THE INFLUENCE OF SOME PHYTOBIOTICS ON OXIDATIVE STRESS AT *OREOCHROMIS NILOTICUS* GROWN IN AN INTENSIVE RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The purpose of this paper was to evaluated the influence of several phytobiotics on oxidative stress, at Oreochromis niloticus species, reared in an intensive recirculating aquaculture system. The phytobiotics from this experiment were administered in feed, in a concentration of 1%/kg feed, and consist in: thyme (Thymus vulgaris) in V2, fenugreek (Trigonela foenum graecum) in V3, neem (Azadirachta indica) in V4 and V1 represented the control variant. A total number of 180 fish, with an average initial weight of 125.41±34.33 g/fish, were randomized distributed into four rearing units. The oxidative stress was determined by analysing the lipid peroxidation indice (malondialdehyde – MDA), total antioxidant capacity (TAC) and reduced glutathione (GSH). The analyses were measured spectrophotometrically in the research laboratory of Aquaculture, Environmental Science and Cadastre Department, University "Dunarea de Jos" Galati. The MDA concentration recorded the highest values at V2 – thyme (9.45 nmol/ml tissue; 7.75 nmol/ml gut). The same variant (V2) also recorded the highest value of GSH concentration (1.075 µmol/dl). Regarding TAC concentration, the lowest values was recorded at V3 - fenugreek and V4 - neem (plasma, tissue, liver, gut). In conclusion, the results of this study demonstrate that thyme is an important additives, for conferring wellfare, in commercial diets of Oreochromis niloticus.

Key words: phytobiotics, oxidative stress, *Oreochromis niloticus*, intensive recirculating aquaculture system

INTRODUCTION

Nowadays, the main aim of intensive aquaculture is to ensure the welfare and growth performance of biological material, through noninvasive techniques. As shown in recent years, research were focused on replacing antibiotics with different plant extracts (such as medical and aromatic plants), with immunostimulatory properties, that are also considered growth promoters.

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" [11].

To limit the harmful effects of oxidative stress, due to free radicals, the body has an antioxidant system composed of enzymatic antioxidants (catalase, glutathione peroxidase, superoxide dismutase, etc.) and non-enzymatic antioxidants (vitamins E, A and C, reduced glutathione and uric acid). The main free radicals are represented by reactive oxygen and nitrogen species (ROS, RNS). Antioxidants are defined as molecules capable to inhibit the oxidation of other molecules.

Oxidative stress can be characterized by an oxidative activity or a rapid production of ROS quantities, like superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen and hydroxy peroxid radical [9], so this plays an important role in the pathogenesis of many diseases like cancer, anemia, diabetes, inflammation, liver disease, heart disease, Alzheimer's, Parkinson's and HIV disease and also accelerates the aging process [7].

Quantification of oxidative stress is made, among others, by analyzing lipid peroxidation

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(MDA), total antioxidant capacity (TAC) and reduced glutathione (GSH).

Determination of lipid peroxidation plays an important role because it is responsible for plasma membranes damage [17]. Malondialdehyde is the analysis which commonly quantifies lipid peroxidation.

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 [13].

GSH is a tripeptide, composed of glutamic acid, cysteine and glycine, that has antioxidant properties. In healthy cells and tissues, reduced glutathione is found in a percentage of 90% from total glutathione. However, at fish, GSH values are relatively smaller compared to those of mammals [14].

The purpose of current research is to determine the influence of thyme, fenugreek and neem, on oxidative stress, on *Oreochromis niloticus* species, reared in an intensive aquaculture recirculating system.

MATERIAL AND METHOD

The experiment was made during six weeks, starting from 20.06.2012 to 1.08.2012, at pilot recirculating system, of Aquaculture, Environmental Science and Cadastre Department, from "Dunarea de Jos " University of Galati.

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 aeration and oxygenation units) [3]. The important physicochemical parameters of technological water (oxygen, the concentration of nitrites, nitrates, ammonia, pH and temperature) were maintained between normal limits.

The biological material used in the experiment was composed of 10 months old Nile tilapia, with an average individual biomass of 125.41 ± 34.33 g. A number of 45 fish were randomly distributed in each rearing unit. Fish were fed with SOPROFISH pelleted feed. The biochemical composition of feed is shown in Table 1.

