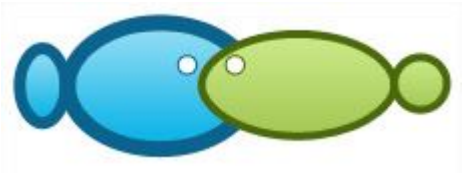

# Effects of some phytobiotics on oxidative stress in *Oreochromis niloticus* reared in a recirculating aquaculture system

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**Abstract**. The aim of this research was to determine the influence of some phytobiotics on oxidative stress at *Oreochromis niloticus* species reared in a recirculating aquaculture system. The experiment was made during fourteen weeks and the experimental variants were: V1 – control, V2 – 1% rosemary (*Rosmarinus officinalis*)/kg feed, V3 – 1% sea buckthorn (*Hippophae rhamnoides*)/kg feed and V4 – 1% ginger (*Zingiber officinale*)/kg feed. Analysis of oxidative stress was performed by measuring the following indicators: reduced glutathione (GSH) – from blood, lipid peroxidation (malondialdehyde – MDA) and total antioxidant capacity (TAC) - from plasma, muscle tissue, liver and gut. The analyses were performed at the beginning and at the end of the experiment. Lipid peroxidation registered a significant increase ($p < 0.05$, $p = 0.004$) in blood plasma in V3 and V4 variants, and a significant reduction ($p < 0.05$, $p = 0.018$) in liver in V3 variant. In terms of total antioxidant capacity, there was an insignificant reduction ($p > 0.05$) in blood plasma ($p = 0.721$) and liver ($p = 0.615$) and a significant reduction ($p < 0.05$) in muscle tissue ($p = 0.023$) and gut ($p = 0.022$), mostly in variant V3, respectively in V4. Results of reduced glutathione from blood showed an insignificant increase ($p > 0.05$, $p = 0.652$) in variants V2 and V3. In conclusion, based on the results from lipid peroxidation analysis, we can say that oxidative stress was reduced after administration of sea buckthorn and ginger in feed of *O. niloticus* species.

**Key Words**: aquaculture, *Hippophae rhamnoides*, *Oreochromis niloticus*, *Rosmarinus officinalis*, *Zingiber officinale*.

**Introduction**. Oxidative stress is defined as a situation when steady-state reactive oxygen species (ROS) concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak 2011).

This is a harmful condition in which increases in free radical production, and/or decreases in antioxidant levels can lead to potential damage. Indicators of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products. Oxidative stress is known to play a large role in the pathology of several diseases (Thannickal & Fanburg 2000).

Reactive oxygen species (ROS) are continually produced in animals during normal aerobic metabolism that can damage most cellular components leading to cell death (Livingstone 2001). Fish, like other vertebrates, possess an antioxidant defense system to reduce the negative effects of ROS. Fish, like all aerobic organisms, are susceptible to ROS (Trenzado et al 2006). However, most of data about oxidative defense in fish are focused on natural and anthropogenic toxicological aspects, especially environmental factors and xenobiotics (Winston & Di Giulio 1991).

In different studies, the effect of different diets on fish antioxidant defense had been assayed (Huang et al 2010; Chen et al 2013). Since 1993 many interesting tests have been proposed in order to measure the total antioxidant capacity (TAC) of a biological sample (blood, saliva, urine, feces), food or vegetable extract or of living tissues and organs (Cao et al 1993; Miller et al 1993; Benzie & Strain 1996; Ferrari 2000).

There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional incidence and their role in health and disease (Pieroni et al 2002; Couladis et al 2003).

Phytobiotics possess a lot of phytochemical constituents with antioxidant activities, including phenolic compounds and carotenoids (Huang et al 2010; Awah et al 2012) which have antioxidant properties such as chain breaking antioxidants. Intake of carotenoids has shown a significant reduction in the risk of several chronic and degenerative diseases (Rao & Rao 2007). Phenolic compounds are usually found in medicinal plants and food products and mainly consisted of phenolic acids, flavonoids and tannins. These compounds have a wide range of antioxidant activities (Martins et al 2011; Qin et al 2011).

