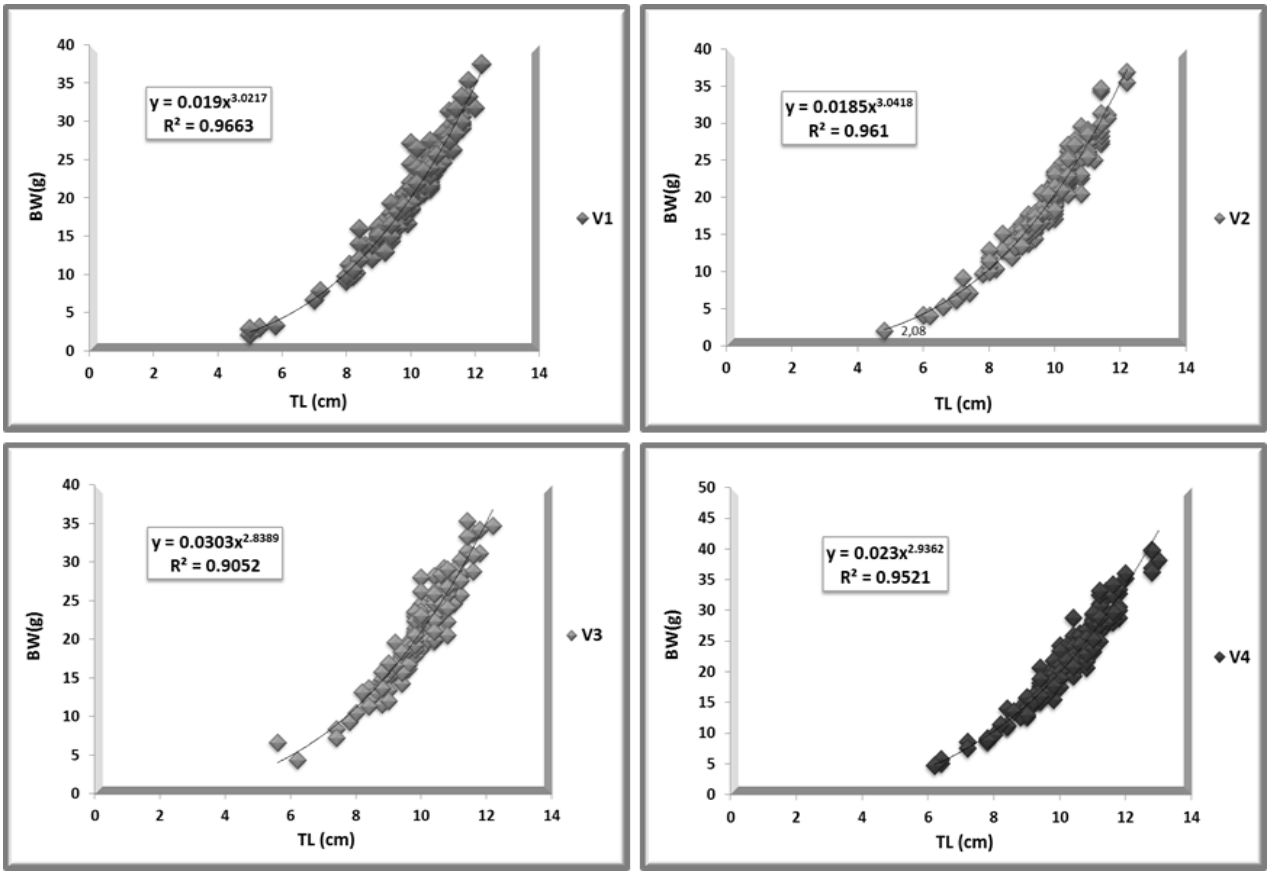

Figure 2. Regression total length–body weight at Nile tilapia in terms of sea buckthorn, vitamin E and sea buckthorn with vitamin E administration in feed

Oxidative stress

Oxidative stress analysis was performed by determining MDA and TAC from liver, muscle tissue and gut. The results are transposed in Table 4. After statistical analysis of the results, Levene test showed that the variance of values from experimental variants are not homogeneous in case of MDA values obtained in gut ($p<0.05$; $p=0.016$) and liver ($p<0.05$; $p=0.037$), and in case of TAC values obtained in liver ($p<0.05$; $p=0.003$).

