EVALUATION OF THE IMMUNOMODULATORY EFFECTS OF Silymarin EXTRACT (Silybum Marianum) ON SOME IMMUNE PARAMETERS OF RAINBOW TROUT, Oncorhynchus mykiss (Actinopterygii: Salmoniformes: Salmonidae)

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Background. Herbal medicines are increasingly used for their effects on the immune system. Silymarin, a mixture of flavonolignans from the seed of milk thistle (Silybum marianum), is used in treatment of liver disease, food and drug poising, and foetal diseases such as viral hepatitis, diabetes, and ischemia. Although, immunostimulant effects of the dietary silymarin were studied on different experimental animals, no information is available about the effects of silymarin on immune parameters of fish. The presently reported study was conducted to investigate the immunomodulatory effects of silymarin on some immunological and haematological parameters of rainbow trout.

Materials and methods. Juvenile rainbow trout, Oncorhynchus mykiss (Walbaum, 1792), were maintained in 1000 L fiberglass tanks at 15 ± 2°C supplied with systems of water recirculation and aeration. Silymarin extract incorporated into diets (0.0, 0.1, 0.4, and 0.8 g of per 1 kg of feed) of fish. The trout were fed silymarin-supplemented diet for 30 days. Haematological parameters, such as red blood cell count (RBC), white blood cell count (WBC), haematocrit (Hct), haemoglobin (Hb), differential leukocyte- and immunological parameters such as peroxidase, lysozyme, and complement activities, total protein, albumin and globulin levels were measured on day 7, 15, and 30 days of silymarin treatment.

Results. The results indicated that oral administration of silymarin in fish, after 15 and 30 days of experimental periods, might enhance haematological and immunological parameters including lysozyme and complement activities, total protein and globulin levels, compared to the controls.

Conclusion. The results suggest that oral administration of silymarin may be useful to strengthen the immune system in rainbow trout.

Keywords: silymarin, rainbow trout, haematological parameters, immunological parameters

INTRODUCTION

In fish therapy efforts reported from many parts of the world, chemical drugs in aquaculture industry have been replaced by herbal medicine (Dügenci et al. 2003, Citaras et al. 2006). The role of plant extracts in stimulating the fish immune system challenged with bacterial-, parasitic-, and fungal agents has been the subject of many studies (Sivaram et al. 2004, Vasudeva Rao and Chakrabarti 2005, Citarasu et al. 2006, Vasudeva Rao et al. 2006, Divyagneswari et al. 2007). However, the effect of herbal drugs on the health status of fish has been overlooked in most of these studies. Consequently, not much is known about usage of commercial herbal medicine in aquaculture. Therefore, investigating the effects of these compounds after administration seems a necessity.

Liver failures and diseases, food and drug poising and foetal diseases such as viral hepatitis (El-Kamary et al. 2009), diabetes (Soto et al. 2003), ischemia (Oliveira et al. 2001, Canbek et al. 2008), etc. in human and laboratory animals can traditionally be treated by one of the most important herbal medicines called milk thistle, Silybum marianum (Family: Asteraceae; known also as Compositae). The...
active constituents of *S. marianum* may strengthen or stimulate the immune response by interacting with various parameters of the immune system. The reported positive effect of herbal medicine has been expressed by a number of mechanisms, e.g., inhibition of tumor necrosis factor TNF-α, interferon IFN-γ, interleukin IL-2, IL-4, and nuclear factor-kappa B (NF-κB) activation in rat (Wilarsusme et al. 2002, Ardestani and Yazdanparast 2007), inhibition of fibrosis in rat (Jia et al. 2001), inhibition of inflammation (Kaur and Agarwal 2007, Ramasamy and Agarwal 2008), immunomodulation in mice (Schümann et al. 2003), inhibition of mitochondrial injury in rat (Rolo et al. 2003), inhibition of P450 activity in human liver microsomes (Beckmann-Knopp et al. 2000), antioxidant properties and inhibition of lipid peroxidation in rat and fish (Han et al. 2007, Toklu et al. 2007, Banaee et al. 2011, Banaee unpublished*), enhancement of RNA, DNA (Sonnenbichler et al. 1984), protein synthesis in liver tissue of rainbow trout (Banaee et al. 2011), regulation of cell permeability (Kiruthiga et al. 2007, Basiglio et al. 2009), and adjustment of enzyme levels activity in plasma (Banaee et al. 2011).

Although the effect of oral administration of silymarin on blood biochemistry of fish has previously been studied (Banaee et al. 2011), no information is available on possible effect of silymarin, used as a feed supplement on different parameters of immune system of fish. Measuring the alterations in innate immunity and non-specific immune parameters of fish treated with herbal derivatives may be a good method to evaluate the effects of a herbal drug on immune system of fish. Therefore, the aim of the presently reported study was to assess the effect of oral administration of silymarin on some non-specific immune parameters in blood of rainbow trout.

