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Effects of *Rheum rebis* Extract on the Blood Parameters and Responses of *Rutilus frisii kutum* under Heat Stress

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Abstract: It is well know that certain blood parameters serve as reliable indicators of fish health. So, in the present investigation we focus on hematological parameters as indicator to For evaluation of the effect of extract from *rheum rebis* on health and resistance of Kutums to high temperature stress. Kutum fingerlings were divided randomly into four groups: a control group on basal diet, three treatment groups fed a basal diet supplemented with 0.5%, 1% and 2% anthraquinone extract, respectively. After 8 weeks feeding, blood samples were obtained in two stages (before and after stress) for CBC test and cortisol evaluation. Water temperature was set up at 31 ± 0.5°C. We concluded that 0.5%-2% extract from *Rheum ribes* that added to the basal diet of *Rutilus frisii kutum* can have significant effect on the blood parameters and improve responses of *Rutilus frisii kutum* to heat stress.

Key words: *Rutilus frisii kutum* · *Rheum ribis* · Blood Parameters · Temperature Stress

INTRODUCTION

Kutum, *Rutilus frisii kutum,* is one of the most important and economical fish of the south shores of the Caspian Sea. The meat of this fish has a very good taste and assigned to itself the great share of people's food in Iran. Each year millions of artificially propagated larvae of this fish are released into the rivers for rehabilitation. Unfortunately, during previous years, its resources have been declined because of various reasons such as overexploitation of brood stocks, changes of rivers, decrease of water flow of rivers, pollution increase, gravel and sand removals in rivers which are all caused the decrease of natural breeding of fishes and Kutums in Iran, are released in rivers with one gram weight. Meanwhile, high percentage of larvae are killed before reaching the fingerling stage and some of larvae take a long time to reach this fingerling stage and some of them are also underdeveloped [1]. Also Kutums during culture and transportation face with kinds of stressors that can affect its growth and resistance to the types of deceases.

The response to stress in fish is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and subsequently metabolic and physiological changes [2, 3]. Under conditions of stress, the body of fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNC) and the release of the stress hormones, cortisol and catecholamines, into the bloodstream by the endocrine system [4]. Secondary responses occur as a consequence of released stress that causing changes in blood and tissue chemistry [5].

Cortisol is the principal corticosteriod in teleost fishes and its concentrations in blood rise dramatically during stress [6]. On the other hand, the hematological parameters of fish can be used as indicators of physiological conditions and monitoring diseases and the stress caused by handling [7,8], pollutants, metals, hypoxia, etc [9-11]. The study of blood parameters in fishes has been widely used for detection on physiopathological alterations in different conditions of stress [12].

Rheum ribes L. belongs to the family polygonaceae. It's used for medicinal purposes and its fresh stems and petioles are also consumed as a vegetable. It is commonly found in eastern Turkey, Lebnon and Iran [13]. In mammal, *R. ribes* roots have been used as a laxative and antipsoriatic drug in Iran [14]; the roots of the species are

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also used to treat diabetes, hypertension, obesity and diarrhea [15, 16]; The young shoots and petioles of *R. ribes* are used against diarrhea and as a stomachic and antiemetic treatment [13]. The medicinal properties of this species are due to its antraquinone components [13, 17]. The roots and leaves extracts have demonstrated significant antimicrobial activities [18]. There are more than 40 Rheum species grown in China [19]. However, people found that only three species (*Rheum palmatum*, *R.tanguticum* and *R.officeinale*) among the rhubarbs have most effective medicinal activities [19]. Lately, Xie *et al*. [20] used anthraquinone extract of *R. officeinale* on common carp that promoted the growth and raised the capability this fish. So, with respect to the effect of *Rheum ribis* on the blood parameter (for example its role in treat of diabetes and hypertension) of human and having antraquinone components, we hypothesis that *Rheum ribis* can have effect on blood parameters of fish too that no studies have investigated on effects of it on fish. So, the objective of this study was to evaluate the effect of *R. rebis* extract on the blood parameters of *Rutilus frisii kutum* and its changes after creation temperature stress.

MATERIAL AND METHODS

Experimental Fish and Diets: Kutums (*Rutilus frisii kutum*) were selected from Voshmgir dam (one of breeding centers) in iran. A total of 180 healthy fingerlings of Kutum allocated to 12 aquavarium (70 × 30 × 20 cm) and acclimatized for 30 days. Then kutums (an average initial body weight of 4.88) randomly divided into four groups i.e., a control group on a basal diet and three treatment groups fed a basal diet supplemented with 0.5%, 1.0% and 2.0% anthraquinone extract, respectively.

