Original Article

# Effect of tunceli garlic (*Allium tuncelianum*) on the haematological and immunological parameters of scaly carp (*Cyprinus carpio*)

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**Abstract**

The effects of Tunceli garlic oil on the hemato-immunological mechanisms of Scaly carp (Cyrinus carpio) were examined. After fed with0.1%, 1.0% and 10% of Tunceli garlic*(Alliumtuncelianum)* oil, blood was taken from the caudal vein of anesthetized fish and hematocrit and leucocrit levels, red blood cells (RBC) and white blood cells (WBC) values, phagocytic activity and index, NBT adherent, protein and total immunoglobulin levels were determined on days 3, 7, 14 and 21. The same procedure was conducted on a control group. No differences were found in the levels of total protein and total immunoglobulin levels between the control and experimental groups ($P > 0.05$). However, there were considerable increases in the other immune parameters and differences were detected between the control and experimental groups, while MHC and MCHC decreased. This study demonstrated that different doses of Tunceli garlic were associated with different effects on the hemato-immunological parameters in scaly carp.

**Keywords:** tunceli garlic (Allium tuncelianum)

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Received Date: 18/08/2016 Revised Date: 22/09/2016 Accepted Date: 10/11/2016

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| Quick Response Code: | Website: www.statperson.com |
| | DOI: 08 December 2016 |

## INTRODUCTION

Fish culture is an important and developing industry worldwide. However, intensive fish stocking in ponds affects the health of fish. Consequently, the physiological condition of cultured fish will be affected by environmental conditions. Thus, fish farmers have to practice careful husbandry techniques (Sakai, 1999). The use of antibiotics and chemotherapeutants to control diseases of fish, but they are not recommended since improper and continuous use of antibiotics may lead to potential development of antibiotic resistant bacteria, environmental pollution and accumulation of toxic residues in fish (Syahidah *et al*., 2015).The herbal extracts as a crucial role in the defense immune system. It is also well known that the innate immune system in fish can be triggered by many herbal immunostimulants, such as *S. Trilobatum(*Divyagnaneswari *et al*. 2007), *Toona sinensis*(Wu *et al*., 2010), *Astragalus* sp. (Yin *et al*. 2009), *Ocimum sectum, Emblica officinalis, Cynodon dactylon, and Adathoda vasica*(Selvaraj *et al*., 2005), *Epinephelus tauvina*(Sivaram *et al*., 2004). *Allium tuncelianum*, called Tunceli garlic, is only common in Tunceli province of Turkey, especially aroundMunzur Mountains in Ovacık district, and it is known as an endemic to this region (Yazar, 2006). Unlike other garlics withmultiple-cloved bulb, *A. tuncelianum* has single-cloved bulbs and has small formations like a small bulbs, and it can also produce fertile flowers and seeds (Taşkın *et al*., 2013). Garlic (*Alliumsativum*) has shown antibacterial effects in fish (Ranjan *et al* 2012). Also, dietary intake of garlic significantly enhanced the non-specific immune responses in rainbow trout (Nya and Austin, 2009; Nya and Austin, 2011). The garlic are found to increase resistance against infection in African catfish (*Clariasgariepinus*), rainbow trout (*Onchorhynchus mykiss*), and hybrid tilapia

How to cite this article: Engin Seker, Saliha Akkan. Effect of tunceli garlic (Allium tuncelianum) on the haematological and immunological parameters of scaly carp (Cyprinus carpio). *International Journal of Recent Trends in Science and Technology.* December 2016; 21(2): 122-128. http://www.statperson.com (accessed 10 December 2016).

(*Oreochromisniloticus* x *Oreochromis aureus*) (Ndong and Fall, 2007; Nya and Austin, 2009; Thanikachalam *et al*., 2010). There are so many studies on effect to immune response in the fish of *Allium sativum*. There is no published result to use in the fish of Tunceli garlic. The *aim* of the present study was therefore to *determine* the *effect* of Tunceli garlic on hematological and immune parameters.

## MATERIALS AND METHODS

**Fish**

Fish samples, weighing 29.92 ±1.03g and total length 11.89 ±0.07cm, were obtained from the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. The fish were kept in a 250L fibreglass tank. Before each experiment, the fish were acclimated in experimental units for 14 days. During this period, they were fed a commercial diet to apparent satiation once daily. At the end of the acclimation period, fish were randomly selected and stocked at rates of 20 fish/tank for experiments. The use of the fish and the experimental protocol were approved by the Animal Experimentation Ethics Committee of the Firat University (Elazığ, Turkey).