**MATERIALS AND METHODS**

**Fish and experimental procedure.** Healthy rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), average weight 90 ± 15 g were obtained from a private farm (Rainbow trout farm, Kordan village, Karaj, Iran.). They were maintained in closed water recirculating systems (1000 L) at the optimal laboratory conditions (temperature 15 ± 2°C; pH: 7.4 ± 0.2; photoperiods: 16L:8D) in a 1000 L aquarium at the optimal laboratory conditions (temperature 15 ± 2°C; pH: 7.4 ± 0.2). Water was changed twice a week and water quality parameters were maintained according to the manufacturer’s instructions. Fish were randomly divided into four groups by triplicate that each contained 12 fish. Fish were fed commercial diets (Behparvar Co. Karaj, Iran) twice a day, equivalent to 2% of their body weight. During 30-day experiment, fish were fed commercial feed, two types of commercial feed powder to achieve doses of 0.1, 0.4, and 0.8 g per kg of fish feed.

During the trials, the fish appetite was assessed based on the volume of the digestive system contents. Immunostimulatory activity was evaluated on day 7, 15, and 30 of the experimental periods; 12 fish per treatment were captured and anesthetized within aquatic solution of clove powder (as powder of dried flower) (1 : 5000). Fish from each group (experimental and control) were bled from the caudal vein into sterilized glass vials at 4°C containing the anticoagulant (1% EDTA). The blood was centrifuged for 15 min at 4000 × G, 4°C. Plasma were immediately stored at −78°C until biochemistry and immunostimulatory activity analysis.

**Haematological parameters.** The blood was immediately used to determine the number of red blood cells (RBC) and white blood cells (WBC) by means of a haemocytometer slide at a magnification of 400 ×. Subsequently, blood was diluted to 10⁻² and 10⁻³ in phosphate buffered saline (PBS), at pH 7.2 (Sarder et al. 2001). Haematocrit (Hct) was determined by the microhaematocrit method described by Brown (1988). Haemoglobin (Hb) concentration was conducted by using the cyanohaemoglobin method (Azizoglu and Cengizler 1996). To differentiate blood cell type, blood smears from triplicate samples were prepared according to Banaee et al. (2008) and examined at a magnification of 400 ×.

**Alternative complement activity.** Alternative complement activity (ACH50) was evaluated following the procedure of Yano (1992) using rabbit rabbit red blood cells (RbRBC). Briefly, RaRBC were washed and adjusted to 2 × 10⁸ cell·mL⁻¹ in ethylene glycol tetra-acetic acid magnesium-gelatine veronal buffer (0.01 M). 100 µL of the RaRBC suspension was lysed with 3.4 mL of distilled water and the absorbance of the haemolsate was measured at 414 nm against distilled water to acquire the 100% lysis value. The test plasma was appropriately diluted, and different volumes ranging from 0.1 to 0.25 mL were made up to 0.25 mL total volume before being allowed to react with 0.1 mL of RaRBC in test tubes. After incubation at 20°C for 90 min with occasional shaking, 3.15 mL of a 0.9% (v/v) saline solution was added to each tube with centrifugation at 1600 × G for 10 min at 4°C. The absorbance (A) of supernatant was measured using a spectrophotometer at 414 nm. A lysis curve was obtained by plotting the percentage of haemolysis against the volume of plasma added. The volume of plasma producing 50% haemolysis (ACH50) was determined and the number of ACH50 units·mL⁻¹ was obtained for each fish.

**Lysozyme activity.** The turbidimetric assay for lysozyme activity was carried out according to Lange et al. (2001) with minor modifications. Thus, plasma (50 µL) was added to 2 mL of a suspension of *Micrococcus lysodeikticus* (Actinobacteria: Micrococcaceae) (0.2 mg mL⁻¹) in a 0.05 M sodium phosphate buffer (pH 6.2). The reaction was carried out at 25°C and absorbance was measured at 570 nm after 0.5 min and 4.5 min by spectrophotometer. PBS was used as the blank. Lysozyme of sample calibrated using a standard curve determined with hen’s egg white lysozyme (Sigma) in PBS. The specific activity (units/ml plasma) for lysozyme was determined.

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* Banaee M. 2010. تأثير سيلیمارین در کاهش استرس اکسیدیویی آد اید شدید ناشی از نیزه درمان ماهی گسل آلایه رانگین کمان (Oncorhynchus mykiss). [Influence of silymarin in decline of sub-lethal diazinon-induced oxidative stress in rainbow trout (Oncorhynchus mykiss)]. PhD Thesis, Aquaculture and Environmental Department, Natural Resource Faculty, Natural Resource and Agriculture Collage, Tehran University, Iran. [In Persian.]
**Peroxidases content.** The total peroxidase content present in plasma was measured according to Cuesta et al. (2007) with modification. Briefly, 10 µL of plasma was diluted with 100 µL of Hank’s balanced salt solution (HBSS). Then, 50 µL of 20 mM 3,3′,5,5′-tetramethylbenzidine hydrochloride and 2.5 mM H₂O₂ were added. The colour change reaction was stopped after 2 min by adding 50 µL of 2 M sulphuric acid and the optical density (OD) was read at 450 nm. Standard samples without plasma were also analyzed. The peroxidase activity (units · mL⁻¹ plasma) was determined defining one unit of peroxidase as that which produces an absorbance change of 1 OD.

**Blood biochemical parameters.** Plasma total protein and albumin levels were measured by using the total protein and albumin kit (Parsazma Co. Iran). Globulin levels were calculated by subtracting albumin values from plasma total protein.