Extraction of *R. rebis*: The stems and the roots of *R. ribes* were obtained from Sabzevar in Iran. Then, we washed and dried them in a free weather away from sunshine [21]. The roots and the stems of *R. ribes* were extracted to soxhelet method [22].

Rearing Management: Kutums were acclimatized in aquavariums (15 fish in each aquavarium) for 30 days and then fed by trial diet with the feeding amount about 2-4.5% body weight (BW). Feeding was conducted twice a day, 0ne at 8:00-9:00 in the morning, the other at 16:00-17:00 in the afternoon. The water in each aquavarium exchange twice a week; a tow-third volume was exchanged each time as the water quality. The water quality in the experiment was as follows: average water temperature 20±2°C, DO?5 mg/L, PH 6.5-7.5. The amount of feeding was adjusted according to BW measurement every 15 days. After 60 days rearing experiment, feeding was suspended 48 h prior to testing and fish were not fed during the experiments. Blood samples were collected before and after stress.

Stress Experiment: Water temperature was set up at 31±0.5°C. We selected 12 aquavariums and during one week temperatures were set up. After feeding period and 48 h suspended feeding, 8 fish of each replication randomly were exposed to the temperature stress. After 24 hour exposure to temperature stress three blood samples were taken from each replication.

Hematologic Parameters: Three blood samples from each replication were obtained from caudal artery. Fish were subjected to the anesthesia material (clove flower) but to avoid of affecting this material on blood parameters, Fish were not anesthized completely and we used of the results of the publication by Imanpour *et al*. [23]. Then, blood samples immediately (less than 1 h) transferred to the Landa lab (Gorgan, Iran) and Total leukocytes (WBC), erythrocytes (RBC), hematocrit (Hct), hemoglobin (Hb), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) were counted in this lab.

For cortisol determination, Plasma was separated by centrifugation (1000 g for 10 min), removed, aliquoted into 1.5mL eppendorf tubes and frozen at -20°C until analysis of cortisol [24]. Then plasma was thawed (allowed to reach ambient temperature) and cortisol was measurement with ELISA (modle Plate Screen-Italy) according to description of Monobind Cortisol EAI kit (Product code: 3625-300).

Data statistics and analysis: We used SPSS (version 16.0) software Duncan's multiple range tests to determine the differences between groups. All the results were expressed as means±standard error (SE.).

RESULTS

Before the stress the red blood cells had a tendency to increase in the groups that supplemented with extract (Fig. 2A). The red blood cell significantly increased in the group supplemented with 1% *R. ribis* extracts before stress compared with control group ($P < 0.05$, Fig. 2A). After 31±0.5°C high temperature stress, the red blood cell significantly increased in the groups supplemented with 0.5% and 1 % *R. ribis* extract compared with the control ($P< 0.05$, Fig. 2A).

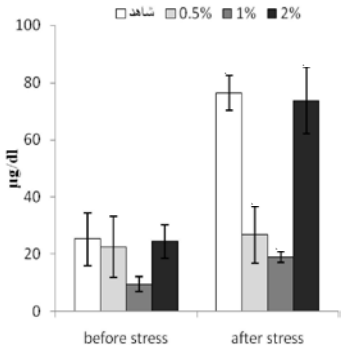

Fig. 1: Blood cortisol level of Kutums (Mean ± S.E) fed with experimental diets for 60 days. Dissimilar letters indicate significant differences within groups in to condition (before stress and after stress).

