The Influence of Rosemary, Sea Buckthorn and Ginger on Oxidative Stress at *Oreochromis niloticus* Reared in a Recirculating Aquaculture System

Alina ANTACHE, Victor CRISTEA, Iulia R. GRECU, Sândiţa (PLĂCINTĂ) ION, Mirela (CREŢU) MOCANU

Department of Aquaculture, Environmental Science and Cadastre, "Dunărea de Jos" University of Galati, Romania; antache_alina@yahoo.com

Abstract. The purpose of this study was to determine the influence of some phytobiotics on oxidative stress at *Oreochromis niloticus* reared in a recirculating aquaculture system. 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), lipid peroxidation (malondialdehyde-MDA) and total antioxidant capacity (TAC). The analyses were measured using the spectrophotometer SPECORD 210 Analytikjena, at the beginning and at the end of the experiment from the blood, plasma, muscle tissue, liver and gut. Regarding lipid peroxidation significant differences (p<0.05), in statistical terms, were recorded in plasma and gut level. Concerning the total antioxidant capacity significant differences (p<0.05) were recorded in plasma. Results obtained in determination of reduced glutathione from blood showed between initial and final experimental variants significant differences (p<0.05). At the end of experiment, it was observed a correlation between MDA and TAC in the gut level. In conclusion, based on the results from lipid peroxidation analysis, we can say that oxidative stress was reduced after administration of sea buckthorn in feed of *Oreochromis niloticus* species.

Keywords: phytobiotic, oxidative stress, *Oreochromis niloticus*, recirculating aquaculture system

INTRODUCTION

To prevent oxidation and damage at cellular level, the body has developed an antioxidant defense system against free radicals. The phytobiotics were introduced in fish diet for ensuring the welfare status due to its antioxidant properties.

Recent researches had shown that the introduction of relatively small concentrations of vitamins, probiotics, prebiotics and newest phytobiotics in animal diet lead to ensuring some specific demands and/or to influencing in a positive way (direct or indirect) the growth performances and the welfare status (Antache et al., 2012; Denev, 2008; Maurillio, 2011; Pop, 2006).

Due to various stress factors exert on fish from aquaculture, there are trying by different methods to improve or suppress the effects on fish welfare. In recent years was proven the beneficial effect on health status of phytobiotics introduced in fish diet. Newest, their effect can be investigated by analyzing the oxidative stress.

In 1985, Sies defined oxidative stress expression as an alteration of the balance between pro-oxidants versus antioxidants, but Jones in 2006 redefined oxidative stress as "a disruption of redox signaling control".

Halliwell and Gutterdige (2007) have characterized the oxidative stress as an oxidative activity or a rapid production of ROS quantities, like superoxide radical, hydrogen...
peroxide, hydroxyl radical, singled oxygen and hydroxy peroxide radical, for this reason plays an important role in the pathogenesis of many diseases.

Determination of oxidative stress is achieved through a series of sensitive and reliable biomarkers. They can provide information on the health of organisms and can be used as early warning signals for general or particular stress (Korte et al., 2000). Doherty et al. (2010) shows that cellular biomarkers represent early diagnostic tools since they can identify changes at sub-organism level (cellular, molecular, etc.) before becoming evident at higher level of biological organization. Quantification of oxidative stress at fish it is made among others by analyzing lipid peroxidation (malondialdehyde-MDA), total antioxidant capacity (TAC) and reduced glutathione (GSH) (Antache et al., 2013; Lupoaie et al., 2011).

The purpose of this study was to determine the influence of some phytobiotics on oxidative stress at *Oreochromis niloticus* reared in a recirculating aquaculture system.

**MATERIALS AND METHODS**

The experiment was made during six weeks, starting from 17.08.2012 to 28.09.2012, in pilot recirculating system, of Aquaculture, Environmental Science and Cadastre Department, from “Dunarea de Jos” University of Galati.

A total number of 168 pieces of *Nile tilapia*, with an initial average weight of 280.07±54.03 g/fish, were randomly distributed in 4 rearing units. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein. The feed biochemical composition and the recirculating system design were presented in the paper of Antache et al. (2013). Fish were fed four times per day (09:00, 12:00, 15:00, 18:00) with a daily ration of 2 % of fish body weight.

The phytobiotics used in this experiment were rosemary, sea buckthorn and ginger, as a powder, and were purchased from a Plafar market. The introduction of phytobiotics in feed was performed using an aqueous solution of gelatin with 2% concentration. The feed was sprayed, mixed and then dried at 25ºC. The experimental variants were organized as follows: 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.