**Statistical analyses.** Statistical analyses were performed using SPSS (Release 15) software. Data are presented as mean ± standard deviation. For all data, normal distribution was confirmed by the Kolmogorov–Smirnov test. Data were analyzed by one-way analysis of variance (ANOVA). Means were compared by Tukey’s test and a P < 0.05 was considered statistically significant.

### Results

The effects of oral administration of 0.4 and 0.8 g of silymarin on RBC were statistically significant on day 30 (P < 0.05). Hb concentrations increased significantly when fish were treated with diets enriched with 0.8 g of silymarin on days 15 and 30 when compared to control (P < 0.05). Hb was significantly higher in fish which were fed with 0.4 g of silymarin supplementary food on day 15 (P < 0.05). Hct value of fish fed 0.1 g of silymarin supplement was significantly higher than its value in control group on day 30. There was no significant difference in haematological parameters between all of treatments and control groups on day 7 (Table 1).

Leukocyte counts significantly increased in fish fed 0.1 g of silymarin-enriched diets on day 15. Thrombocyte count was significantly higher in fish fed 0.8 and 0.1 g of silymarin (P < 0.05) as compared to controls on day 15 and 30, respectively. Nevertheless, no significant changes in neutrophils and monocytes were observed during the experiments (Table 2).

No significant change in peroxidase activity in plasma of fish fed silymarin supplement was observed when compared with control group during experimental periods. ACH50 levels increased significantly when fish were treated with diets enriched by 0.4 g of silymarin during the experiments. Generally, lysozyme activity increased significantly in fish fed 0.1 and 0.4 g of silymarin on day 15 and 30 (P < 0.05) (Table 3).

Total protein levels significantly increased in the fish fed enriched by 0.1 g of silymarin on day 7, 15, and 30 of treatment when compared with control group (P < 0.05). In addition, a significant increase was observed in total protein plasma levels of treated fish by 0.4 and 0.8 g of silymarin on day 15 and 30 (P < 0.05). Albumin levels in plasma of fish fed feed enriched by 0.1 g of silymarin per kg was significantly higher than control group on day 15 (P < 0.05). There was a significant elevation in the globulin levels in plasma of fish fed by food enriched with 0.1 g of silymarin during experimental periods (P < 0.05).

### Table 1

Principal haematological parameters of *Oncorhynchus mykiss* fed diet containing silymarin

| HP       | Treatment [g·kg⁻¹] | 7         | 15        | 30         |
|----------|--------------------|-----------|-----------|------------|
| RBC [10⁶·µL⁻¹] | Control   | 1.14 ± 0.06ᵃ | 1.30 ± 0.13ᵃᵇ | 1.18 ± 0.12ᵃ |
|          | 0.1      | 1.18 ± 0.08ᵃ | 1.22 ± 0.11ᵃ | 1.23 ± 0.10ᵃᵇ |
|          | 0.4      | 1.21 ± 0.11ᵃ | 1.46 ± 0.16ᵇ | 1.40 ± 0.09ᵇ |
|          | 0.8      | 1.16 ± 0.03ᵃ | 1.39 ± 0.15ᵃᵇ | 1.38 ± 0.13ᵃ |
| WBC [10⁴·µL⁻¹] | Control   | 12.07 ± 0.31ᵃ | 12.38 ± 0.20ᵃ | 12.37 ± 0.94ᵇ |
|          | 0.1      | 12.54 ± 0.53ᵃ | 12.92 ± 1.08ᵃᵇ | 14.29 ± 2.74ᵃ |
|          | 0.4      | 12.79 ± 0.73ᵃ | 14.13 ± 1.70ᵇ | 13.41 ± 2.17ᵃ |
|          | 0.8      | 12.28 ± 0.62ᵃ | 12.50 ± 0.51ᵃᵇ | 13.93 ± 1.36ᵃ |
| Hb [g·dL⁻¹]  | Control   | 10.10 ± 1.90ᵃ | 8.35 ± 1.63ᵃ | 8.78 ± 0.93ᵇ |
|          | 0.1      | 11.24 ± 2.19ᵃ | 9.23 ± 1.21ᵃᵇ | 9.97 ± 2.17ᵇ |
|          | 0.4      | 11.17 ± 1.01ᵃ | 10.01 ± 0.41ᵃᵇ | 11.56 ± 1.19ᵇ |
|          | 0.8      | 10.94 ± 1.01ᵃ | 10.74 ± 0.90ᵇ | 12.01 ± 0.92ᵇ |
| Hct [%]    | Control   | 39.32 ± 5.21ᵃ | 40.18 ± 2.56ᵃ | 38.88 ± 2.88ᵇ |
|          | 0.1      | 42.32 ± 2.41ᵃ | 42.85 ± 1.76ᵇ | 43.65 ± 3.03ᵃ |
|          | 0.4      | 38.63 ± 4.10ᵃ | 44.48 ± 4.01ᵇ | 40.85 ± 2.00ᵃᵇ |
|          | 0.8      | 43.10 ± 1.97ᵃ | 43.98 ± 3.22ᵃᵇ | 41.33 ± 1.86ᵃᵇ |

HP = haematological parameter; RBC = erythrocyte count; WBC = leukocyte count; Hct = haematocrit; Hb = haemoglobin; Treatment values express g of silymarin per 1 kg of feed; values not sharing identical superscript letters are significantly different (one-way ANOVA, P < 0.05; mean ± standard deviation).
Consumption of food containing 0.8 g of silymarin supplements had a significant effect on globulin concentrations in plasma of experimental fish on day 30 (Table 3).