The feeding ratio was 3.4%, administered four times per day, at 09:00, 12:00, 15:00, 18:00.

The phytobiotics were administered in a concentration of 1%/kg feed and consists of: thyme (*Thymus vulgaris*), fenugreek (*Trigonela foenum graecum*) and neem (*Azadirachta indica*). The experimental variants were organized as follows: V1 – control, V2 - 1% thyme, V3 - 1% fenugreek and V4 - 1% neem.

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.	

To quantify the oxidative stress, lipid peroxidation was determined by the concentration of malondialdehyde (MDA nmol/ml), total antioxidant capacity (TAC % inhibition) and reduced glutathione (GSH µmol/dl). MDA and TAC were determined from tissue, liver, gut and plasma, and the GSH was determined from blood. The three mentioned parameters were analyzed spectrophotometrically.

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% inhibition) was measured spectrophotometrically, 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 and Van Den Berg in 1999 [21]. The

inhibition degree was calculated with the following formula:

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

where:

AbsP - sample absorbance,

AbsB - blank absorbance.

The reduced glutathione was determined in accordance with Ellman's method, modified by Seldak and Sinsdary [5].

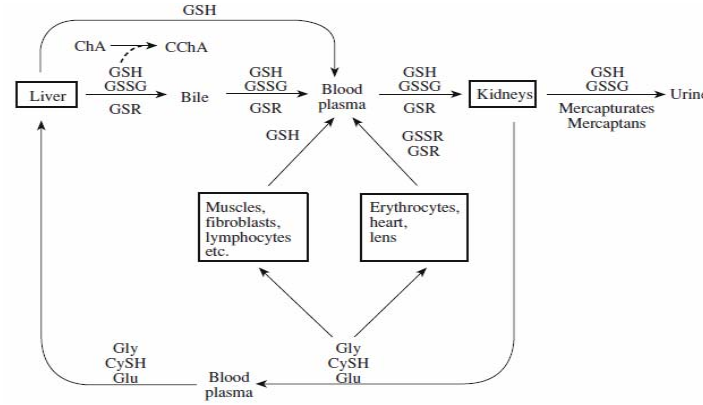

Fig. 1 Reduced glutathione metabolism; ChA – cholic acid, cChA – conjugated cholic acid [12]

Prior to sampling, fish were anesthetized with 2-phenoxyethanol.

The data were statistically analyzed, in Microsoft Excel, using descriptive statistics and ANOVA test.

RESULTS AND DISCUSSIONS

To prevent oxidation and damage at cellular level, the body has developed an antioxidant defense system that includes defense at both molecular and enzymatic level, against free radicals.

Oxidative stress occurs when there are more pro-oxidants and therefore, antioxidant defense yield, reactive species of oxygen becoming unable to be removed [22, 23]. It is assumed that the way antioxidants act is particularly, by destroying free radicals and adjusting antioxidant enzyme activity [15].

Results on malondialdehyde (MDA) dynamics are presented in Table 2. Comparing to V1, V3 and V4 experimental variants, at V2 variant can be observed an increase of MDA concentration, at plasma, tissue and gut level. In contrast, when we speak about liver, we can say that an increase of MDA can be observed at V3 (5.03 ± 0.14 nmol/ml), comparing with V1 (4.69 ± 0.11 nmol/ml).

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

MDA (nmol/ml)					
Experimental variant		**V1**	**V2**	**V3**	**V4**
Initial plasma	M±ES	6.25±0.46			
Final plasma	M±ES	4.48±0.46	9.45±1.71	4.55±0.24	4.83±0.39
tissues	M±ES	3.96±0.37	5.18±0.72	4.93±0.67	4.60±0.36
liver	M±ES	4.69±0.11	4.96±0.40	5.03±0.14	4.79±0.16
gut	M±ES	5.76±0.13	7.75±0.07	5.41±0.51	6.55±1.40

Note: Experimental variants: V1 – control, V2 – thyme, V3 – fenugreek,V4 – neem; M±ES - mean ± standard error

The fact that at V2 plasma and gut MDA concentration increased, compared to V1, by 210.94% and respectively 134.55%, can be explained by the increasing oxidative stress and thus, by free radicals generation.

However, the literature states that intense changes of MDA concentration take place both at tissue and liver level.

