BIOCHEMICAL DETERMINATIONS AND OXIDATIVE STRESS EVALUATION ON *ONCORHYNCHUS MYKISS* GROWN IN RECIRCULATING SYSTEM

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Abstract

The paper presents the results of the oxidative stress evaluation on rainbow trout (Oncorhynchus mykiss, Fam. Salmonidae) through measures of the lipid peroxidation indice (MDA), total antioxidant capacity (TAC) and analysis of the glucose content (GLU), total protein (PT) and immunoglobulin M(IgM) from blood plasma. The purpose of the experiment consist in the technological performance of the growth on Oncorhynchus mykiss in the biosecurised recirculation system with different stocking density. The biological material was represented by immature rainbow trout with 80±10 g body weight, means ± SEM. The used methods for the TAC and MDA analysis was performed by the spectrophotometric technique and the biochemical samples were read at the Vitros TP and Imola devices. Biochemical indices are in comparison limits with the speciality literature: GLU=69,5÷124 mg/dl; PT=3,45÷4,3g/dl; IgM=49,72÷87,72mg/dl. The MDA concentration has the highest value in L_1(5,64nmol/ml plasma) at a value of 42,72% echiv. Trolox TAC. The changes the two indices expect from the oxidative stress (MDA and TAC) and the analysed biochemical indices offer informations about stocking density knowing that the ontogenetic level of the rainbow trout grown in the recirculation system.

Key words: oxidative stress, lipidic peroxidation, rainbow trout, recirculation system

INTRODUCTION

The molecular oxidation affects the normal functions of the cells and maybe leads towards of a cellular degradation wich in many cases gets the death of it. Free radicals represent the major factor involved in this oxidation, in this case they could brake the biological activity of the proteins, lipids and nucleic acids wich can't be replaced as a matter of fact this is bad for the functions of the cell. The biology of the free radicals are involved in many aspects of the present human living, from the food industry, where the stoppage of the oxidation is crucial in the food conserving to the medical science like cardiology, neurology or other engineering sciences from where takes its places the acvaculture.

All the free radicals derived of the oxygen and of their other forms creates reactive oxygen species(ROS). The lipids are considerate to be one of the most vulnerable macromolecules to be attacked by ROS, wich leads to a waterfall of chemistry reactions. The most harmful effect of the lipid peroxidation is the bothering to the structure and the function of the plasmatic membrane. The most resistant and detectable final products of the lipid peroxidation are malondialdehyde (MDA) and 4-hidroxinenalul (4-HNE). Malondealdehyde is soluble in the water state and can react with ADN and proteins [7;15].

The way the lipidic peroxidation reacts is presented in Fig.1 and consists in the obtaining of a final product of the oxidative stress.

The oxidative stress may be determinate by a large number of factors wich may result seriously diseases like cancer, atherosclerosis

os the acceleration aging process [8;11]. Many experiments of the oxidative stress took place on mamels. However, some researchers suggest that the cells of the fish may be sensible at the oxidative stress. Therefore, the fish have been and are studied for the xenobiotics effect from the aquatic enviroment, the oxygen variation from the water, fast changes of the temperature and pH, etc.. Because the xenobiotics have a toxic effect by ROS growing, many species of fish have been used and as biomarkers in the environmental monitoring [12;18].

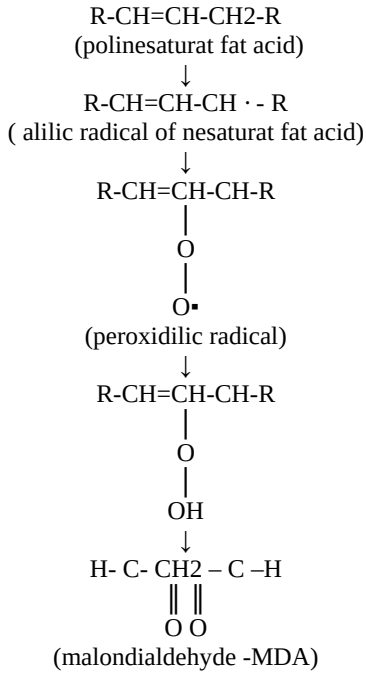

Figure 1. Lipidic peroxidation [1]

The direct opinion of the intensity product ROS is hard therefore we considered, in the present paper, that we can gather indirectly the MDA changes in the same time the answer capacity of the organism through the antioxidant total capacity evaluation (TAC).