**DISCUSSION**

The presently reported study investigated the immunostimulatory properties of silymarin—a milk thistle extract containing a large number of flavonolignans—including silybin, isosilybin, silydianin, silychristin, and dehydroisochristin, dehydrosilychristin, neosilyhermin, silyhermin, and silybinome (Banaee et al. 2011) on haematological- and immunological parameters of rainbow trout (Oncorhynchus mykiss). Recent studies have shown that herbal supplements to feed increased disease resistance in fish and improved survival and growth in rats, which may be attributed to improvement of immune functions (Christybapita et al. 2007, Divyagnaneswari et al. 2007, Ardó et al. 2008, Cheng et al. 2008).

Haematology, based on erythrocyte count, leukocyte count, haemoglobin concentration, and haematocrit has provided valuable information for fishery biologists in the assessment of fish health (Banaee et al. 2008). Our results suggest that oral administration of silymarin for at least 15 to 30 days may increase the number of erythrocytes and leukocytes as well as haematoctrit and haemoglobin values. In other words, silymarin may effect the function of haematopoietic organs such as spleen and head kidney which play important role in blood cell formation. The results of this research revealed no significant alternations in differential leukocytes (lymphocytes, monocytes, and neutrophils) in the experimental fish when compared with control group. Increase in haemoglobin content (Hb), haematocrit, and numbers of leukocytes and thrombocyte were reported in Nile tilapia (Shalaby et al. 2006), hybrid tilapia (Ndong and Fall 2011) fed diet enriched by garlic. These results are in agreement with previous research in which feeding with other herbal supplemented feed led to an increase in erythrocyte- and leukocyte count, haemoglobin level and haematocrit (Martins et al. 2002, Ji et al. 2007).

Lysozymes are a family of enzymes with antibacterial activity characterized by the ability to damage the cell wall of bacteria. Thus, the significant increase in lysozyme activity in plasma of fish after 15 and 30 days of feeding with diets enriched by 0.1 and 0.4 g of silymarin may indicate an improvement of defence mechanisms against bacterial agents. Yet, feeding for 7 days did not reveal a significant difference in its activity relative to the controls. Ardó et al. (2008) reported an increase in lysozyme activities of Nile tilapia, Oreochromis niloticus (L.), fed for 7 days with Chinese herbs, Astragalus membranaceus (Fabaceae) and Lonicerajaponica (Caprifoliaceae). Furthermore, according to other reports the use of Astragalus radix ((Fabaceae; see Yin et al. 2006), Eclipta alba (Asteraceae; see Christybapita et al. 2007), and Ganoderma lucidum (Agaricomycetes: Ganodermataceae; see Yin et al. 2009) incorporated into the fish diets, fed for 14 to 60 days, led to a significant increases in the lysozyme activity.

Complement includes over 20 different plasma proteins that are produced by a variety of cells including, hepatocytes, macrophages, and gut epithelial cells. Some

### Table 2

Leukocyte- and thrombocyte counts in blood of Oncorhynchus mykiss fed diet containing silymarin

| HP Treatment [g·kg⁻¹] | Day of experiment |
|-----------------------|-------------------|
|                        | 7                 | 15                | 30                |
| **Lymphocytes**        |                   |                   |                   |
| Control                | 84.99 ± 1.02 a    | 86.66 ± 1.02 a b  | 83.66 ± 1.01 a    |
| 0.1                    | 83.49 ± 1.01 a    | 88.83 ± 1.01 b    | 83.99 ± 1.02 a    |
| 0.4                    | 84.83 ± 1.01 a    | 86.80 ± 1.03 a b  | 84.82 ± 1.02 a    |
| 0.8                    | 86.13 ± 1.03 a    | 84.49 ± 1.02 a    | 85.16 ± 1.01 a    |
| **Monocytes**          |                   |                   |                   |
| Control                | 7.11 ± 1.15 a     | 6.43 ± 1.18 a     | 7.63 ± 1.11 a     |
| 0.1                    | 7.65 ± 1.07 a     | 5.31 ± 1.10 a     | 6.77 ± 1.17 a     |
| 0.4                    | 6.63 ± 1.13 a     | 6.03 ± 1.26 a     | 6.95 ± 1.14 a     |
| 0.8                    | 6.78 ± 1.15 a     | 5.44 ± 1.18 a     | 7.61 ± 1.14 a     |
| **Neutrophils**        |                   |                   |                   |
| Control                | 1.62 ± 1.73 a     | 1.59 ± 1.43 a     | 2.00 ± 1.00 a     |
| 0.1                    | 2.00 ± 1.00 a     | 1.41 ± 1.46 a     | 1.90 ± 1.76 a     |
| 0.4                    | 1.91 ± 1.43 a     | 1.74 ± 1.36 a     | 1.62 ± 1.73 a     |
| 0.8                    | 1.41 ± 1.46 a     | 2.14 ± 1.18 a     | 1.78 ± 1.33 a     |
| **Thrombocytes**       |                   |                   |                   |
| Control                | 5.86 ± 1.28 a     | 5.01 ± 1.33 a     | 6.63 ± 1.13 a b   |
| 0.1                    | 6.80 ± 1.12 a     | 4.19 ± 1.33 a     | 6.91 ± 1.19 a     |
| 0.4                    | 6.46 ± 1.13 a     | 5.38 ± 1.27 ab    | 6.27 ± 1.17 ab    |
| 0.8                    | 5.28 ± 1.35 a     | 7.76 ± 1.17 ab    | 5.25 ± 1.21 a     |