Fish is an important aquatic organism. Fish products are an important source of protein for human consumption (Duran & Talas 2009). Aquatic organisms can provide model systems for investigation of how ROS damage cellular compounds, how cells respond, how repairing mechanisms reverse this damage, and how oxidative stress can lead to disease.

The purpose of this study was to determine the influence of rosemary (*Rosmarinus officinalis*), sea buckthorn (*Hippophae rhamnoides*) and ginger (*Zingiber officinale*) on oxidative stress in Nile tilapia, *Oreochromis niloticu*s species reared in a recirculating aquaculture system.

## Material and Method

***Experimental design***. The research was performed during fourteen weeks in a recirculating aquaculture system at Aquaculture, Environmental Science and Cadastre Department, from "Dunarea de Jos" University of Galati. The recirculating system design includes four rearing units, with a volume of 1 $m^3$ each, and a series of water quality conditioning units (mechanical and biological filters) (Cristea et al 2002).

During the experiment, the feed was supplemented with three phytobiotics: rosemary, sea buckthorn and ginger, in a concentration of 1% phytobiotic/kg feed. Thus, the experimental variants were: V1 – control with no phytobiotic, V2 – 1% rosemary/kg feed, V3 – 1% sea buckthorn/kg feed and V4 – 1% ginger/kg feed. The phytobiotics were purchased from a local Plafar market and the introduction in feed was performed using an aqueous solution of gelatin with 2% concentration. The feed was sprayed, mixed and then dried at 25ºC.

A total number of 168 Nile tilapia individuals, were randomly distributed in four rearing units. The initial average weight was 280.40±57.53 g $fish^{-1}$ in V1, 279.83±55.58 g $fish^{-1}$ in V2, 280.05±48.13 g $fish^{-1}$ in V3, and 279.98±52.51 g $fish^{-1}$ in V4. Fish were fed four times per day (09:00, 12:00, 15:00, 18:00), with a daily ration of 2% body weight, with SOPROFISH pelleted feed (fish meal, soybean protein content, corn, wheat), with 38% crude protein. The biochemical composition of feed is presented in Table 1.

Table 1

The biochemical composition of SOPROFISH 38/7 pelleted feed (Antache et al 2013)

| *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 $kg^{-1}$) | 15000 |
| Vitamin D (IU $kg^{-1}$) | 2500 |
| Vitamin E (mg $kg^{-1}$) | 90 |
| Vitamin C (mg $kg^{-1}$) | 200 |
| Lysine (%) | 2.3 |
| Methionine+Cysteine (%) | 1.2 |

***Oxidative stress analyses***. To quantify the oxidative stress, the following parameters were determined:

- lipid peroxidation (malondialdehyde-MDA nmol $mL^{-1}$) was performed in accordance with Draper & Hadley (1990) method, at an optical density of 532 nm;
- total antioxidant capacity (TAC mM Trolox) using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) in accordance with the method described by Re et al (1999), at an optical density of 734 nm;
- reduced glutathione (GSH µmol $dL^{-1}$) was determined in accordance with Ellman (1959).

MDA, TAC and GSH were measured using the spectrophotometer SPECORD 210 Analytikjena, at an optical density of 532 nm, 734 nm, respectively 490 nm.

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

***Statistical analysis***. The results were statistically analyzed using descriptive statistics and One Way ANOVA test (Tukey – Duncan, significance level $p \leq 0.05$). Programs used were Microsoft Excell 2010 and IBM SPSS Statistics 20.0. The results were presented as minimum, maximum and mean±standard deviation.