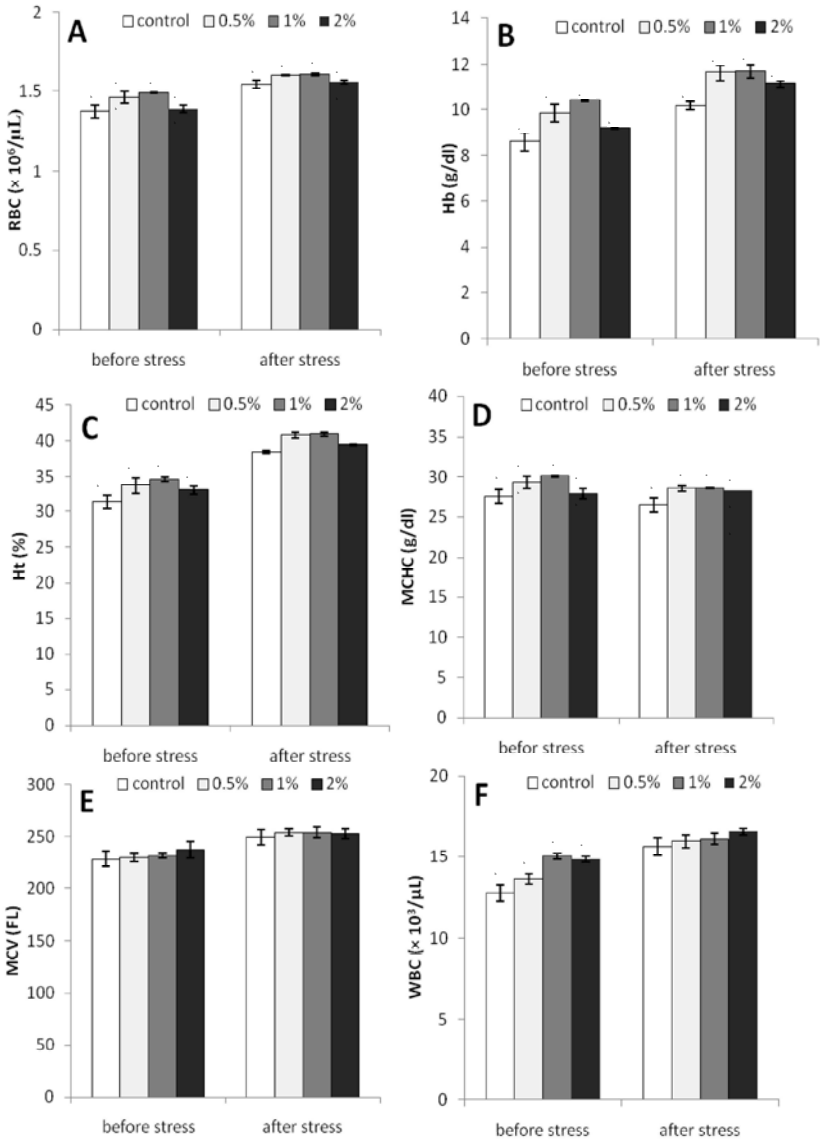

Fig. 2: *Rutilus frisii kutum* red blood cell count (RBC), hematocrit (Ht), hemoglobin concentration (Hb), mean cell volume (MCV) and mean cell hemoglobin concentration (Mchc) fed with experimental diets for 60 days. Control = commercial diet, 0.5%, 1%, 2% extract from *Rheum ribis*. Bars indicate mean±SD. Different letters indicate significant differences (p ? 0.05).

The hemoglobin level of blood increased in treated groups and compared with control significantly increased in groups fed with 0.05% and 1% extract (Fig. 2B). After stress the hemogobin levels all groups compared with it levels before stress were increased. After stress the hemoglobin levels significantly increased in treatment groups with 0.05% and 1% extract compared with control (Fig. 2B).

The hematocrit of the test groups supplemented with extract had an increasing trend compared with control and this increasing was significant in the test group supplemented with 1% extract ($P< 0.05$, Fig. 2C). After high temperature stress the hematocrit of all groups had tendency to increase but no significant differences were found between groups (Fig. 2C).

The mchc value showed significant difference in group fed with 1% extract and was higher in this group compared with control (Fig. 2D). After stress mchc significantly increased in groups fed with 0.5% and 1% anthraquinone extract from *R.ribes*.

Before and after no differences were found in MCV of all groups (Fig. 2E).

The WBC levels significantly increased in the groups supplemented with 1% and 2% extracts before stress compared with control (Fig. 2F). After stress, no differences were found between groups (Fig. 2F).

Although before stress, the cortisol level in group fed with *R. ribis* extract was lower in compared with control group, but it was not significant between groups. After temperature stress the difference in cortisol level was clearer, as the level of cortisol in groups that fed with 0.5% and 1% extract of *R. ribis* significantly was low compared with control group ($P < 0.05$, Fig. 1).