HP = haematological parameter; Treatment values express g of silymarin per 1 kg of feed; values not sharing identical superscript letters are significantly different (one-way ANOVA, P < 0.05; mean ± standard deviation).
Complement proteins bind to immunoglobulins or to membrane components of cells. The complement system is an essential and effective part of the innate immune system. It can rapidly distinguish and opsonize bacteria for phagocytosis by specialized phagocytes or destroy them directly by membrane disorder (Rooijakkers and van Strijp 2007). Thus, the increase of the complement activity (ACH50) in plasma of fish may help to identify and eliminate bacteria agents by phagocytosis. The enhancement of complement activity (ACH50) in plasma of fish fed the feed enriched with 0.4 g of silymarin may indicate an improvement of the capabilities of the fish immune system during the experimental period. In line with the results of the present study, several authors have reported an increase in complement activity following administration of different immunostimulants such as herbal derivatives (Jian and Wu 2003, 2004, Christybpapita et al. 2007), sodium alginate (Bagni et al. 2005, Cheng et al. 2008), and vitamins C and E (Ortuño et al. 1999, 2001).

Peroxidases are a large family of enzymes which play important role as natural antibacterial agent in animal immune system, e.g., myeloperoxidase (Clark and Klebanoff 1975). Although, oral administration of silymarin did not significantly affect peroxidase activity in plasma of fish when compared with control group, peroxidase activity in plasma of fish fed 0.1 g of silymarin was higher than in fish given 0.8 g of silymarin after 30 days of feeding. Christybpapita et al. (2007) recorded an increase in myeloperoxidase activity in tilapia fed diets supplemented with different levels of aqueous extract of Eclipta alba, for 1 week, whereas they did not report any significant changes in myeloperoxidase activity after 2 or 3 weeks.

In the presently reported study, the potential enhancement of total protein by using silymarin-supplemented feed was investigated in fish. Banaee et al. (2011) reported that oral administration of silymarin might improve protein synthesis in fish liver tissue. Consequently, a significant increase of the total protein levels in plasma in

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### Table 3

Blood biochemical parameters of *Oncorhynchus mykiss* fed diet containing silymarin

| BP      | Treatment [g·kg⁻¹] | Day of experiment |            |            |            |
|---------|--------------------|------------------|------------|------------|------------|
|         |                    | 7                | 15         | 30         |            |
| Px [U·mL⁻¹] | Control     | 121.33 ± 7.00ᵃ   | 115.83 ± 8.47ᵃ | 111.50 ± 7.82ᵃ |
|         | 0.1            | 124.00 ± 7.10ᵃ   | 119.00 ± 10.02ᵃ | 124.00 ± 7.64ᵇ |
|         | 0.4            | 116.67 ± 6.15ᵃ   | 112.83 ± 7.57ᵃ | 117.33 ± 7.23ᵇ |
|         | 0.8            | 115.67 ± 7.47ᵃ   | 110.50 ± 12.34ᵃ | 106.67 ± 11.99ᵃ |
| Ac [U·mL⁻¹]  | Control     | 311.17 ± 6.88ᵃ   | 307.50 ± 9.50ᵃ | 311.67 ± 14.38ᵃ |
|         | 0.1            | 326.17 ± 11.84ᵃ  | 323.50 ± 10.84ᵇ | 330.50 ± 14.53ᵇ |
|         | 0.4            | 337.17 ± 13.01ᵇ  | 340.50 ± 11.78ᵇ | 343.33 ± 17.51ᵇ |
|         | 0.8            | 310.33 ± 10.78ᵇ  | 316.00 ± 12.18ᵇ | 323.67 ± 7.66ᵇ |
| Ly [U·mL⁻¹]  | Control     | 113.67 ± 5.54ᵃ   | 117.00 ± 5.02ᵃ | 112.17 ± 7.88ᵃ |
|         | 0.1            | 118.83 ± 7.33ᵃ   | 122.83 ± 6.49ᵇ | 125.00 ± 3.74ᵇ |
|         | 0.4            | 122.33 ± 14.18ᵇ  | 130.33 ± 12.63ᵇ | 135.17 ± 8.70ᵇ |
|         | 0.8            | 111.83 ± 7.68ᵇ   | 107.83 ± 9.62ᵇ | 102.83 ± 7.25ᵇ |
| Pr [mg·dL⁻¹] | Control     | 3.95 ± 0.16ᵃ     | 3.95 ± 0.16ᵃ | 4.08 ± 0.16ᵃ |
|         | 0.1            | 4.87 ± 0.66ᵇ     | 5.12 ± 0.92ᵇ | 4.65 ± 0.20ᵇ |
|         | 0.4            | 4.75 ± 0.75ᵇ     | 4.23 ± 0.21ᵇ | 4.62 ± 0.34ᵇ |
|         | 0.8            | 4.47 ± 0.41ᵇ     | 4.22 ± 0.19ᵇ | 4.72 ± 0.27ᵇ |
| Al [mg·dL⁻¹] | Control     | 1.95 ± 0.16ᵃ     | 1.92 ± 0.15ᵃ | 1.95 ± 0.08ᵃ |
|         | 0.1            | 2.37 ± 0.33ᵇ     | 2.43 ± 0.45ᵇ | 2.17 ± 0.26ᵇ |
|         | 0.4            | 2.30 ± 0.36ᵇ     | 2.12 ± 0.21ᵇ | 2.17 ± 0.12ᵇ |
|         | 0.8            | 2.23 ± 0.39ᵇ     | 1.93 ± 0.08ᵇ | 2.12 ± 0.13ᵇ |
| Gl [mg·dL⁻¹] | Control     | 2.00 ± 0.00ᵃ     | 2.03 ± 0.08ᵃ | 2.13 ± 0.16ᵃ |
|         | 0.1            | 2.50 ± 0.35ᵇ     | 2.68 ± 0.49ᵇ | 2.48 ± 0.12ᵇ |
|         | 0.4            | 2.45 ± 0.40ᵇ     | 2.12 ± 0.35ᵇ | 2.45 ± 0.29ᵇ |
|         | 0.8            | 2.23 ± 0.26ᵇ     | 2.28 ± 0.16ᵇ | 2.60 ± 0.24ᵇ |