**Preparation of Tunceli garlic oil and experimental feeds**

Tunceli garlic was collected locally in Ovacik. The garlic was washed in distilled water, dried in room temperature, powdered in a ceramic mortar, and stored at −20 °C until used. Tunceli garlic powdered (100g) was extracted with 1L of corn oil. The extraction was performed by a cold maceration process for 7 days with twice a day. This solution were stored at −20 °C until used for the experiment. The experimental diets were prepared from the basal feed by mixed with 0%, 0.1%, 1.0%, and 10.0% of Tunceli garlic oil were sprayed to the basal diet. The pellets was air dried. The diets were *stored at −20°C until used*.

**Experimental design and collection of blood**

The entire experiment was repeated two in dependent times; each replicate for each group contained 20 fish, for a total of 280 fish. The fish were divided into seven groups as follows:

- Group 1 (C), the control group, was maintained in tap water and fed commercial basal diet without Tunceli garlic.
- Group 2 (T1) was maintained in tap water and fed 0.1% Tunceli garlic supplemented diet.
- Group 3 (T2) was maintained in tap water and fed 1.0% Tunceli garlic supplemented diet.
- Group 4 (T3) was maintained in tap water and fed 10.0% Tunceli garlic supplemented diet.
- Group 5 (T4) was maintained in tap water and fed 0.1% corn oil supplemented diet.
- Group 6 (T5) was maintained in tap water and fed 1.0% corn oil supplemented diet.
- Group 7 (T6) was maintained in tap water and fed 10.0% corn oil supplemented diet.

The diets were reformed into pellets, spread to dry and stored at +4 °C for the feding experiment. The remade pellets were given to the fish manually at a rate of approximately 2% fish body weight per day for 21 days. Control group received the normal pellet diet, which not contained *A. tuncelium* oil.The analysis were performed with five fish from each groups at 3,7, 14 and 21 days of exposure. No feeding was done on sampling days.

**Sampling and blood collection**

The fish were anesthetized in 50 ppm benzocaine solution. Blood was collected in EDTA tubesfor hemato-immunological parameters from the caudal.

**Hematological parameters**

The erythrocyte count was performed with the technique described by Wintrobe (1934) in hemacytometer using a Natt and Herrick solution (1952). Hemoglobin (Hb) contents were determined spectrophotometrically at 540nm using the cyanomethemoglobin method (Drabkin, 1946). The hematocrit (Ht) was determined by the volume occupied by erythrocytes in heparinized microhematocrit, and the hematimetric indicates how secondary indices, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration), were determined using standard formulas described by Wintrobe (1934). White blood cells (WBC) count were realized according to Tavares-Dias and Moraes (Tavares-Dias and Moraes, 2006).

**Immunological parameters**

The heparinized blood was immediately used for the phagocytic assay. Briefly, 1 x $10^7$ cells of *Staphylococcus* sp. in 0.1 mL of PBS were added to 0.1 mL of blood samples in a microplate and incubated for 30 min after through mixing in the well. After incubation, the plate was mixed gently and 0.05 mL of this suspension was smeared on the glass slide. After air-drying, the smears were fixed in ethanol, and cells and phagocytosed bacteria were counted. For the detection of metabolic activity of neutrophils (NBT activity by spectrophotometric assay), 0.1 mL of blood was put into a microtiter plate well, and then an equal amount of 0.2% NBT solution was added. After incubation at room temperature for 30 min, 0.05 mL of the NBT-blood cell suspension was removed and added to a glass tube containing 1.0 mL of N, N dimethyl formamide. After centrifugation, reading in a spectrophotometer at 620 nm in a 1.0 mL cuvette was performed. The total protein

level was determined through the Biuret method. Serum (0.1 ml) and 0.9 mL of distilled water were pipetted into a spectrophotometer tube and mixed thoroughly by inversion. The Biuret reagent (4.0 ml) was added, and the mixture was incubated for 30 min at room temperature in dark. The sample absorbance was read at 540 nm with a spectrophotometer (Siwicki *et al*., 1994; Ispir *et al*. 2009). The total immunoglobulin level was determined by following the method previously published by Siwicki *et al*. (1994). The assay is based on applying a microprotein determination method to measure the total serum protein content prior to and after immunoglobulin precipitation with a 12% solution of polyethylene glycol. The difference in protein content before and after depletion is the serum total immunoglobulin content.

**Statistical analysis**

Themeans (±SD) of assayed parameters were calculated for each group of fish. One-way ANOVA and Duncan's tests were to compare values from individual experimental fish groups with those from controls.