## Results and Discusions

### *Lipid peroxidation*

*Blood plasma*. MDA concentration has recorded significant differences ($p < 0.05$) between the experimental variants at the level of blood plasma. The results obtained in variants in which the diet was supplemented with sea buckthorn (V3) and ginger (V4) were significantly higher ($p = 0.004$) than the results obtained in the control variant (V1) and in variant in which the diet was supplemented with rosemary (V2) (Figure 1). Thus, compared to V1, the MDA concentration increased with 29.97% in V3, 27.71% in V4, and decreased with 11.21% in V2.

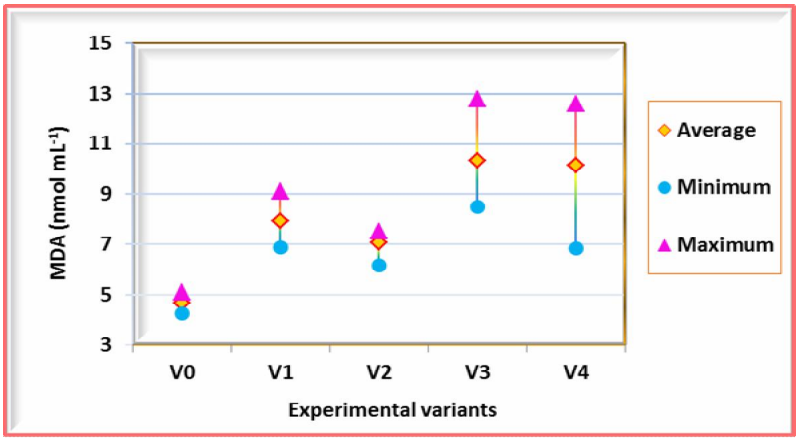


Figure 1. The results of MDA concentration obtained from blood plasma (V0 – values obtained at beginning of the experiment).

In the end of the experiment the MDA concentration from V3 and V4 was significantly higher ($p < 0.05$, $p = 0.000$) than the value recorded at the beginning of the experiment (4.67±0.41 nmol $mL^{-1}$).

*Liver*. After fourteen weeks of phytobiotics administration, a significant reduction ($p < 0.05$, $p = 0.018$) of lipid peroxidation was observed at the level of liver versus the control variant. The greatest effect was observed in V3, then in V2 and in V4 variants (Figure 2). The reduction was of 39.20% (V3), 32.58% (V2), and 24.56% (V4) compared with control variant (V1 - 5.74±0.38 nmol $mL^{-1}$).

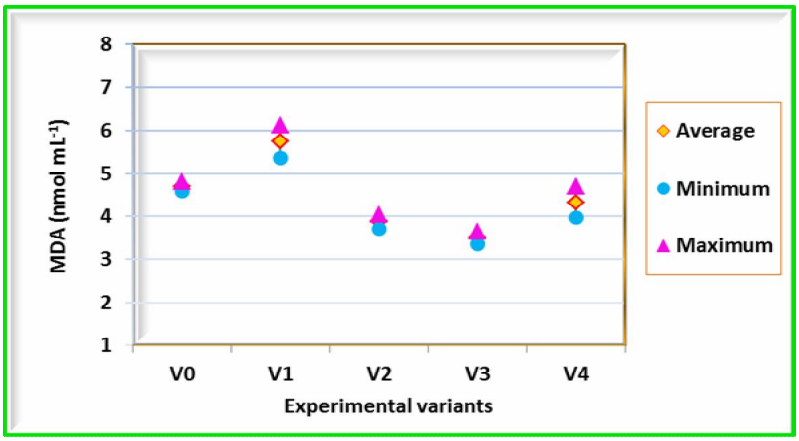


Figure 2. The results of MDA concentration obtained from liver (V0 – values obtained at beginning of the experiment).

The values recorded in variants in which the diet was supplemented with phytobiotics were significantly lower ($p < 0.05$; $p = 0.009$) compared to the value recorded at the beginning of the experiment (4.69±0.11 nmol $mL^{-1}$).