The blood of the fish together with the hematological device are present in the permanent metabolic processes assureing the gas changes and an accommodation to the new climate, like the stress forms. In this study have been analysed biochemical indices in plasma (GLU-glucose,TP-total protein, Ig M-imunoglobulin M) wich can show us some changes in the metabolic processes even in the ROS influention even under the influence of the protective antiradical systems.

The objective of the present study is analisys oxidative stress determinate by different stocking density by biosecured recirculating system.

MATERIALS AND METHODS

Juvenile rainbow trout weighting 80±10g growth until this weight in the Pilot Recirculating System from the Laboratory of the Acvaculture, Enviroment Science and Cadastre Departament from the Dunarea de Jos University of Galati.

There have been used 4 units of growing of 0,336 m^3/buc.(0,35x0,80x1,20 m) by glass, part from a recirculating system [5] having 3 pomps, UV of sterilization lamp, mechanic and biological filter. The fish were monitorised over 7 days. Temperature was 15±1°C, dissolved oxygen value at 5,9÷6,7 mg l^{-1} and pH de 7,4±0,2.

The tank wre illuminated artificial with photoperiod a 12h : 12h. Fish were fed daily with commercial fish pellets (Nutra Pro MP-T) with 1.7 mm and the next biochemical composition: raw protein 50%,gross fats and oils 20%, fibres 10%, gross ash 8%, phosphor 1,2%, calcium 1%, sodium 0,4%, vitamine A 6000UI/kg, vitamine D_3 1200UI/kg.

An amount of pellets was given every day at 08:00, 12:00 and 16:00, corresponds of a ration 3% total body weight. Every tank was populated with different biomass: LM (control group) = 4200g, L_1=7300g, L_2=6000g and L_3=4700g. MDA concentration was performed using method described by Drapper and Hadley(1990) and Bîrcan (2008) [3;6]. After the reactions between MDA and other samples wich react with TBRAS, one MDA molecule join with two TBA molecules giving birth of a pink pigment, with measurable density of Spectrophotometrically at 532 nm.

The TAC determination was done by spectrophotometrically method ABTS [16], and the results are represented in Trolox echiv.. The inhibition percentage was calculated with the formula:

%inhibition=1-(Abs_{734}P(S) / Abs_{734}B) x 100;

where,

P-sample, S-standard,B-blank.

Biochemical determination (GLU-glucose,TP-total protein,Ig M-imunoglobulin M) were realized in the laboratory of the Medicine and Pharmacy Faculty of the University Dunarea de Jos Galati with Vitros TP device using slides of dry and wet biochemical with the Imola device (Imola Clinical Chemistry Analyzer).

To find the significant differences, the means were analysed by t-Student unequal test. We needed to use Microsoft Excel to show the statistical performance. The results were shown as a mean ± standard deviation.

RESULTS AND DISCUSSIONS

Throughout the experiment period there haven't been losses of biological material and the blood samples analisys was realized quickly after the harvesting.

The means values of the biochemical indices obtained are presented in Table 1 and the MDA analisys respective TAC in Table 2.

The plasmatic glucides represented by glucose can be found,normally, at values between 40÷90-mg /100ml in the blood of the fish and at the healthy rainbow trout fits at values like 71mg/100ml blood [10]. In this experiment, in control group (LM) with the lowest stocking density, the glucose has a mean value of 69.5 mg/dl , as the speciality literature. This value is lower in comparison with L_1-L_3. The semnificative growth is registred in L_1 with 78.41% besides LM. Glucose is an important marker for different types of stress at fish. The manipulation stress can go toa a significant growth of the glucose until 137mg/dl value [2]. In this case the density population is a factor that influences the plasmatic glucose values.

After some authors [17] normal values of the total proteins at fish are between 3.5÷5.5g/dl. However, this values present extensive variations that differs from a species to other, age,sex, water temperature, season, quality and amount of feeding[14]. Total protein from the fish's blood of sample, L_1-L_2, follow downward line in comparison with control group. So, at the highest stocking density, the level of the total proteins decreases with 15,85% but fits in the normally limits. This fact can be the cause of a high proteic contain from feed of 50% raw protein.

In the ontogenetic development the first class of imunoglobuline wich appears is the imunoglobuline M class(IgM) and represents the antibody wich appear after the first contact with the antigen. The IgM values at fish can be influenced by a series of exogen and endogen factors and can fit between 1-100mg/dl [14]. The highest value of IgM in our experiment is L_1 of 87.72mg/dl, with a growth of 76,42% in comparison with the control group. In L_3 growths were of 19,67% and L_2 of 28,72%. Probably, the stress due by a high stocking density starts the reaction of the organism but in thay remain in some normal values.