## RESULTS

There was a significant difference in hematocrit value in supplemented groups of 1.0% and 10% Tunceli garlic and control throughout the experimental period. The hematocrit level of fish fed the 0.1% supplemental Tunceli garlic oil diet had lower throughout the experimental period (Table 1). RBC levels on days 3, 7 and 14 in the experimental group was similar on all experimental groups. There was a significant decrease in RBC ($P < 0.001$) in supplemented groups of 0.1% Tunceli garlic oil (Table 1). When fish were fed Tunceli garlic diets, WBC significantly increased as dietary Tunceli garlic level increased. Tunceli garlic oil had no effect on Hb values. Hemoglobin values of fish fed the diet supplemented was significantly lower than that of fish fed the control diet in 21 days.The MCV, MCH and MCHC varied significantly among the treatments. The lowest MCV, MCH and MCHC in Tunceli garlic oil groups while the highest values in control group. The significant increases of the NBT reduction in bloodTable 2were observed in the garlic (10.0% garlic group) and corn oil groups of experimental fishes compared to control values since the 21$^{rd}$ day. Maximum increase in the neutrophil metabolic activity was seen on the 21$^{nd}$ day. In the corn oil supplemented groups, activity of the NBT reduction in blood was also significantly higher than in Tunceli garlic group fishes ($p < 0.05$) since the 21$^{rd}$ day. In the Tunceli garlic oil treatment group there was an increase in phagocytic activity. In the experimental group showed a significant increase in phagocytic ability Table 2. In phagocytic activity, the maximum numbers of observed cells were 87.66 and 63.33 for the experimental and control groups, respectively. Changes in protein levels in plasma of carp are shown in Table 2. No significant differences were observed among the values for fish fed dietary Tunceli garlic. Total immunoglobulin level was lower in fish fed the diet with 0.1% of Tunceli garlic than those of the other experimental groups in 21 daysTable 2. In other groups, no significant differences were observed among the values for fish fed dietary Tunceli garlic and corn oil.

**Table 1:** Hematological parameters of Scaly carp control (C) and experimentl groups fed with Tunceli garlic oil supplementationfeeds (T1, T2 and T3) and corn oil (T4, T5 and T6)