The lowest value of MDA concentration was registered in V3 variant (3.49±0.15 nmol $mL^{-1}$) in which the diet was supplemented with sea buckthorn. Composition of sea buckthorn range of bioactive substances has led the development of interest on this plant, finding always new and interesting effects (Brad et al 2002). It should be noted that all parts of sea-buckthorn are a rich source of bioactive components, their highest concentration being found in fruit (vitamins A, C, E, K, carotenoids, organic acids etc.) (Yang & Kallio 2001).

Also, Csep et al (2010) have shown at *Cyprinus carpio* species the potential of sea buckthorn administration on welfare status.

*Tissue*. Determination of malondialdehyde concentration in muscle tissue revealed a significant reduction ($p < 0.05$; $p = 0.036$) of the results obtained after fourteen weeks of experiment (V1 - 4.21±0.59 nmol $mL^{-1}$, V2 - 3.86±0.47 nmol $mL^{-1}$, V3 - 3.82±0.40 nmol $mL^{-1}$, V4 - 3.27±0.05 nmol $mL^{-1}$) versus those recorded at the beginning of the experiment (5.83±0.93 nmol $mL^{-1}$).

In the end of the experiment, it was registered a reduction of the malondialdehyde concentration in variants in which phytobiotics were administered with 8.31% in V2, 9.26% in V3, respectively with 22.33% V4 compared with V1 (4.21±0.59 nmol $dL^{-1}$), but insignificant from a statistical point of view ($p > 0.05$, $p = 0.547$). Results are presented in Figure 3.

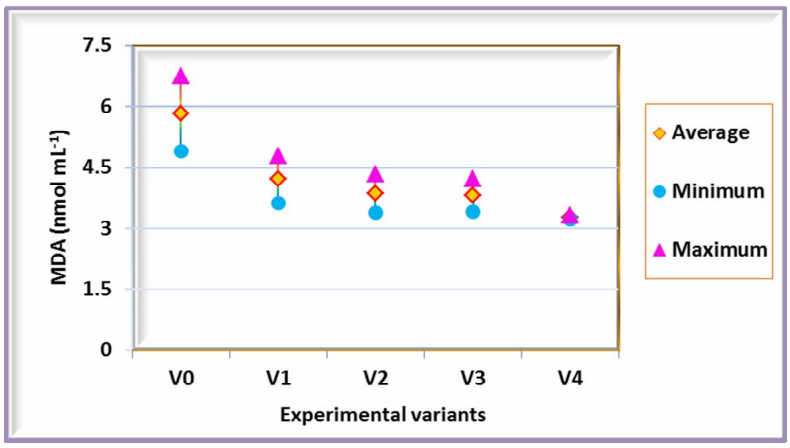


Figure 3. The results of MDA concentration obtained from muscular tissue (V0 – values obtained at beginning of the experiment).

*Gut*. The lipid peroxidation process recorded a reduction in variants with phytobiotics after fourteen weeks of experiment. The results registered (V1 - 4.91±1.39 nmol $mL^{-1}$, V2 - 4.04±0.06 nmol $mL^{-1}$, V3 - 3.28±0.33 nmol $mL^{-1}$, V4 - 4.53±0.76 nmol $mL^{-1}$) are presented in Figure 4, and they were significantly lower ($p < 0.05$, $p = 0.005$) than those recorded at the beginning of the experiment (6.37±0.80 nmol $mL^{-1}$).