MDA values obtained by us are: 2,27nmol/ml at LM, 2,84nmol/dl at L_3, 4,01nmol/dl at L_2 si 5,64nmol/dl at L_1. It can be observed a significant growing in L_1 of 248,45% in comparing with LM. This high percenteage can be explained with a growing of the oxidative stress and produceing of the free radicals. The raised up biomass from L_2 seems to influence the growth of the lipid peroxidation in a percenteage of 76,65 in comparison with the control group. Usualy the MDA content is modified because of the actions of other stress factors. So, after some authors the external factors like pesticides in some concentrations can activate the lipid peroxidation marker in ascendent way until 390% from control groups [9].

This thing leads us at the hypothesis that the the highest stocking density from L_1 (7300g / 0,336m^3) changes the MDA, but, much little in comparison with the xenobiotic influence.

To challenge the toxic effects ROS the aerobe organisms use enzymatic and nonenzymatic mechanism for destroying the free radicals.[13]. However, a growing of ROS can overcome the capacity of the antioxidant cells and keep the oxidative stress.

Table 1- Biochemical evaluation at rainbow trout growth in recircuating system

Sample	Indici statistici	GLU mg/dl	TP g/dl	Ig M mg/dl
LM	X±ES	69.5±1.29	4.1±0.08	49.72±5.31
	n	5	4	5
L_1	X±ES	124±14.7	3.45±0.44	87.72±9.26
	n	5	5	5
	±M%	+78.41	-15.85	+76.42
L_2	X±ES	83.5±10.5	3.67±0.55	64.0±10.94
	n	5	4	5
	±M%	+20.14	-10.48	+28.72
L_3	X±ES	74.5±4.50	4.3±0.21	59.50±4.99
	n	5	4	4
	±M%	+7.19	+4.65	+19.67

Note: LM- control ,L_1-L_3-experimental groups, X±ES- standard error, n-number of the sample, ±M%-the percentage difference between group and group control, $p<0.05$(significance threshold).

Table 2- Analisis of the MDA and TAC content of the blood plasma fish

Sample	Statistical indicies	MDA nmol/ml plasma	TAC % inhibiţie echiv. Trolox
LM	X±ES	2.27±0.29	76.07±3.25
	n	4	5
L_1	X±ES	5.64±0.56	42.72±2.51
	n	4	5
	±M%	+248.45	-43.84
L_2	X±ES	4.01±1.13	62.40±3.66
	n	5	5
	±M%	+76.65	-17.97
L_3	X±ES	2.84±0.68	67.8±3.08
	n	4	5
	±M%	+25.11	-10.87

Note: LM-control ,L_1-L_3- experimental group, X±ES-standard error, n-number of the sample, ±M%- the percentage difference between group and group control, $p<0.05$(significance threshold).

In the same time with MDA,the use of the TAC test and the obtained results show the adaptative complexity of the changes produced as an answer at the growth of the free radicals oxygen production [4]. The inhibition percenteage, exprimed in Trolox echiv., of TAC found by us, is 76,07 in LM. The antioxidant capacity nonenzymatic from the experimental groups(L_1-L_3) we see a descrease until 43,84%, So, we can appreciate that once with the MDA growth takes places a significant descrease of TAC as a possible influence of the difference of biomass.

There are known many stocking densities for optim growing of family Salmonidae. Some authors recommend a maximum density of 16 kg/m^3 (Shepherd, 1984) and others (Wedemeyer, 1976) lead us at higher values that are bettween 13-61kg/m^3 in case of the rainbow trout[19]. Stocking densities experimented by us between 12,5kg ÷ 17,9kg /m^3 offer ther promising result of a superintensive growing of the rainbow trout.

CONCLUSION

Our researches put in evidence one type of oxidative stress that throughout the biochemical analisys of the blood plasma. The high values of the lipidic peroxidation marker, on this way and the low values of the total antioxidant capacity, on other way, shows the working of some physiological mechanism of self defense. These mechanisms are more efficient in fish experimental case from L_2 and L_3 and can

show a first step in the further investigations and prescript of the stocking densities, depending of ontogenetic stage of the *Oncorhynchus mykiss* species, like the possibility of growing in recirculating system.

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