| Days | Hematological parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | C | T1 | T2 | T3 | T4 | T5 | T6 |
| 3 Days | Ht | 22.03±1.79 | 18.86±14.33 | 29.46±4.65 | 32.06±2.20 | 29.96±0.66 | 30.10±6.69 | 29.53±2.89 |
| | Hb | 6.60±1.08 | 5.86±2.83 | 8.26±2.23 | 8.70±0.45 | 8.20±0.60 | 7.96±1.46 | 7.73±0.85 |
| | RBC | 1.0±0.04 | 0.82±0.63 | 1.35±0.24 | 1.52±0.06 | 1.37±0.06 | 1.42±0.29 | 1.47±0.12 |
| | WBC | 116.60±55.93 | 113.67±20.63 | 122.79±25.53 | 127.32±13.38 | 179.24±23.30 | 109.54±27.83 | 111.62±11.57 |
| | MCV | 220.03±10.80 | 214.16±25.55 | 218.96±12.11 | 214.93±3.82 | 218.90±6.84 | 226.63±7.90 | 200.83±7.25 |
| | MCH | 65.73±7.96 | 66.23±5.22 | 61.00±12.37 | 56.30±0.51 | 59.86±4.22 | 64.93±9.98 | 52.56±2.37 |
| | MCHC | 29.83±3.01 | 26.83±1.96 | 27.76±4.47 | 26.13±0.63 | 27.33±1.52 | 28.60±3.46 | 26.16±0.28 |
| 7 Days | Ht | 22.40±1.27 | 28.43±3.27 | 29.76±1.86 | 24.16±2.82 | 31.66±3.36 | 29.00±1.37 | 29.20±1.25 |
| | Hb | 7.13±0.15 | 7.30±0.91 | 7.10±0.45 | 6.26±0.65 | 8.46±0.92 | 7.76±0.41 | 7.73±0.85 |
| | RBC | 1.13±0.05 | 1.47±0.19 | 1.54±0.14 | 1.19±0.13 | 1.58±0.26 | 1.37±0.11 | 1.43±0.04 |
| | WBC | 95.50±13.81 | 118.34±16.84 | 163.63±15.84 | 123.07±13.40 | 140.32±19.04 | 131.30±32.76 | 144.56±11.00 |
| | MCV | 220.16±10.88 | 193.40±5.18 | 193.66±8.39 | 202.56±2.31 | 201.63±15.97 | 211.10±7.74 | 203.40±11.17 |
| | MCH | 64.06±7.79 | 49.60±3.11 | 46.23±2.70 | 52.53±1.16 | 53.83±3.46 | 56.50±1.85 | 52.76±6.26 |
| | MCHC | 29.16±0.76 | 25.66±1.02 | 23.83±0.46 | 25.96±0.83 | 26.43±1.05 | 26.76±0.25 | 26.03±3.95 |
| | Ht | 22.53±1.73 | 24.93±0.05 | 22.20±0.17 | 26.23±0.98 | 27.26±0.56 | 28.73±0.96 | 30.96±0.32 |
| | Hb | 7.0±1.01 | 5.06±0.92 | 5.26±1.09 | 6.63±0.30 | 8.06±0.77 | 9.33±1.55 | 8.40±0.30 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 14 Days | RBC | 1.10±0.12 | 1.46±0.46 | 1.35±0.24 | 1.34±0.07 | 1.42±0.11 | 1.70±0.04 | 1.37±0.10 |
| | WBC | 79.16±4.43 | 193.34±0.57 | 167.29±1.12 | 138.94±2.19 | 111.09±7.38 | 131.02±21.48 | 136.42±14.06 |
| | MCV | 220.83±12.27 | 226.56±2.29 | 198.10±6.17 | 196.13±7.00 | 249.66±1.15 | 224.10±15.98 | 251.56±1.20 |
| | MCH | 63.76±11.27 | 46.13±0.98 | 57.93±0.80 | 49.40±1.03 | 56.50±1.34 | 54.90±9.76 | 60.03±3.28 |
| | MCHC | 30.70±3.22 | 22.00±0.86 | 26.00±0.86 | 25.30±0.34 | 25.96±0.80 | 25.96±0.80 | 26.43±0.50 |
| | Ht | 25.26±0.56 | 14.10±8.87 | 28.13±3.02 | 27.46±2.21 | 35.10±12.41 | 25.03±4.89 | 25.10±5.52 |
| | Hb | 8.96±0.11 | 3.76±1.64 | 7.06±0.56 | 7.43±0.68 | 9.44±1.00 | 8.33±0.37 | 9.20±0.50 |
| 21 Days | RBC | 1.64±0.15 | 0.84±0.04 | 1.42±0.17 | 1.4±0.12 | 1.65±0.28 | 1.51±0.06 | 1.43±0.07 |
| | WBC | 100.66±18.99 | 72.67±21.60 | 168.92±36.36 | 162.78±17.58 | 104.32±10.49 | 91.40±11.44 | 95.23±19.97 |
| | MCV | 204.80±19.89 | 206.73±0.63 | 220.73±0.63 | 195.33±3.59 | 190.83±13.45 | 223.43±16.46 | 240.23±32.35 |
| | MCH | 62.60±9.80 | 62.00±0.86 | 48.86±3.84 | 53.06±3.90 | 51.20±0.52 | 54.93±1.11 | 64.36±5.88 |
| | MCHC | 30.20±1.92 | 24.60±2.70 | 25.10±1.21 | 27.06±0.91 | 21.00±0.86 | 25.73±1.41 | 31.73±0.23 |

**Table 2:** Immunological parameters of Scaly carp control (C) and experimentl groups fed with Tunceli garlic oil supplementationfeeds (T1, T2 and T3) and corn oil (T4, T5 and T6)