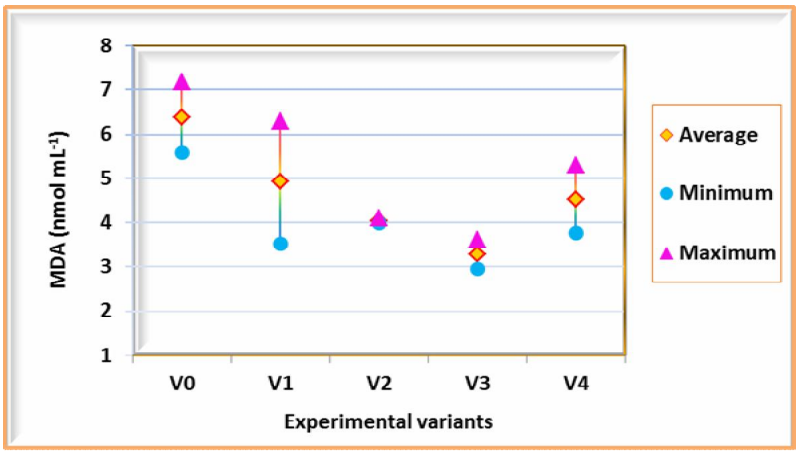


Figure 4. The results of MDA concentration obtained from gut (V0 – values obtained at beginning of the experiment).

With the continuation of the experimental period it can be observed a reduction of the lipid peroxidation process in the liver, muscle and gut in variants in which the diet were supplemented with phytobiotics.

***Total antioxidant capacity***

*Blood plasma*. After fourteen weeks of experiment it was registered a decrease of TAC with 8.75% in V2; 16.97% in V3 and 9.53% in V4 compared with control (V1 – 7.66±1.46 mM Trolox), but insignificant from statistical point of view ($p > 0.05$, $p = 0.721$) (Figure 5).

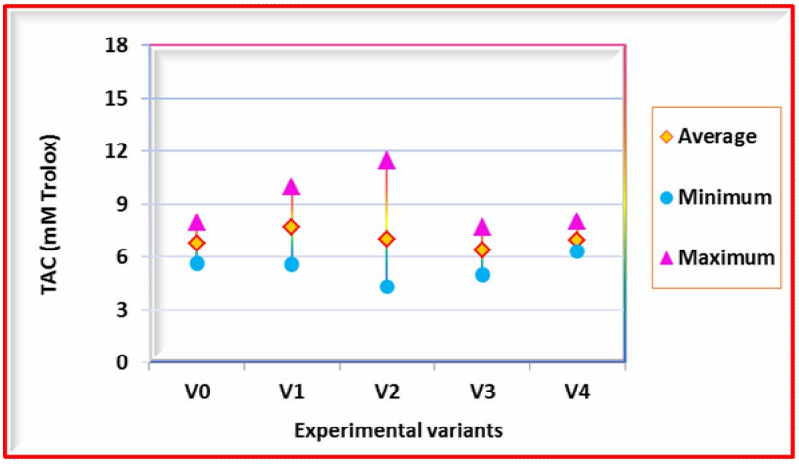


Figure 5. The results of TAC concentration obtained from blood plasma (V0 – values obtained at beginning of the experiment).

It is known that malondialdehyde concentration values are indirectly proportional with total antioxidant capacity concentration (Metwally 2009), this also being observed in our experiment.

This process can be explained by the fact that there may be a compensatory mechanisms at the level of body to overcome lipid peroxidation (MDA concentration) by

in vivo growth of antioxidants that can maintain the oxidant/antioxidant ratio within normal limits without the appearance of oxidative stress (Suresh et al 2010).

*Liver*. After fourteen weeks of experiment, a reduction of total antioxidant capacity concentration was observed in variants in which phytobiotics were administered (with 20.77% in V2, 21.48% in V3, 10.83% in V4) compared with control (Figure 6). However, the differences between the experimental variants were not significant ($p > 0.05$, $p = 0.615$), nor from the value obtained at the beginning of the experiment ($p > 0.05$, $p = 0.228$).

