Journal of
Aquaculture Engineering and Fisheries Research

1(3): 133-139 (2015) E-ISSN 2149-0236 doi: 10.3153/JAEFR15014

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

SHORT COMMUNICATION KISA MAKALE

DETERMINATION OF SOME HAEMATOLOGICAL AND NON-SPECIFIC IMMUNE PARAMETERS IN NILE TILAPIA (*Oreochromis niloticus* L., 1758) FED WITH SPIRULINA (*Spirulina platensis*) ADDED DIETS

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Received: 22.01.2015

Accepted: 10.03.2015

Published online: 16.04.2015

This research was supported by the Academic Research Projects Unit of Cukurova University (Project Number: SUF2009 BAP11).

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Abstract:

In this study, growth performance, haematological and non specific immune system parameters of Nile Tilapia (*Oreochromis niloticus* L., 1758) fed with diets which contain different levels of (0; 5.0; 7.5 and 10.0 g/kg) spirulina (*Spirulina platensis*) were investigated. At the end of the feeding experiment (75 days), total erythrocyte (RBC) and leukocyte counts (WBC), hematocrit (Hct), hemoglobin (Hb), leukocyte cell types (monocyte, lymphocyte, neutrophil, eosinophyl), RBC indices (MCV, MCH, MCHC) and phagocytic activity of blood taken from fish were determined in a certain period. According to the results, the addition of 5.0 g/kg spirulina in diet highly increased the phagocytic activity of neutrophils and monocytes and also the addition of 7.5 g/kg of spirulina in diet increased the amounts of RBC and WBC.

Consequently, these two levels of spirulina can be used as a protective agent against diseases by supporting the fish immune system.

Keywords: *Spirulina platensis*, Haematology, Non-Specific Immune Response, *Oreochromis niloticus*.

Introduction

In fish hatcheries, the indiscriminate use of antibiotics in prophylactic treatment has led to the development of the resistent strains and the need to swicth over to other antibiotics. The antibiotics also may reduce the larval growth and inhibit defence mechanisms of the fish larvae (Citarasu, 2010).

For this reason, prevention of insensible application of medication, focusing on prophylactic drug applications, improving the immune system of the organism and to include high protein content alternative additives in fish food rations is very important from the health and the growth performance of the organism.

Spirulina (*Spirulina platensis*), used in this research, is a blue-green filamentous algae and play an important role on phagocytic activity of macrophages, stimulate of antibodies, antibacterial effect and non-specific immune system (Watanuki et al., 2006; Hirohashi et al., 2002).

In this study investigated growth, the non-specific immune function and haematological effects of spirulina (0; 5; 7.5 and 10 g/kg) in Nile Tilapia (*O.niloticus*).

Materials and Methods

Experimental design and fish materials

This experiment was conducted in Dr. Nazmi Tekelioglu Freshwater Research Station of Cukurova University, Faculty of Fisheries. The three hundred of *Oreochromis niloticus* (initial mean weights; 10.00 ±0.98 g) were randomly 20 fish stocked into 12 net cages (1x1x1 m sizes) as three replicates during 75 days. The fish were fed two times a day with different levels of spirulina (0; 5.0; 7.5 and 10.0 g/kg) diets during the whole experimental period.

Experimental diets

Spirulina culture: Microalgae *Spirulina platensis* stock cultures were maintained at 24 ±2°C on continuous illumination with flourescent (Philipswhite, 36 watt) lights in erlenmayers (250 mL, 500 mL, 2 L) and carboys (5 L and 10 L) in laboratory conditions. Their radiance, as measured by a Radiation Sensor LI-COR (LI-250), was 80 µmol. Photon. $m^{-2}.s^{-1}$. The stock cultures were grown in spirulina medium (Boussiba et al., 1992).

Formulation of the experimental diets are presented in Table 1. Four isonitrogenic (35% crude protein) and isolipidic (10% crude lipid) diets were used. Also, different levels (0; 5.0; 7.5 and 10.0 g/kg) of *Spirulina platensis* powder added to diets, air dried and stored at 4 °C during the experiment.

Moisture and ash contents were determined according to AOAC, 1995. The protein and lipid contents of samples were determined using Kjeldahl, Blingh and Dyer (1959) methods (chloroform/methanol; 2:1, vol/vol), respectively (Mattisek et al., 1988).

Somatic indexes, haematological and non-specific immune analysis

After 75 days of feeding, 15 fish from each experimental diet were collected then weighed their viscera and livers in order to find somatic indexes. Before the haematological and non-specific immune analysis, fish in all groups were anaesthetized with Quinaldine *sulphate* (20 ml/L 4-5 dk.) (Sigma Chemical Co., Germany).