| Days | Experimental groups | NBT Activity | Phagocytic Activity | Total Protein Levels | Total Immunoglobulin |
|---|---|---|---|---|---|
| 3 Days | 1 | $29.40\pm9.20^{a,b,A}$ | $63.33\pm10.69^{a,A}$ | $28.96\pm6.46^{a,A}$ | $19.96\pm4.72^{a,A}$ |
| | T1 | $16.66\pm10.69^{a,A}$ | $67.33\pm12.50^{a,A}$ | $27.40\pm3.76^{a,A}$ | $18.86\pm3.04^{a,A}$ |
| | T2 | $37.20\pm9.35^{b,A}$ | $68.66\pm7.50^{a,A}$ | $28.70\pm2.42^{a,A,B}$ | $19.26\pm1.50^{a,A,B}$ |
| | T3 | $28.73\pm0.63^{a,b,A}$ | $46.00\pm35.51^{a,A}$ | $27.43\pm4.47^{a,A}$ | $17.90\pm4.17^{a,A}$ |
| | T4 | $14.20\pm1.03^{a,b,A}$ | $63.33\pm3.78^{a,A}$ | $26.00\pm1.73^{a,A}$ | $15.33\pm1.15^{a,A}$ |
| | T5 | $15.72\pm2.11^{a,A}$ | $67.33\pm14.84^{a,A}$ | $27.10\pm1.15^{a,A}$ | $17.13\pm1.96^{a,A}$ |
| | T6 | $22.13\pm7.44^{a,b,A}$ | $75.66\pm13.57^{a,A}$ | $26.66\pm1.15^{a,A}$ | $16.66\pm1.15^{a,A}$ |
| 7 Days | 1 | $24.96\pm9.56^{a,A}$ | $65.66\pm4.16^{a,A}$ | $28.00\pm7.93^{b,c,A}$ | $20.00\pm4.00^{a,A}$ |
| | T1 | $17.56\pm11.49^{a,A}$ | $86.00\pm4.35^{a,B}$ | $28.06\pm0.55^{c,A}$ | $19.40\pm0.36^{a,A}$ |
| | T2 | $32.46\pm14.61^{a,A}$ | $71.00\pm24.24^{a,A,B}$ | $26.33\pm2.34^{a,b,A}$ | $18.03\pm1.78^{a,A}$ |
| | T3 | $30.73\pm17.11^{a,A}$ | $71.00\pm24.24^{a,A}$ | $25.96\pm3.20^{a,A}$ | $18.66\pm2.57^{a,A}$ |
| | T4 | $14.53\pm2.19^{a,A}$ | $83.66\pm4.93^{a,C}$ | $28.16\pm3.95^{a,b,A,B}$ | $20.00\pm0.72^{a,B}$ |
| | T5 | $16.05\pm2.87^{a,A}$ | $73.33\pm8.50^{a,A}$ | $29.56\pm4.55^{a,b,A}$ | $20.43\pm3.20^{a,A,B}$ |
| | T6 | $21.80\pm8.19^{a,A}$ | $90.00\pm4.00^{a,B}$ | $27.16\pm2.41^{a,A}$ | $19.06\pm1.95^{a,A}$ |
| 14 Days | 1 | $29.73\pm9.25^{a,b,A}$ | $66.66\pm11.84^{a,b,A}$ | $31.96\pm2.31^{a,A}$ | $22.60\pm1.83^{b,c,A}$ |
| | T1 | $20.86\pm0.75^{a,A,B}$ | $74.66\pm0.57^{a,b,A,B}$ | $35.20\pm1.03^{a,A}$ | $24.93\pm0.80^{c,B}$ |
| | T2 | $42.20\pm1.03^{a,b,A}$ | $87.66\pm0.57^{b,B}$ | $30.76\pm0.63^{a,B}$ | $21.83\pm0.57^{b,B}$ |
| | T3 | $18.86\pm0.80^{a,A}$ | $73.66\pm0.57^{a,A}$ | $27.13\pm0.98^{a,A}$ | $18.76\pm0.66^{a,A}$ |
| | T4 | $61.63\pm44.71^{b,B}$ | $80.33\pm5.68^{a,A,B}$ | $30.43\pm1.88^{a,A,B}$ | $21.56\pm1.25^{a,b,B,C}$ |
| | T5 | $56.53\pm37.29^{a,b,B}$ | $76.66\pm5.68^{a,b,A}$ | $29.16\pm2.13^{a,A}$ | $20.43\pm0.89^{a,b,A,B}$ |
| | T6 | $60.73\pm25.66^{b,B}$ | $79.66\pm6.65^{a,b,A,B}$ | $28.16\pm3.95^{a,A}$ | $19.63\pm3.10^{a,b,A}$ |
| 21 Days | 1 | $35.40\pm3.01^{a,b,A}$ | $64.33\pm15.14^{a,A}$ | $32.36\pm3.25^{a,A}$ | $23.60\pm2.20^{b,A}$ |
| | T1 | $34.33\pm2.02^{a,b,B}$ | $68.66\pm1.52^{a,b,c,A}$ | $22.76\pm13.32^{a,A}$ | $21.16\pm1.98^{a,b,A}$ |
| | T2 | $26.53\pm6.20^{a,A}$ | $66.00\pm4.00^{a,b,A,B}$ | $26.86\pm1.78^{a,A}$ | $19.10\pm1.35^{a,A}$ |
| | T3 | $43.30\pm10.65^{b,c,B}$ | $62.00\pm10.44^{a,A}$ | $29.20\pm1.31^{a,A}$ | $21.10\pm1.24^{a,b,A}$ |
| | T4 | $71.70\pm22.69^{b,B}$ | $74.33\pm9.07^{a,b,c,B,C}$ | $31.76\pm2.60^{a,B}$ | $23.16\pm1.92^{b,C}$ |
| | T5 | $67.80\pm7.81^{b,C}$ | $75.00\pm5.56^{b,c,A}$ | $21.46\pm16.03^{a,A}$ | $22.33\pm1.40^{a,b,B}$ |
| | T6 | $50.23\pm7.82^{c,B}$ | $78.33\pm4.72^{c,A}$ | $29.23\pm2.67^{a,A}$ | $21.03\pm1.92^{a,b,A}$ |