For this reason, in many research papers, the determination of MDA was made only from tissue and liver.

Together with MDA, the determination of total antioxidant capacity (TAC) provides information regarding the complexity of adaptive changes, produced as a response to the increasing production of free oxygen radicals [1]. The dynamics of total antioxidant capacity can be observed in Table 3.

An significant increase ($p<0.05$) of total antioxidant capacity at plasma ($p=0.012$) and liver ($p=0.029$) level can be seen at V2 variant. In contrast, in tissue and gut, TAC provided a greater degree of inhibition in case of V1 variant. Recent studies show that the presence of antioxidants from phytobiotics, such as polyphenols and flavones, might influence the body total antioxidant capacity against free radicals leading, as a consequence of a certain oxidative stress attenuation [6, 18, 19].

However, a decrease of serum and plasma total antioxidant capacity does not necessarily represent an undesirable situation, this only if the measurement reflects a decrease of reactive species [2].

The TAC of fish might reflect the resistance capacity to oxidation and is associated with health status [16].

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

TAC (%inhibition)					
Experimental variant		**V1**	**V2**	**V3**	**V4**
Plasma	M±ES	30.93±0.72	34.96±1.55	18.50±3.65	19.79±1.19
Tissues	M±ES	52.12±5.33	46.63±7.93	39.42±0.52	40.03±4.18
Liver	M±ES	45.19±8.10	59.51±2.15	27.02±3.37	26.11±5.57
Gut	M±ES	79.96±2.65	75.62±1.29	56.24±1.78	57.64±0.81

Note: Experimental variants: V1 – control, V2 – thyme, V3 – fenugreek,V4 – neem; M±ES - mean ± standard error.

At V2 variant can be observed an increased levels of malondialdehyde and a decrease of total antioxidant capacity, in both gut and tissue. This phenomenon is caused by the 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 [8, 17].

Regarding malondialdehyde concentration from liver, no significant differences are observed ($p>0.05$, $p=0.74$), however the total antioxidant capacity presents higher values in case of V2 variant (1% thyme/kg feed).

In terms of reduced glutathione, results indicated an increase at V2 - 1.075 ± 0.42 µmol/dl, followed by a decrease in V3 case - 0.951 ± 0.13 µmol/dl, V4 - 0.932 ± 0.03 µmol/dl and also V1 - 0.838 ± 0.038 µmol/dl, Figure 2. Thereby, an increase of reduced glutathione (GSH) blood concentration was found in variants were phytobiotics were given, comparing to control variant.

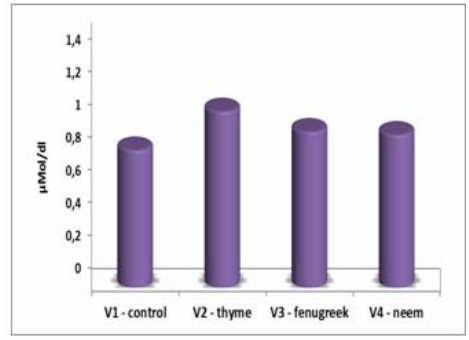

Fig. 2 Reduced glutathione values (µmol/dl) at the end of the experiment

In case of *Oreochromis niloticus* species, the exposure to four different concentrations of sodium selenite (Na_2SeO_3) caused the GSH concentration from liver to decrease, compared to the control variant [10]. Monteiro et al, in 2009, showed that a decrease of GSH makes the fish body cells more susceptible to toxic compounds attack [20].

CONCLUSIONS

The current research has shown that the administration of thyme, fenugreek and neem in *Oreochromis niloticus* diets led to changes

of malondialdehyde concentration, total antioxidant capacity and reduced glutathione, from various tissues and also from blood.

We can say that the functioning of some physiological defense mechanisms is confirmed by high values of lipid peroxidation while low values of total antioxidant capacity. This is observed at the gut and tissue level, in case of V2 variant.

In conclusion, the results of this study shows that thyme, in a concentration of 1%/kg feed, is an important additive in *Oreochromis niloticus* diets, with the effect of conferring a certain welfare to the biological material. We can say, to some extent, the same thing about fenugreek, pointing that the investigations regarding the optimal concentration that must be administered in feed might continue. Research should also be continued on the administration of neem at *Oreochromis niloticus* species.

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