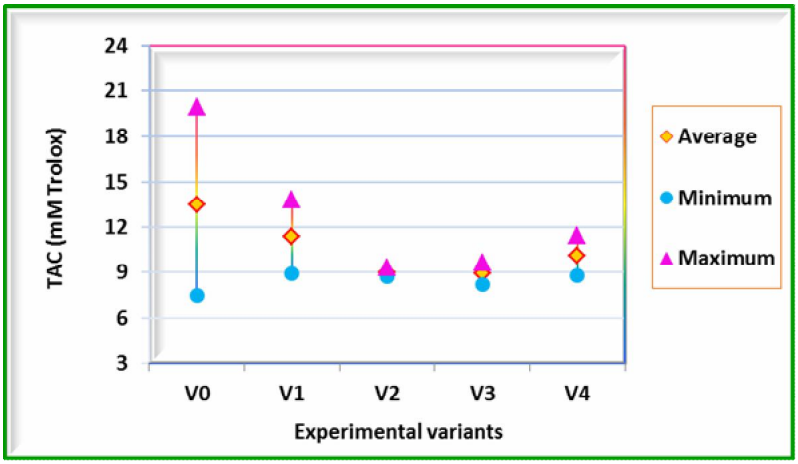


Figure 6. The results of TAC concentration obtained from liver (V0 – values obtained at beginning of the experiment).

*Tissue*. After fourteen weeks, a significant reduction ($p < 0.05$, $p = 0.023$) of total antioxidant capacity concentration was obtained in variants in which phytobiotics were administered (Figure 7). Compared to the control (19.09±0.32 mM Trolox) there was a reduction with 20.20% in V2, 30.03% in V3 and 25.69% in V4.

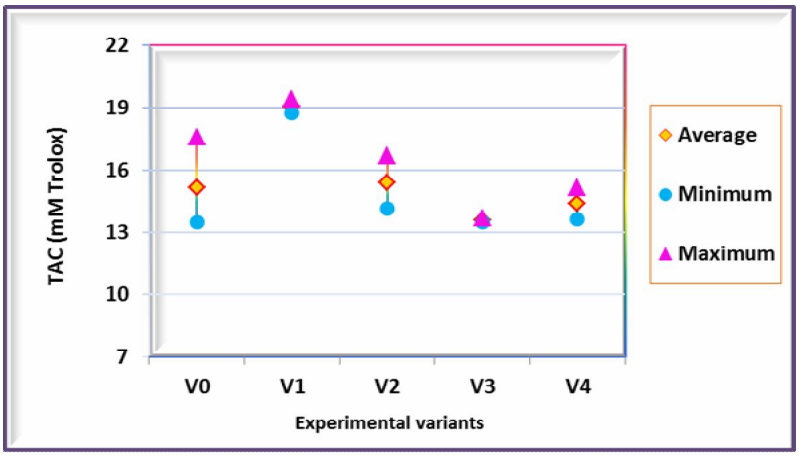


Figure 7. The results of TAC concentration obtained from muscular tissue (V0 – values obtained at beginning of the experiment).

*Gut*. At the end of the fourteen weeks of the experiment at the level of gut, as well as the muscle tissue, was registered a significant reduction ($p < 0.05$, $p = 0.022$) of TAC concentration in variants in which phytobiotics were administered compared to the control. The smallest value was recorded in V3 (17.36±0.78 mM Trolox) (Figure 8). At the same time significant differences ($p < 0.05$, $p = 0.013$) were also observed against the value obtained at the beginning of the experiment (22.46±3.02 mM Trolox).