a,b,c The values (mean ± SE) in the same column with different letters are statistically significant ($p<0,05$).
A, B, C The values (mean ± SE) in the same line with different letters are statistically significant ($p<0,05$).

## DISCUSSION

The red blood physiological indices, such as Ht, Hb and RBC,may be an indicator of fish health, because erythrocytes are one of the major production sites of free radical and some of them can trigger peroxidation of sutured fatty acids in their membrane phospholipids, therefore alteringtheir quality and quantity (Pearce *et al.,*2003, Kiron *et al.,* 2004).Shalaby *et al* (2006),also reported that*Oreochromis niloticus* fed garlic and onion were observed to have significantly increased hematocrit than fish fed the control diet. Kalyankar *et al*. (2013), reported that higher haematocrit value were observed in *Xiphophorus helleri* fed a diet containing garlic.However, Saleh *et al*. (2015), were showed in *Dicentrarcus labrax*, hematocrit of fish fed high level of garlic was lower than

that of fish fed control diet.Our results for this parameter showed similarity to the results of Kalyankar *et al*. (2013). In the this study, RBC levels of fish fed of garlic oil were significantly higher than those of fish the control group. Significant increase in RBC, Hb and Htc of Nile tilapia fed with garlic is reported by Shalaby *et al*. (2006). Felicitta *et al*. (2013) reported a increase of RBC value in juvenile Tilapia after fed with garlic.The results in this study are in agreement with the results in previous investigations. Generally, leukocyte counts are useful as indicators of disease condition or response to infection and significantly elevated or depressed values are obtained in abnormal conditions (Rashidi *et al*., 2012). Martins *et al*. (2002) verified that the addition of garlic to fish diets increased the leukocyte number. In the present study, the WBCvalues of the control individual as well as the thosesupplemented with Tunceli garlic oil were significantly increased($p<0.05$) (Table 1). The other some studies suggest thata garlic doses (0.5 g/kg) had significantly improved leukocyte count (Ndong and Fall 2011). The present study demonstrated that administration of garlic induced a considerable increase in Hb concentration. Increment in Hb contents was recorded in Asian sea bass when fed with garlic diets (Talpur and Ikhwanuddin, 2012). Similar findings were reported by Thanikachalam *et al*. (2010). The erythrocyte indexes MCV and MCH have a wide range of physiological variation (Vázquez and Guerrero, 2007). We found increase in MCV and decrease in MCH and MCHC levels.Fazlolahzadeh *et al* (2011), found that supplementing garlic (*Allium sativum*) in rainbow trout diet decreases MCV but no significant differences observed in MCH and MCHC. Jeney and Anderson (1993) reported that the total number of NBT-positive cells in blood kept rising after treatment with immunostimulant. In this study, an increase in NBT values was detected in fish of the experiment compared with the control group. This suggests that the effect in the phagocytic activity of Tunceli garlic oil. An increase of NBT activity has been reported in tilapia after treatment with the garlic for one month. Phagocytosis is one of the main mediators of non-specific immunity to pathogens including bacteria, viruses, and parasites in fish. The most important cells involved in this defence are the phagocytes. The Allium species showed immune enhancing activities that included promotion of lymphocyte-synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo *et al*., 2001). Sahu *et al*., (2007) studied the effect of garlic on immune response of *Labeo rohita*. Their study documented that supplementation of garlic had bactericidal activity of phagocytic cells. Ndong and Fall (2011) observed that hybrid tilapia fed with 0.5 g/kg supplementation of garlic showed an increased of phagocytic activity.The present study indicated that phagocytic activity of leucocytes increased significantly in scaly carp fed with Tunceli garlic oil.Our results for this parameter showed similarity to the results in previous studies. Serum or plasma proteins have a variety of functions, starting with regulation of the water balance in fish (Wedemeyer andYasutake, 1977), and enable protective effects through the role of acute phase proteins in limiting the dispersal of infectious agents by repairing tissue damage and killing micro-organisms (Larsen *et al*.,2001). Sahu *et al*.,(2007) recorded enhanced total protein levels in rohu fed with 0.1, 0.5 and 1% garlic. Similarly, the highest serum protein level was recorded in rainbow trout fed for 14 days with garlic(Nya and Austin, 2009). But, in our study with fish showed that dietary Tunceli garlic oil concentrations had no effect on total protein and immunoglobulin levels.