After the blood samples were taken from the caudal vein using a syringe from anaesthetized fish and transferred into tubes with EDTA and stored at 4°C. RBC and WBC were counted using Natt-Herrick solution and Thoma microslide. Cyanmethaemoglobin and microhaematocrit methods were used to determine Hb and Hct (Blaxhall and Daisley, 1973; Tanyer, 1985). MCV (Mean Corpuscular Volume) (μ^3), MCH (Mean Corpuscular Haemoglobin) (pg) and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated according to Mumford et al., 1994.

The oxidative radical production of phagocytes was measured by spectrophotometric assay of nitroblue tetrazolium (NBT) activity (Siwicki and Anderson, 1993). Peripheric blood smears (PBS) were stained with the mixture of May-Grünwald and Giemsa. Percentages of leukocyte cell types were determined using these preparations (Mumford et al., 1994).

Table 1. Experimental Diet Groups

Ingredients (g/kg)	Control	Spirulina		
		5.0 (g/kg)	7.5 (g/kg)	10 (g/kg)
Fish meal	425	425	425	425
Wheat meal	245	245	245	245
Corn meal	130	130	130	130
Dextrin	80	80	80	80
Fish oil+Sunflower oil (1:1)	50	50	50	50
CMC	20	15	12,5	10
DCP	10	10	10	10
Vit Mix	20	20	20	20
Min Mix	20	20	20	20
Spirulina	**0**	**5.0**	**7.5**	**10**
Chemical composition (% in dry matter)				
Ash	12.37	11.91	11.91	11.84
Moisture	6.30	5.86	6,42	5.71
Protein	34.70	35.20	35.67	35.91
Lipid	10.45	10.12	10.13	10.34

Table 2. Some Growth Parameters of Tilapia (average initial body weight, 10.00±0.98 g)

Parameters	Control	Spirulina		
		5.0 (g/kg)	7.5 (g/kg)	10 (g/kg)
FBW	49.88±1.67[b]	43.21±4.45[a]	44.67±1.08[a]	42.58±2.38[a]
FE[2]	0.71±0.04[b]	0.83±0.16[c]	0.65±0.05[a]	0.78±0.14[b]
SGR[3]	2.14±0.04[b]	1.94±0.13[a]	1.99±0.03[ab]	1.92±0.07[a]
VSI[4]	8.88±0.58	7.87±0.51	7.92±0.55	7.78±0.52
HSI[5]	3.87±0.25	3.43±0.46	3.95±0.35	3.32±0.27

[1] Values are means± SD. Values in the same line with different superscript are significantly different ($p < 0.05$).
[2]Feed Efficiency=Wet weight gain/dry feed intake
[3]Specific Growth Rate=100×[ln(final body weight ((FBW))-ln(initial body weight (IBW))]/day
[4]Viscerasomatic Index=100 × (Visceral area lipid weight/FBW). Initial level was: 7,95±1,08
[5]Hepatosomatic Index=100×(Liver weight/FBW). Initial level was: 1,16±0,47

Statistical analysis

The data obtained from the experimentation were evaluated using SPSS 15.0 Windows software package.

Results and Discussion

Results

The oxygen level, water temperature and pH values were respectively measured as 6.9 ±1 mg/L, 23.3 ±1 and 7.5 ±1 ºC.

Table 2, shows the final body weight (FBW), feed efficiency (FE), specific growth rate (SGR), visceral somatic index (VSI) and hepatosomatic index (HSI) of tilapia with the experimental diets featuring different spirulina levels during 75 days. According the results, FBW and SGR values were statistically different in between control and experimental groups among the groups ($P<0.05$). The other parameters (FE, VSI and HSI) showed no significant differences among the groups ($P>0.05$).

Haematological and Non-Specific Immune Parameters

The values of health indicators in fish such as the RBC, Hct and Hb levels and the RBC indices were displayed in the Table 3. The highest RBC amount was determined in fish fed with 7.5g of spirulina. Hct amount was significantly higher in groups fed with 10 g spirulina ($P<0.05$). The MCV was identified to significantly lowest in the group fed with 7,5 g spirulina and MCH was found to significantly lowest in the groups fed with 5 and 7.5 g spirulina ($P<0.05$). MCHC was found to be significantly lowest in groups fed with 5.0 and 10 g spirulina ($P<0.05$).

The highest NBT activity values were obtained at 5.0 g/kg of dietary spirulina, mean while the lowest value was obtained at 10 g/kg. The NBT activity of fish fed diets containing 5% spirulina was significant difference from those at the all groups ($P<0.05$).

Leukocytes, which are the cellular elements of the immune system in fish, were determined to be present in elevated levels in fish fed with 7.5 g/kg spirulina whereas the lymphocyte and eosinophil cell concentrations were significantly higher in the groups fed with 5.0 and 10 g/kg of spirulina. The concentration of neutrophils functioning in phagocytosis did not display a significant difference between different groups whereas the monocyte level was lower in the group fed with 5.0 g/kg of spirulina (Table 4). The stained blood photos were shown in Figure 1.