BP = Biochemical parameter; Px = peroxidase; Ac = ACH50; Ly = lysozyme; Pr = protein; Al = albumin; Gl = globulin; Treatment values express g of silymarin per 1 kg of feed; values not sharing identical superscript letters are significantly different (one-way ANOVA, P < 0.05; mean ± standard deviation).
treated fish is similarly reflected increase of protein synthesis in liver tissue. Similarly, the highest serum protein level was recorded in Nile tilapia fed yellow leader and Japanese honeysuckle (Ardó et al., 2008), ginger, mistletoe, and stinging nettle (Dügenci et al. 2003). Proteins include albumin and globulin; some globulins are produced in the liver, while others are made by the immune system (Sandnes et al. 1988). Globulin is made up of subunit of α1, α2, β, and γ globulins, which are considered as the source of almost all the immunologically active proteins in the blood (Jha et al. 2007). Commonly, increases in the levels of plasma total protein, albumin and globulin in fish are thought to be associated with a stronger innate response (Wiegertjes et al. 1996). Although albumin did not increase in most of the treatment groups in the present study, globulin tended to respond similarly to total protein, which significantly increased in all experimental groups on day 15 and 30. Since albumin plays an important role in transport of some compounds such as drugs in blood, minor increase albumin levels in plasma of experimental fish may aid the transport of silymarin in blood. Therefore, the increase of globulins in plasma of fish treated by silymarin may indicate an enhancement of immune system of fish.

In conclusion, the results indicated that the use the incorporation of silymarin as immunostimulants into fish feed might lead to enhanced health- and immune parameters in blood of the fish stimulated this way. Haematological studies recorded increases in RBC, Hct, and Hb following the administration of the silymarin-supplemented feed.

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REFERENCES

Ardestani A., Yazdanparast R. 2007. Down regulation of NF-kappa B as a therapeutic strategy for type 1 diabetes: effect of flavonoids. Iranian Journal of Medical Hypotheses and Ideas 1 (1): 1–7.

Ardó L., Yin G., Xu P., Váradi L., Szigeti G., Jeney Z., Jeney G. 2008. Chinese herbs (Astragalus membranaceus and Lonicera japonica) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against Aeromonas hydrophila. Aquaculture 275 (1–4): 26–33.

DOI: 10.1016/j.aquaculture.2007.12.022

Azizoğlu A., Cengizler I. 1996. An investigation on determination of some haematologic parameters in healthy Oreochromis niloticus (L.). Turkish Journal of Veterinary and Animal Sciences 20 (6): 425–431.

Bagni M., Romano N., Finoia M.G., Abelli L., Scapigliati G., Tiscar P.G., Sarti M., Marino G. 2005. Short- and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (Dicentrarchus labrax). Fish and Shellfish Immunology 18 (4): 311–325.

DOI: 10.1016/j.fsi.2004.08.003

Banaee M., Mirvagefei A.R., Rafie G.R., Majazi Amiri B. 2008. Effect of sub-lethal Diazinon concentrations on blood plasma biochemistry. International Journal of Environmental Research 2 (2): 189–198.

Banaee M., Sureda A., Mirvaghefi A.R., Rafie G.R. 2011. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (Oncorhynchus mykiss). Fish Physiology and Biochemistry 37 (4): 885–896.

DOI: 10.1007/s10695-011-9486-z

Basiglio C.L., Sánchez Pozzi E.J., Mottino A.D., Roma M.G. 2009. Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. Chemico-Biological Interactions 179 (2–3): 297–303.

DOI: 10.1016/j.cbi.2008.12.008

Beckmann-Knopp S., Rietbrock S., Weyhenmeyer R., Böscher R.H., Beckurts K.T., Lang W., Hunz M., Fuhr U. 2000. Inhibitory effects of silibinin on cytochrome P-450 enzymes in human liver microsomes. Pharmacology and Toxicology 86 (6): 250–256.