This study demonstrates for the first time that after Tunceli garlic oil supplementation, hematological and immunological parameters was significantly increased compared to control groups in fish.

## REFERENCES


1. Drabkin DL. 1946. Spectrometric studies, XIV: the crystallographic and optimal properties of the hemoglobin of man in comparison with those of other species. Journal of Biological Chemistry164:703-723.
2. Divyagnaneswari M, Christybapita D, Dinakaran-Michael R. Enhancement of nonspecific immunity and disease resistance in Oreochromis mossambicus by Solanum trilobatum leaf fractions. Fish Shellfish Immunol. 2007;23:249–259.
3. Fazlolazade, F., Keramati, S. Nazifi, S. Shirian, S. Seifi, "Effect of Garlic (Allium sativum) on Hematological Parameters and Plasma Activities of ALT and AST of rainbow trout in Temperature Stress", Australian Journal of Basic and Applied Science, vol. 5, pp. 84-90, 2011
4. Felicitta, J., Manju, R. A., Ronald, J., Sakthika, T., Nagarajan, R., and Chelladurai, G. (2013). Effect of Different Concentrations Garlic (Allium sativum) and Onion (Allium cepa) on Growth, Survival, and Hematology of Juvenile Tilapia (Oreochromis mossambicus). ISRAELI JOURNAL OF AQUACULTURE-BAMIDGEH, 65.
5. Jeney, G., Anderson, D.P., 1993. Glucan injection or bath exposure given alone or incombination with a bacterin enhance the nonspecific defence mechanisms in rainbow trout (Oncorhynchus mykiss). Aquaculture 116, 315–329.
6. Kalyankar, A.D., Gupta, R.K., Bansal, N., Sabhlok, V.P., Singh, D., 2013. Effect of garlic (Allium sativum) against Aeromonas hydrophila and health management of Swordtail, Xiphophorus helleri A.D. J. Environ. Sci. Sustainability JESS 1 (2), 41–48.
7. Kiron V., J. Puangkaew, K. Ishizaka, S. Satoh, and T. Watanabe (2004) Antioxidant status and nonspecific immune responses in rainbow trout (Oncorhynchus