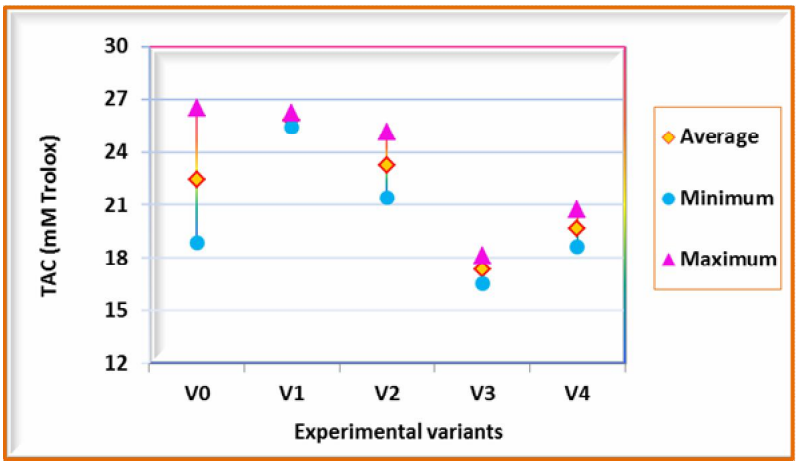


Figure 8. The results of TAC concentration obtained from gut
(V0 – values obtained at beginning of the experiment).

***Reduced glutathione***. Another analysis performed was reduced glutathione from blood (GSH - µmol $dL^{-1}$). During the experiment, there was an increase in the reduced glutathione concentration in variants in which the fish diet was supplemented with phytobiotics (Figure 9).

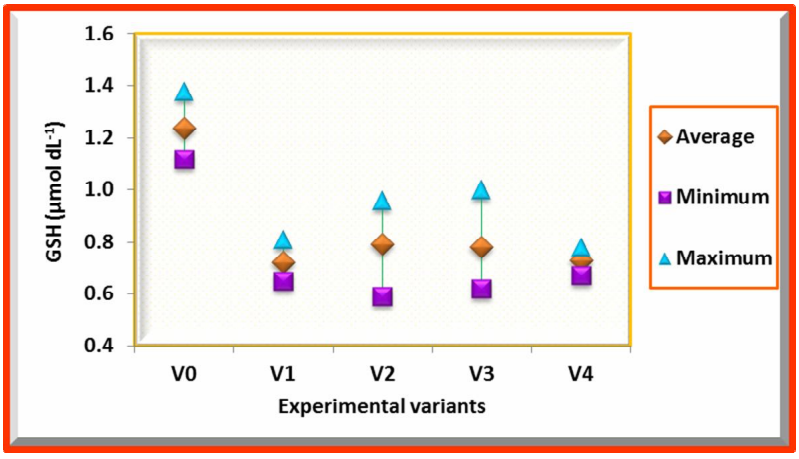


Figure 9. The results of reduced glutathione
(V0 – values obtained at beginning of the experiment).

After fourteen weeks of the experiment the increase of reduced glutathione concentration was no significant ($p > 0.05$, $p = 0.652$) in variants in which phytobiotics were administered (Figure 9). The highest values were recorded V2 (0.79±0.013 µmol $dL^{-1}$) and V3 (0.78±0.013 µmol $dL^{-1}$).

Research has shown that exposure of fish to various stressors leads to reduced glutathione concentration and also reduces the enzymatic activity of reduced glutathione (Wu et al 2004).

Todoran (2015) reported that sea buckthorn flour has proven to be most effective, followed by rosehip (*Rosa canina*) lour, both bringing production increases and increasing the percentage of survival in case of trout (*Salvelinus fontinalis*) growth in high densities.

**Conclusions**. This research has shown that the supplementation of Nile tilapia feed with rosemary, sea buckthorn and ginger in 1% concentration led to changes in malondialdehyde concentration, total antioxidant capacity and reduced glutathione from various tissues. Thus we can say that:

- sea buckthorn administration in fish feed led to a significant reduction of the MDA concentration in the liver;

- phytobiotics administered in fish feed led to a significant reduction of total antioxidant capacity concentration at the level of intestine and muscle tissue;

- the dietary supplementation with phytobiotics, especially rosemary and sea buckthorn, has led to an increase in the concentration of reduced glutathione and implicitly to the reduction of oxidative stress.

Based on the results obtained for the malondialdehyde concentration and the total antioxidant capacity of the different studied tissues, we can once again emphasize the effectiveness of the phytobiotics administration in fish diet on the reduction of the oxidative stress.

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