DOI: 10.1111/j.0901-9928.2000.860602.x

Brown B.A. 1988. Routine hematology procedures. Pp. 7–122. In: Brown B.A. (ed.) Hematology: Principles and procedures. Leo and Febiger, Philadelphia, PA, USA.

Canbek M., Uyanoglu M., Bayramoglu G., Senturk H., Erkasp N., Koken T., Uslu S., Demirustu C., Aral E., Can Basiglio C.L., Sánchez Pozzi E.J., Mottino A.D., Roma M.G.

DOI: 10.1016/j.clipping.2007.11.022

Cheng A.-C., Chen Y.-Y., Chen J.-C. 2008. Dietary administration of sodium alginate and κ-carrageenan enhances the innate immune response of brown-marbled grouper Epinephelus fuscoguttatus and its resistance against Vibrio alginolyticus. Veterinary Immunology and Immunopathology 121 (3–4): 206–215.

DOI: 10.1016/j.clipping.2007.09.011

Christyabipati D., Divyagnaneswari M., Dinakaran Michael R. 2007. Oral administration of Eclipta alba leaf aqueous extract enhances the non-specific immune responses and disease resistance of Oreochromis mossambicus. Fish and Shellfish Immunology 23 (4): 840–852.

DOI: 10.1016/j.fsi.2007.03.010

Citrusus T., Sivaram V., Immanuel G., Rout N., Murugan V. 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, Penaeus monodon with reference to haematological, biochemical and immunological changes. Fish and Shellfish Immunology 21 (4): 372–384.

DOI: 10.1016/j.fsi.2006.01.002

Clark R.A., Kblemanoff S.J. 1975. Neutrophil-mediated tumor cell cytotoxicity: Role of the peroxidase system. The Journal of Experimental Medicine 141 (6): 1442–1447.

DOI: 10.1084/jem.141.6.1442
Immunomodulatory effects of silymarin extract on rainbow trout

Cuesta A., Vargas-Chacoff L., García-López A., Arjona F.J., Martínez-Rodríguez G., Meseguer J., Manera J.M., Esteban M.A. 2007. Effect of sex-steroid hormones, testosterone and estradiol, on humoral immune parameters of gilthead seabream. Fish and Shellfish Immunology 23 (3): 693–700. DOI: 10.1016/j.fsi.2007.01.015

Dügenci S.K., Arda N., Candan A. 2003. Enhancement of non-specific immunity and disease resistance in Oreochromis mossambicus by Solanum trilobatum leaf fractions. Fish and Shellfish Immunology 23 (2): 249–259. DOI: 10.1016/j.fsi.2006.09.015

Dügenci S.K., Arda N., Candan A. 2003. Some medicinal plants as immunostimulant for fish. Journal of Ethnomedecology 16 (5): 391–400. DOI: 10.1016/j.phymed.2009.02.002

Han M.H., Yoon W.K., Lee H., Han S.-B., Lee K., Park S.-K., Jia J.-D., Bauer M., Cho J.J., Ruehl M., Milani S., Boigk G. 2001. Antifibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagenα1(I) and TIMP-1. Journal of Hepatology 35 (3): 392–398. DOI: 10.1016/S0165-2427(01)00264-1

Jia J.-D., Bauer M., Cho J.J., Ruehl M., Milani S., Boigk G., Riecken O.E., Schuppan D. 2001. Fibrotic effect of silymarin in rat secondary biliary fibrosis is mediated by downregulation of procollagenα1(I) and TIMP-1. Journal of Hepatology 35 (3): 392–398. DOI: 10.1016/S0165-2427(01)00264-1

Jian J., Wu Z. 2003. Effect of traditional Chinese medicine on non-specific immunity and disease resistance of large yellow croaker, Pseudosciaena crocea (Richardson). Aquaculture 218 (1–4): 1–9. DOI: 10.1016/S0044-8486(02)00192-8

Jian J., Wu Z. 2004. Influence of traditional Chinese medicine on non-specific immunity of Jian Carp (Cyprinus carpio var. Jian). Fish and Shellfish Immunology 16 (2): 185–191. DOI: 10.1016/S1050-4648(03)00062-7

Kaur M., Agarwal R. 2007. Silymarin and epithelial cancer chemoprevention: How close we are to bedside? Toxicology and Applied Pharmacology 224 (3): 350–359. DOI: 10.1016/j.taap.2006.11.011

Lange S., Gudmundsdottir B.K., Mangadottir B. 2001. Humoral immune parameters of cultured Atlantic halibut (Hippoglossus hippoglossus L.). Fish and Shellfish Immunology 11 (6): 523–535. DOI: 10.1006/fsim.2000.0333

Martins M.L., Moraes F.R., Miyazaki D.M., Bruin C.D., Onaka E.M., Fenerick J.jr., Bozzo F.R. 2002. Alternative treatment for Anacanthorus penilabiatus (Monogenea: Dactylorhidae) infection in cultivated pacu, Piaractus mesopotamicus (Osteichthyes: Characidae) in Brazil and its haematological effects. Parasite 9 (2): 175–180.

Ndong D., Fall J. 2011. The effect of garlic (Allium sativum) on growth and immune responses of hybrid tilapia (Oreochromis niloticus × Oreochromis aureus). Journal of Clinical Immunology and Immunopathology Research. 3 (1): 1–9.