In various published studies have been shown significant therapeutic effects of Spirulina or its extracts on animals and humans (Watanuki et al., 2006). Latest, Spirulina has been suggested as immunomodulatory, especially, associated with non-specific immune system (Duncan and Klesius, 1996; Watanuki et al., 2006; Abdel-Tawwab and Ahmad, 2009). Blackmoli (*Poecilia latipinna*) larvae were tested using four different types of foods. Larvae were fed microalgae Spirulina, rotifer *Brachionus plicatilis*, Artemia and an artificial diet. At the end of 21 days of culture period the best performance in the form of live weight gain and survival rate of larvae was observed on spirulina diet (Tekelioğlu et al., 2005).

Growth performance results from this study indicated that *Oreochromis niloticus* had an optimum growth. Ogunji et al. (2008) have used the same species (initial weight; 2.85 g) in their study. They found the final body weight between 11g to 16 g in 56 days experimental period. Generally feed efficiency values for tilapias range between 0.65 and 0.80 for fish fed well prepared feeds (De Silva and Anderson, 1995). Our results have an acceptable growth and feed utilization values compared with the previous results.

Spirulina platensis is widely used in many countries as a health food due to its protein content and biochemical substances for immune system (Richmond, 1992). *Clarias gariepinus*, which received feed 5% spirulina had higher values for red and white blood cell counts and the lysozyme activity. The present study found that fish fed with 5% spirulina exhibited higher red and white blood cell counts and a higher immunity stimulating capacity (Promya and Chitmanat, 2011).

RBC, Hb and Hct values were determined to be very high in Tilapia fed with 7.5 and 10 g of spirulina. The studies conducted in recent years indicated the positive effects of feeding 7.5 and 10 g of spirulina on health indicators of fish such as the RBC indices and amounts (Duncan and Klesius, 1996).

Table.3 Haematological Parameters of Tilapia

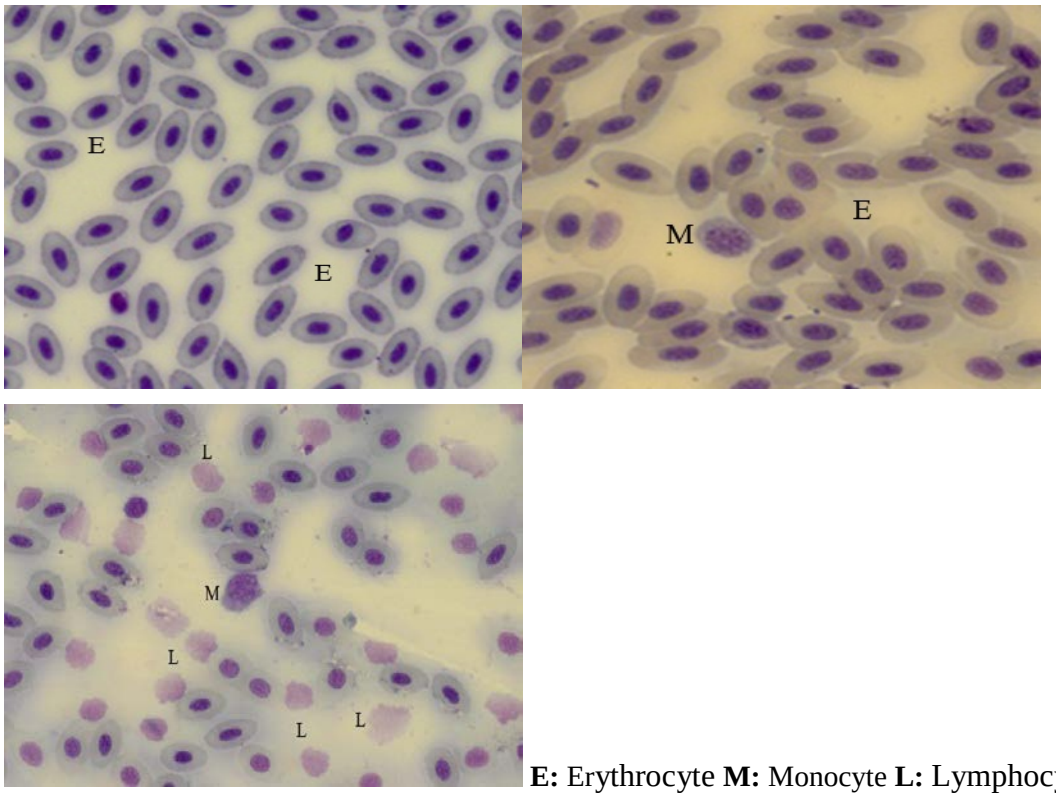
Parameters	Control	Spirulina		
		5.0 (g/kg)	7.5 (g/kg)	10 (g/kg)
RBC (10^6/mm^3)	2.34 ±0.27^a	3.15 ±0.25^b	3.61 ±0.05^c	3.08 ±0.18^b
Hct (%)	33.08 ±2.37^a	34.79 ±2.58^a	34.37 ±3.35^a	38.00 ±1.70^b
Hb (g/dL)	10.34 ±0.59^b	9.71 ±0.79^a	10.57 ±0.60^b	10.69 ±0.52^b
MCV (μ^3)	142.75 ±18.76^d	111.04 ±10.45^b	95.25 ±9.50^a	123.80 ±9.74^c
MCH (Pg)	44.63 ±5.66^c	31.08 ±3.93^a	29.27 ±1.58^a	34.77 ±2.00^b
MCHC (% g)	31.35 ±2.23^b	28.11 ±3.55^a	30.98 ±3.36^b	28.17 ±1.80^a