mykiss) fed two levels of vitamin E along with three lipid sources. Aquaculture 234: 361–379
8. Kyo, E.; Uda, N.; Kasuga, S.; Itakura, Y., 2001: Immunomodulatory effects of aged garlic extract. Journal of Nutrition 131, 1075S–1079S.
9. Natt MP, Herrick CA. 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Pollution Science31:735-738.
10. Martins, M.L., Moraes, F.R., Miyazaki, D.M., Brum, C.D., Onaka, E.M., Fenerick Jr., J., Bozzo, F.R., 2002. Alternative treatment for Anacanthorus penilabiatus (Monogenea: Dactylogyridae) infection in cultivated pacu, Plaractus mesopotamicus (Osteichthyes: Characidae) in Brazil and its haematological effects. Parasite 9, 175–180.
11. Ndong D, Fall J. The effect of garlic (Allium sativum)on growth and immune responses of hybrid tilapia (Oreochromis niloticusx*Oreochromis aureus). Document Scientifique du CRODT, 2007;14:1-22.
12. 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 Immnunology and Immunopathology Research 3(1): 1-9.
13. Nya, E. J.,and Austin, B. (2009). Use of garlic, Allium sativum, to control Aeromonas hydrophila infection in rainbow trout, (Oncorhynchus mykiss Walbaum). Journal of Fish Diseases, 32(11), 963-970.
14. Nya, E. J.,and Austin, B. (2011). Development of immunity in rainbow trout (Oncorhynchus mykiss, Walbaum) to Aeromonas hydrophila after the dietary application of garlic. Fish and shellfish immunology, 30(3), 845-850.
15. Ispir, U., Gokhan, H. B., Ozcan, M., Dorucu, M., and Saglam, N. (2009). Immune response of rainbow trout (Oncorhynchus mykiss) to selected antigens of Yersinia ruckeri. Acta Veterinaria Brno, 78(1), 145-150.
16. Pearce, J., J. E. Harris, and S. J. Davies (2003). The effect of vitamin E on the serum complement activity of the rainbow trout, (Oncorhynchus mykiss Walbaum). Aquacul Nut. 9: 337–340.
17. Ranjan, S., Dasgupta, N., Saha, P., Rakshit, M., and Ramalingam, C. (2012). Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish. Adv. Appl. Sci. Res, 3(1), 495-501.
18. Rashidi, Z., Khara, H., and Mousavi-Sabet, H. (2012). Hematological profile of the mature Rutilus frisii kutum (Cyprinidae) migrated to the Tajan River in the Southern Caspian Sea. World J. Fish mar. Sci, 4, 665-671.
19. Sahu, S.; Das, B. K.; Mishra, B. K.; Pradhan, J.; Sarangi, N., 2007: Effect of Allium sativum on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Journal of Applied Ichthyology 23, 80–86.
20. Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture. 172, 63–92 pp.
21. Saleh, N.E., Michael, F.R. and Toutou, M.M. (2015): Evaluation of garlic and onion powder as phyto-a dditives in the diet of sea bass (Dicentrarcus labrax). Egyptian Journal of Aquatic Research, 41(2), 211-217.
22. Selvaraj V, Sampath K, Sekar V. Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (Cyprinus carpio) infected with Aeromonas hydrophila. Fish Shellfish Immunol 2005; 19:293-306.
23. Shalaby, A. M., Khattab, Y. A., and Abdel Rahman, A. M. (2006). Effects of garlic (Alliumsativum) 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.
24. Sivaram V, Babu MM, Citarasu T, Immanuel G, Murugadass S, Marian MP. Growth and immune response of juvenile greasy groupers (Epinephelus tauvina) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture 2004; 237:9-20.
25. Siwicki, A. K., Anderson D. P. and Ramsey G. L., 1994. Dietary intake of immunostimulants by rainbow trout effects non–specific immunity and protection against furunculosis. Vet. İmmunol. İmmunopathol. 41, 125–139 pp.
26. Syahidah, A., Saad, C. R., Daud, H. M., and Abdelhadi, Y. M. (2015). Status and potential of herbal applications in aquaculture: A review. Iranian Journal of Fisheries Sciences, 14(1), 27-44.
27. Talpur, A.D., Ikhwanuddin, M., 2012. Dietary effects of garlic (Allium sativum) on haemato-immunological parameters, survival, growth, and disease resistance against Vibrio harveyi infection in Asian sea bass, Lates calcarifer (Bloch). Aquaculture 364–365, 6–12.
28. Taşkın, H., Baktemur, G., Kurul, M., and Büyükalaca, S. (2013). Use of tissue culture techniques for producing virus-free plant in garlic and their identification through real-time PCR. The Scientific World Journal, 2013.
29. Tavares-Dias M, Moraes FR. 2006. Hematological parameters for the Brycon orbignyanus Valenciennes, 1850 (Osteichthyes: Characidae) intensively bred. Hidrobiológica 16:271-274.
30. Thanikachalam, K., Kasi, M., Rathinam, X., 2010. Effect of garlic peel on growth, hematological parameters and disease resistance against Aeromonas hydrophila in African catfish Clarias gariepinus (Bloch) fingerlings. Asian Pac. J. Trop. Med. 3 (8), 614–618.
31. Wedemeyer, Gary A., and William T. Yasutake. Clinical methods for the assessment of the effects of environmental stress on fish health. No. 89. US Fish and Wildlife Service, 1977. 18pp.
32. Wintrobe MM. 1934. Variations on the size and hemoglobin content of erythrocytes in the blood various vertebrates. Folia Haematologica51:32-49.
33. Wu, C. C., Liu, C. H., Chang, Y. P., and Hsieh, S. L. (2010). Effects of hot-water extract of Toona sinensis on immune response and resistance to Aeromonas hydrophila in Oreochromis mossambicus. Fish and shellfish immunology, 29(2), 258-263.
34. Vázquez, G. R.,and Guerrero, G. A. (2007). Characterization of blood cells and hematological parameters in Cichlasoma dimerus (Teleostei, Perciformes). Tissue and Cell, 39(3), 151-160.
35. Yazar, E.,Propagation of Tunceli garlic Allium tuncelianum (Kollman, N. ¨Ozhatay, D. Matthew, S¸. S¸iraneci) through in vitro root and shoot tip culture [M.S. thesis], University of Ankara, Turkey, 2006.

36. Yin, G., Ardó, L. Á. S. Z. L. Ó., Thompson, K. D., Adams, A., Jeney, Z., and 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.

Source of Support: None Declared
Conflict of Interest: None Declared