Oliveira C.P.M.S., Lopasso F.P., Laurindo R.M.C., Laudanna A.A. 2001. Protection against liver ischemia–reperfusion injury in rats by silymarin or verapamil. Transplantation Proceeding 33 (6): 3010–3014.

Ortuño J., Esteban M.A., Meseguer J. 1999. Effect of high dietary intake of vitamin C on non-specific immune response of gilthead seabream (Sparus aurata L.). Fish and Shellfish Immunology 9 (5): 429–443. DOI: 10.1016/s0165-2427(01)00264-1

Ramasamy K., Agarwal R. 2008. Multitargeted therapy of cancer by silymarin. Cancer Letters 269 (2): 352–362. DOI: 10.1016/j.canlet.2008.03.053

Rolo A.P., Oliveira P.J., Moreno A.J.M., Palmeira C.M. 2003. Protection against post-ischemic mitochondrial injury in rat liver by silymarin or TUDC. Hepatology Research 26 (3): 217–224.

Rooijakkers S.H.M., van Strijp J.A.G. 2007. Bacterial complement evasion. Molecular Immunology 44 (1–3): 23–32. DOI: 10.1016/j.molimm.2006.06.011

Sandnes K., Lie Ø., Waagbø R. 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, Salmo salar. Journal of Fish Biology 32 (1): 129–136. DOI: 10.1111/j.1095-8649.1988.tb05341.x

Sarder M.R.I., Thompson K.D., Penman D.J., McAndrew B.J. 2001. Immune response of the Nile tilapia (Oreochromis niloticus L.) clones: I. Non-specific responses. Developmental and Comparative Immunology 25 (1): 37–46. DOI: 10.1016/S0145-305X(00)00040-9
Schümann J., Prockl J., Kiemer A.K., Vollmar A.M., Bang R., Tieg G. 2003. Silibinin protects mice from T cell-dependent liver injury. Journal of Hepatology 39 (3): 333–340. DOI: 10.1016/S0168-8278(03)00239-3

Shalaby A.M., Khattab Y.A., Abdel Rahman A.M. 2006. Effects of garlic (Allium sativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (Oreochromis niloticus). Journal of Venomous Animals and Toxins including Tropical Diseases 12 (2): 172–201.

Sivaram V., Babu M.M., Immanuel G., Murugadass S., Citarasu T., Marian M.P. 2004. Growth and immune response of juvenile greasy groupers (Epinephelus tauvina) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture 237 (1–4): 9–20. DOI: 10.1016/j.aquaculture.2004.03.014

Sonnenbichler J., Goldberg M., Hane L., Madubunyi I., Vogl S., Zetl I. 1984. Stimulatory effect of silibinin on DNA synthesis in partially hepatectomized rat livers: Non-response in hepatoma and other malign cell lines. Biochemistry Pharmacology 35 (1): 538–541. DOI: 10.1016/0006-2952(86)90233-9

Soto C., Recoba R., Barrón H., Alvarez C., Favari L. 2003. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 136 (3): 205–212. DOI: 10.1016/S1532-0456(03)00214-X

Toku H.Z., Tunah-Aklay T., Erkanlı G., Yüksel M., Ercan F., Şener G. 2007. Silymarin, the antioxidant component of Silybum marianum, protects against burn-induced oxidative skin injury. Burns 33 (7): 908–916. DOI: 10.1016/j.burns.2006.10.047

Vasudeva Rao Y., Chakrabarti R. 2005. Stimulation of immunity in Indian major carp Catla catla with herbal feed ingredients. Fish and Shellfish Immunology 18 (4): 327–334. DOI: 10.1016/j.sci.2004.08.005

Vasudeva Rao Y., Das B.K., Jyotirmayee P., Chakrabarti R. 2006. Effect of Achyranthes aspera on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Fish and Shellfish Immunology 20 (3): 263–273. DOI: 10.1016/j.sci.2005.04.006

Wiegerijes G.F., Stet R.J.M., Parmentier H.K., van Muiswinkel W.B. 1996. Immunogenetics of disease resistance in fish: a comparable approach. Developmental and Comparative Immunology 20 (6): 365–381. DOI: 10.1016/S0145-305X(96)00032-8

Wilasrusmee C., Kittur S., Shah G., Siddiqui J., Bruch D., Wilasrusmee S., Kittur D.S. 2002. Immunostimulatory effect of Silibum marianum (milk thistle) extract. Medical Science Monitor 8 (11): BR439–BR443

Yano T. 1992. Assay of hemolytic complement activity. Pp. 131–141. In: Stolen J.S., Fletcher T.C., Anderson D.P., Hattari S.C., Rowley A.F. (eds.) Techniques in fish immunology. SOS Publications, Fair Haven, NJ, USA.

Yin G., Ardó L., Thompson K.D., Adams A., Jeney Z., Jeney G. 2009. Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. Fish and Shellfish Immunology 26 (1): 140–145. DOI: 10.1016/j.sci.2008.08.015

Yin G., Jeney G., Racz T., Xu P., Jun X., Jeney Z. 2006. Effect of two Chinese herbs (Astragalus radix and Scutellaria radix) on non-specific immune response of tilapia, Oreochromis niloticus. Aquaculture 253 (1–4): 39–47. DOI: 10.1016/j.sci.2005.06.038

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