To quantify the oxidative stress, lipid peroxidation was determined by the concentration of malondialdehyde (MDA nmol/ml) performed in accordance with Draper and Hadley (1990) method, total antioxidant capacity (TAC % inhibition) using the ABTS-(2,2-azinobis 3-ethylbenzothiazoline-6sulphonic acid) in accordance with the method described by Re and Van Den Berg (1999) and reduced glutathione (GSH µmol/dl) determined in accordance with Ellman method, modified by Seldak and Sindsary (1959). MDA, TAC and GSH were measured using the spectrophotometer SPECORD 210 Analytikjena, at an optical density of 532 nm, 734 nm and respectively 490 nm.

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

The data were statistically analyzed using descriptive statistics and ANOVA single factor test. The results are presented as mean±standard error.

**RESULTS AND DISCUSSIONS**

In recent years, measurement of oxidative damage products in aquatic organisms has received more attention (Oakes et al., 2004; Carney Almroth et al., 2005; McDonagh et al., 2005).
The generation of oxidative stress is mainly due to the existence of reactive species for some chemical elements, which presents an unpaired electron. Such were identified reactive oxygen species (ROS), reactive nitrogen species (SRN) and, more recently, reactive sulfur species (SRS) with important roles in physiological and pathological processes.

The malondialdehyde (MDA) formation often assayed with the thiobarbituric acid assay is the most widely used index of lipid peroxidation. Determination of lipid peroxidation plays an important role because it is responsible for plasma membranes damage (Lupoae et al., 2011).

In our experiment, in case of blood plasma we observed a significant increase (p<0.05, p=0.004) of MDA concentration, in all experimental variants, at the middle of the experiment compared to the initial moment (Fig. 1).

![Fig. 1. Analysis of malondialdehyde from blood plasma at Oreochromis niloticus species](image)

From the four experimental variants the highest value of lipid peroxidation was obtained in variant, which received ginger (V4), but from statistical viewpoint it was insignificant (p<0.05; p=0.115). At the end of the experiment was observed a decrease of malondialdehyde concentration in V3 variant compared to the other experimental variants, but in terms of statistics was not a significant difference (p<0.05; p=0.099).

The obtained results show that the administration of sea buckthorn in Nile tilapia diet led to a decrease in lipid peroxidation at the level of blood plasma. Results on malondialdehyde (MDA) dynamics from the muscle, liver and gut are presented in Table 1.

<table>
<thead>
<tr>
<th>Experimental variant</th>
<th>V0</th>
<th>V1 f.</th>
<th>V2 f.</th>
<th>V3 f.</th>
<th>V4 f.</th>
<th>p value*</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>muscle</td>
<td>5.83±0.93</td>
<td>6.20±0.39</td>
<td>7.88±0.97</td>
<td>8.06±1.92</td>
<td>9.78±0.14</td>
<td>0.22</td>
<td>0.29</td>
</tr>
<tr>
<td>liver</td>
<td>4.87±0.12</td>
<td>6.24±0.68</td>
<td>6.65±0.06</td>
<td>5.93±0.001</td>
<td>6.79±0.45</td>
<td>0.07</td>
<td>0.51</td>
</tr>
<tr>
<td>gut</td>
<td>6.37±0.80</td>
<td>7.75±0.15</td>
<td>7.24±0.19</td>
<td>6.05±0.20</td>
<td>6.98±0.25</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Note:** V0–initially; Final experimental variants: V1–control, V2–rosemary, V3–sea buckthorn, V4–ginger; 
p value is 0.05. For significant differences p<0.05: *between initial (V0) and final variants (V1, V2, V3, V4), **between final variants.
In our case, it was observed that the administration of rosemary, sea buckthorn and ginger has significantly influenced in gut the lipid peroxidation. The lowest concentration of MDA was recorded in V3 variant and the highest concentration in V1. At the muscle level the MDA values were higher in variants that received phytobiotics compared to V1 and V0, but the differences were not significant (p<0.05) from statistical point of view p=0.29, respectively p=0.22. Although, the MDA concentration in the liver was lower in V3 and there was no significant difference compared with the other experimental variants.

Total antioxidant capacity (TAC) provides information regarding the complexity of adaptive changes, produced as a response to the increasing production of free oxygen radicals (Bagnyukova et al., 2006).

The analysis of total antioxidant capacity from blood plasma showed a significant decrease (p<0.05; p=0.001) in V2, V3 and V4 variants compared to V1, after three weeks of experiment (Fig. 2).