Values are means± SD. Values in the same line with different superscript are significantly different ($p<0.05$).

Table.4 Non-Specific Immune Parameters of Tilapia

Parameters	Control	Spirulina		
		5.0 (g/kg)	7.5 (g/kg)	10 (g/kg)
NBT (mg/mL)	1.22 ±0.42^a	1.60 ±0.21^b	1.15 ±0.16^a	0.95 ±0.19^a
Leukocyte (x10^3/mm^3)	23.31 ±0.82^a	25.31 ±1.85^b	35.68 ±1.00^c	24.77 ±1.92^b
Lymphocyte (%)	79.67 ±2.41^a	84.99 ±3.81^b	80.48 ±1.77^a	83.84 ±2.96^b
Monocyte (%)	17.60 ±2.00^b	11.39 ±1.83^a	17.38 ±1.47^b	13.53 ±2.30^c
Neutrophil (%)	2.38 ±0.45^a	2.42 ±1.01^a	1.99 ±0.69^a	1.81 ±0.64^a
Eosinophil (%)	0.60 ±0.88^a	1.97 ±0.46^b	0.71 ±0.21^a	0.82 ±0.38^b

Values are means± SD. Values in the same line with different superscript are significantly different ($p<0.05$).



E: Erythrocyte **M:** Monocyte **L:** Lymphocyte

Figure. 1 The Blood Cells in Tilapia Fed with Different Levels of Spirulina

The innate cellular immune system plays an essential role in host-defense mechanisms. As Siwicki and Studnicka, 1987 indicated that this cellular immune system includes the phagocytic cells such as monocytes/macrophages and neutrophils which play a fundamental role in protection and survival during adverse conditions.

Phagocytosis in fish is the primary mechanism of the non-specific immune response to pathogenic microorganisms. The NBT activity test is one of the methods used to assess the phagocytic activity of neutrophils and monocytes (Siwicki and Anderson, 1993). Abdel-Tawwab and Ahmad (2009) indicated that tilapia fed diets containing 7.5 and 10g/kg spirulina showed a significant increase in NBT values. In the present study, NBT activity was significantly increased 5.0 g/kg of dietary spirulina indicating an improved non specific immune response of the fish. These facts support the present finding that lymphocyte and eosinophils also showed significant difference at 5.0 g/kg of dietary spirulina ($p<0.05$). Watanuki et al. (2006), investigated the effect of feeding carp infected with *Aeromonas hydrophila* with food containing *Spirulina platensis* on the immune system. The results of the study indicated reduced bacterial counts specifically in blood, liver and kidneys upon administration of spirulina. Spirulina was reported to increase phagocytic activity in blood and other tissues and were even useful in establishing immunity upon birth in carps. They highlighted the importance of enriched spirulina diets on the physiological parameters of fish.

Recent studies stressed the therapeutic effects of spirulina as immunostimulant in different animal species. Duncan and Klesius (1996), investigated the specific and non-specific immune response of channel catfish fed with *Spirulina platensis*. The study indicated that spirulina caused increased non-specific immune response and that they aided defense of the fish against *Edwardsiella ictaluri*. Specifically, the percent macrophage from phagocytic cells and the number of red blood cells were shown to display significant increase.

Several studies on Channel catfish, carp and Nile Tilapia reported a reinforcement of the non-specific immune system upon feeding on spirulina and that spirulina acted as an immunostimulator in fish.

Conclusion

The growth performance of tilapia fed on spirulina-supplemented diets was within acceptable limits in the study and the total body nutrient quality was determined to be at optimum levels. Additionally, the administration of spirulina at a concentration of 7.5 g/kg was shown to increase in the erythrocyte and leukocyte concentrations that even a lower dose of 5.0 g/kg was effective in immune response cells.

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