DISCUSSION

Fish are frequently exposed to stressors under culture conditions, which cause a series of physiological responses, known as stress, which are divided in primary, secondary and tertiary responses [25, 26]. Some secondary effects of hormones, such as hyperglycemia, increase of total protein, hematological and plasma ions changes are important parameters to assess the fish health conditions [27]. The hematocrit percentage, hemoglobin rate and erythrocyte count are good indicators for oxygen transportation capacity of fish thus making it possible to establish relationships with the oxygen concentration available in the habitat and the health status of these fish [28]. On the other hand, the White blood cells afford protection against infectious agent caused by microbial and chemical factors [29]. In this study we can see effects of extracts form *R. ribes* on hematological parameters. Without any stressor factors, the red blood cell was higher in group supplemented with 1% extract that can be indices for fish health and ability of fish to contrast with stressor factors, although must be express that the red blood cells in the tow other groups supplemented with extract were higher than control group. Before stress the hematocrit, hemeglobin and mchc values showed affected with the red blood cells values. According to these findings, supplementing 1% of *R.ribes* extracts had the greatest effects on these parameters although the other treated groups were not without effect too. For instance: Kutums fed with 0.05% extracts, morever 1% extracts, significantly had increasing in hemoglobin concentration. Although, this is the first research on *Rhem ribis* effect on blood parameters fish and there are a little information about its effect and need more researches but the role of *R.ribes* extracts on immune system can take and express in different ways. The first way is probable effects on lysozyme activity. This effect of *R. ribis* can occur from its anthraquines compounds. Lysozyme is one of several anti-microbial proteins associated with front line, innate immunity in all vertebrates [30]. Lysozyme available in the lysosomes of neutrophils and macrophages and secreted into the blood by these cells [31-34]. Acute stress causes enhanced lysozyme activity and prolonged stress lowered lysozyme activity [35]. As Xie *et al*. [20] established that anthraquinone extracts can affect on lysozyme activity and increase it so improve fish resistance to stressors. Another way, can focus on it, is effects of anthraquinone exracts on antioxidant enzymes system, superoxidsse dismutase and hepatic catalase and reduce oxidation [20, 35]. A probable way that is more important in increase the red blood cell, hemoglobin, hematocrit and mchc before stress, is the role of *R.ribis* extracts on the intestine environments. It's maybe similar to the role of vitamin C in prevents fish anemia with effect on absorption of iron [27, 36, 37]. Because of laxative effects of *R.ribis* [14], its extract can bias absoption of intestine and hence affect absorption of iron and change hematological parameters. On the other hand, in Publication by Öztürk *et al*. [17] expressed that *R.ribes* affects on bile secretion, that if we suppose this effect for fish then it can modify the GL tract microbial community to enhance non specific immune responses by regulate gut ph similar to one role of prebiotics in fish [38].

In addition to erythrocytes, white blood cells (WBC) are good indicators of physiological stress in fish [27]. From Fig. 2F we found significant increasing in the WBC compared with control before stress so these two doses increase the ability of Kutums to contrast stressors especially in disease conditions. Although, there are no previous study on effects of *R.ribes* on leukocytes in fish but this study may be a reason on the effect of *R.ribes* exract's on leukocytes in *Rutilus frisii kutum* that can induce a protection against diseases and improve the health mechanisms in stress conditions. Moreover after stress high temperature stress, Significant increasing in the red blood cells, hemoglobin and mchc in groups fed with 0.05 % and 1% extracts show the effects of *R.ribis* on blood parameters and improve the ability of *Rutilus frisii kutum* to this stress conditions.

At the end in this research, we evaluated cortisol level that it increase in plasma is one of the most accepted primary responses to stress [39-41]. Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of teleost fish [42]. The primary targets of cortisol action are the gills, intestine and liver, which re?ect the two main adaptive functions of cortisol identi?ed to date: osmoregulation and the maintenance of a balanced energy metabolism [43]. Although, Cortisol appears to play several roles in the stress response including energy mobilization, stimulation of ionoregulatory processes and facilitate-ion of oxygen up take under hypoxic conditions but prolonged cortisol elevation can also have severe, debilitating, consequences for disease resistance, growth and reproduction. For example, the rising cortisol level can cause reducing in the number of lymphocyte and white blood cells [44] and damaging immune organs such as spleen, thymus [45]. Different feeding regimens may alter the cortisol response to stressors. Feeding gilthead sea bream,*Sparus aurata,* with high levels of arachidonic acid reduced the cortisol response to crowding [46]. In this research Fig. 1 indicated that *rheum ribis* extract could affect blood cortisol level of *Rutilus frisii kutum.* Results showed that 0.5% and 1% extract of *R.ribis* had significant effect on cortisol level and reduced its level. Effects of *R.ribis* extract on cortisol level could occur because of its anthraquinone deriverties. As, late research on *common carp* by Xie *et al*. [20] showed that anthraquinone extract reduced cortisol level of this fish after crowding stress. So, with respect to all blood parameters that we evaluated in this research we can see effect of *R. ribis* on health and immune system of kutums.

CONCLUSION

We concluded that 0.5% and 1% extract from *Rheum ribes* that added to the basal diet of *Rutilus frisii kutum* had significant effect on some of blood parameters that we know them as CBC test and 1% and 2% *Rheum ribes* extract had significant effect on cortisol level so we can conclude that 0.5%-2% *Rhuem ribis* extract has effect on health of Kutums and improve responses of them under temperature stress.

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