![Fig. 2. Analysis of TAC from different tissues at Oreochromis niloticus species](image)

V0–initially; Experimental variants: V1–control, V2–rosemary, V3–sea buckthorn, V4–ginger; int–intermediary; f–final

At the end of the experiment, after six weeks, the TAC concentration increased in V2 and V4 variant and decreased in V3 variant compared with control variant, but these changes were insignificant (p<0.05; p=0.086) in statistical terms.

Obtained results concerning the total antioxidant capacity from different tissues are presented in Table 2. The table shows that the administration of rosemary, sea buckthorn and ginger did not significantly affect (p>0.05), in statistical terms, the total antioxidant capacity of the muscle, liver and gut. However, it can be seen in the gut level an increase of TAC concentration at 146.95 % in V3, 131.06 % V2, respectively 125.58 % in V4 compared to V1.

From the specialty literature, it is known that between lipid peroxidation and total antioxidant capacity there is a correlation. So while the malondialdehyde concentration increase, the concentration of total antioxidant capacity decreases and vice versa. This aspect can be observed in our results, especially in blood plasma level. At the gut level was recorded a decrease in MDA concentration and an increase in TAC concentration in variants which were administered the phytobiotics.
Analysis of TAC from different tissues, at *Oreochromis niloticus* species

<table>
<thead>
<tr>
<th>Experimental variant</th>
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<th>V3 f.</th>
<th>V4 f.</th>
<th>p value*</th>
<th>p value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>muscle</td>
<td>15.14±1.49</td>
<td>20.23±0.95</td>
<td>16.46±4.51</td>
<td>19.63±0.54</td>
<td>15.22±0.33</td>
<td>0.37</td>
<td>0.45</td>
</tr>
<tr>
<td>liver</td>
<td>13.51±3.97</td>
<td>13.14±0.89</td>
<td>14.03±0.54</td>
<td>12.40±0.029</td>
<td>15.44±0.46</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>gut</td>
<td>22.46±3.02</td>
<td>18.06±0.77</td>
<td>23.67±5.37</td>
<td>26.54±1.65</td>
<td>22.68±1.67</td>
<td>0.37</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*Note:* V0–initially; Experimental variants: V1–control, V2–rosemary, V3–sea buckthorn, V4–ginger; p value is 0.05. For significant differences p<0.05: *between initial (V0) and final variants (V1, V2, V3, V4), **between final variants.

Suresh *et al.* (2010) showed that there can be 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). In our case this situation can be observed in V3 variant, in muscle and liver, which means that the addition of sea buckthorn in feed makes the body to maintain the normal ratio between oxidants and antioxidants.

One of the most abundant and most important molecular antioxidants in cellular cytoplasm is glutathione (GSH). GSH is used as a reducing equivalent in the metabolism of reactive intermediates, for example reduction of lipid peroxides by the action of glutathione peroxidase (GPx) (Carney Almroth, 2008).

In terms of reduced glutathione, results indicated between initial and final experimental variants significant differences (p<0.05; p=0.0002). At the end of the experiment was obtained a significant increase (p<0.05; p=0.0001) in GSH concentration in V2, V3 and V4 compared to control variant. The dynamics of reduced glutathione values can be observed in *Figure 3.*

![Fig. 3. Reduced glutathione values (µmol/dl) from blood](image_url)

V0–initially; Experimental variants: V1–control, V2–rosemary, V3–sea buckthorn, V4–ginger; f–final
Several authors, among which Monteiro et al. (2009), showed that a decrease of GSH makes the fish body cells more susceptible to toxic compounds attack. Because rosemary, sea buckthorn and ginger increased the GSH concentration, we can say that the appearance of oxidative stress was prevented.

CONCLUSION

This study has shown that the administration of rosemary, sea buckthorn and ginger in Nile tilapia diets led to changes in malondialdehyde concentration, total antioxidant capacity and reduced glutathione, from various tissues.

In conclusion, we can say that:

✓ based on the results from lipid peroxidation analysis from liver, gut and blood plasma, the oxidative stress was reduced after administration of sea buckthorn in feed of Oreochromis niloticus species,

✓ administration of phytobiotics influenced total antioxidant capacity mostly in the gut and then in the blood plasma,

✓ administration of these phytobiotics led to a reduction of the oxidative stress along with increasing the reduced glutathione concentration.

However, to obtain relevant results, in terms of oxidative stress, we suggest that the administration of these phytobiotics should be done for a longer period.

REFERENCES