Table 4. The synthetic table regarding the obtained values of MDA and TAC from liver, muscle tissue and gut at Nile tilapia

Parameter analyzed / experimental variants		V1	V2	V3	V4
MDA (nmol/mL)	liver[b]	8.62±3.42	9.10±2.45	5.42±0.50	6.47±2.07
	muscle tissue[a]	8.44±1.11	8.10±1.54	6.24±1.40	4.33±0.36
	gut[a]	12.66±4.46	7.73±1.78	6.29±1.06	7.10±1.60
TAC (mM Trolox)	liver[b]	13.37±4.65	13.44±3.28	12.13±4.12	14.83±0.52
	muscle tissue[a]	17.68±1.25	14.18±2.33	14.43±1.55	13.97±1.23
	gut[a]	21.87±1.40	17.88±2.54	13.57±0.58	18.52±2.05

"a"-significant differences between experimental variants ($p<0.05$)
"b"-insignificant differences between experimental variants ($p>0.05$)

Regarding the MDA, between the experimental variants significant differences were recorded only in gut ($p<0.05$; $p=0.025$) and muscle tissue ($p<0.05$, $p=0.000$).

TAC concentration was registered significantly differences only in gut ($p<0.05$; $p=0.000$) and muscle tissue ($p<0.05$; $p=0.011$). The highest concentration was recorded in control variant

(V1), 21.87±1.40 mM Trolox, respectively 17.68±1.25 mM Trolox.

At the end of the experiment we can say that the administration of sea buckthorn in combination with vitamin E led to a significant reduction of MDA in muscle tissue and insignificant ($p>0.05$; $p=0.056$) in liver and a slight increase of TAC from liver ($p>0.05$; $p=0.678$). At the same time, it was observed that in V2, V3 and V4 variants were obtained better values of malondialdehyde concentration and it was found a reduction in total antioxidant capacity concentration in muscle tissue and gut compared to the control.

MDA increase is due to formation of free radicals, which initiate chain reactions by forming direct or indirect links with other cellular molecules (nucleic acids, proteins, lipids and carbohydrates), thereby reducing the cellular processes that can culminate with a significant deterioration of cells and even more, to their full destruction [19, 20].

Reducing the occurrence of oxidative stress was performed by obtaining a small values of malondialdehyde in muscle tissue, liver and gut, and an increased of total antioxidant capacity in liver.

From the results obtained we can observe a synergistic influence of sea buckthorn and vitamin E (V4) on growth performance and oxidative stress in juvenile of Nile tilapia.

A number of studies reported the improved of growth performance, immune responses, nutrient digestibility, disease resistance in many fish species as well as terrestrial animals by feeding with higher levels of dietary vitamin E [21-23].

Csep and Bud, 2010 have shown at *Cyprinus carpio* species the potential of sea buckthorn administration on growth performance and welfare [24].

Same thing we have obtained in the experiment in which we administered sea buckthorn but and two other phytobiotics (rosemary and ginger) in a concentration of 1% to *Oreochromis niloticus* species, with an initial average weight of 280.07±54.03 g/fish on growth performance [25] and on oxidative stress [26].

4. Conclusions

This research has shown that the administration of sea buckthorn, vitamin E and sea buckthorn in combination with vitamin E in diets of Nile tilapia juveniles led to changes in growth performance indicator, malondialdehyde concentration and total antioxidant capacity.

In conclusion, we can say that:

- based on the results of FCR, DGR, PER and MDA, from liver, muscle tissue and gut, can be observed the positive influence of vitamin E (500mg/kg feed) administration in Nile tilapia feed;
- the administration of sea buckthorn (1%/kg feed) in combination with vitamin E (500mg/kg feed) led to obtaining a much better growth performance indicators compared to the other experimental variants;
- administration of sea buckthorn in combination with vitamin E led to a reduced occurrence of oxidative stress by decreased values of malondialdehyde in muscle tissue and liver, towards variant that received only sea buckthorn, control variant and even to the variant that received only vitamin E;
- sea buckthorn administration led to obtaining a higher concentration of total antioxidant capacity, in liver and gut, than vitamin E administration.

In conclusion, this experiment reveals that the sea buckthorn in combination with vitamin E shows a synergistic effect leading to an improvement in growth performance and to a reduction of oxidative stress at *Oreochromis niloticus* juveniles.

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