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RESEARCH PAPER

Comparative Study of Some Hematological and Biochemical Parameters of Italian and Turkish Farmed Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract

Blood parameter assessments of fish may be used as quick tools for diagnosing health status. The aim of this research was to undertake a comparative study of hematological and biochemical parameters of Italian and Turkish farmed rainbow trout *Oncorhynchusmykiss*(Walbaum, 1792) to establish baseline values in relation to different areas. Forty Italian trout (32.59±0.25 cm total length, 397.40±6.49 g weight) and forty Turkish trout (33.00±0.24 cm total length, 385.70±3.50 g weight) were examined.

Statistical analysis (unpaired *t*-test) showed no significant differences in weight, length and condition factor between two farmed trout groups. Statistically significant differences ($P < 0.05$) were found in some hematological and biochemical parameters between Italian and Turkish farmed rainbow trout.

Our findings shown that in Italian farmed rainbow trout Red blood cell (RBC), Hematocrit (Hct), Cholesterol and Total protein values were significantly lower than Turkish trout, instead Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentrations (MCHC), Glucose, Triglycerides and Serum albumin shown significantly higher values. No statistically significant differences were found for White blood cell (WBC) and hemoglobin (Hgb).

The results of this study contribute to knowledge of the baseline of hematological and biochemical parameters of rainbow trout farmed in two different habitats suggesting that blood parameters could be an important value in monitoring environmental conditions on fish physiology and fish culture management.

Keywords:Blood parameters, fish farms, Italy, *Oncorhynchusmykiss*, Turkey.

Introduction

Rainbow trout (*Oncorhychusmyakiss*) is a fish belonging to the family of Salmonidae and native to the cold water rivers and lakes of the Pacific coasts of North America and Asia; the habitat and food of rainbow trout determine both their actual color and shape.

Since 1874 *O. mykiss* has been introduced to all continents except Antarctica, for recreational angling and aquaculture purposes, because it is a resistant fish, easy to spawn, fast growing and capable of occupying many different habitats because it can tolerate a wide range of environmental and production conditions better than other trout species (Parisi*et al*., 2014).

The production of rainbow trout has grown exponentially in the 1950s especially in Europe where is one of the main species for aquaculture farmed both for food and sports. Italy is one of the most important European countries of production and breeding of this fish beside Norway, France, Denmark, Spain and Germany. Today, nearly all rainbow trout on the EU market come from aquaculture. Most of the EU supply of trout is locally produced. Currently the largest producers worldwide are Chile, Norway, Iran and Turkey (Woynarovich*et al*., 2011).

In Turkey the modern aquaculture activities began in the 1960s with rainbow trout cultured from eyed eggs imported from Italy. Nowadays, among the main cultured species, the production of rainbow trout currently represents the main source for Turkish aquaculture (followed by European seabass and gilthead sea bream) with an increasing trend. With regard to the production systems concrete pools and off-shore cages are currently the most used in Turkey for rainbow trout farming (Giannetto*et al*., 2015).

Analysis of haematological and biochemical parameters represent a valuable approach for monitoring the health status of farmed animals

because these parameters are specific among different species (Çelik 2004; Asadi*et al*., 2007). Haematological and biochemical studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. A multitude of intrinsic and extrinsic factors cause variations in haematological data such as water quality, temperature (Hrubec *et al*. 1997, Langston *et al*., 2002; Magill and Sayer, 2004), nutritional state (Svetina*et al*., 2002; Lim and Klesius, 2003), stress (Cnaani*et al*., 2004), culture conditions (Hrubec*et al*., 1996), the cycle of sexual maturity, photoperiod (Leonardi and Klempau, 2003), age or sex of the fish can affect some blood values.

One of the difficulties in assessing the state of health of farmed fish population has been the paucity of reliable references of the normal condition (Nicula*et al*., 2010; Fazio *et al*., 2012a, 2015a).

Therefore, to establish normal estimates is difficult for rainbow trout *O. mykiss*as well as for the other fish species.

The main objective of the present study was the comparative investigation of haematological and biochemical parameters of Italian and Turkish farmed rainbow in order to establish baseline values in relation to different geographic locations, to provide a further contribution to the knowledge of adaptive response of farmed fish and to emphasize the fact that changes in blood characteristics are important indices for monitoring the effects of habitat changes on fish physiology.

Materials and Methods

All experimental procedures were carried out in accordance with European legislation regarding the protection of animals used for scientific purposes (European Directive 2010/63).

Italian Farm

The Italian farm, located in PalazzoloAcreide, Siracusa, Sicily (Figure 1a) (Lat. 37° 06' 19"N, Long. 14° 90' 41" E) at an altitude of 679 m above the sea level, consists of 11 open concrete rectangular tanks.

Each rectangular tank was 5 m in width, 20 m in length and 3.5 m deep. The volume of each tank was 80 m^3. Fish stocking density was 25 kg/m^3 for tank.

Turkish Farm

The Turkish farm, located in Fethiye, Mugla (Figure 1b) (Lat. 36˚45' 53" N, 29˚ 24' 03" E) at an altitude of 232m above the sea level, consist of 94 open concrete rectangular tanks.

Each rectangular tank was 3.5 m in width, 20 m in length and 1.7 m deep. The volume of each tank was120 m^3. Fish stocking density was 25 kg/m^3 for tank.

Sampling and Analytic Methods

In this study 80 rainbow trout (*Oncorhyncusmykiss*) in excellent health were investigated in two different commercial farms PalazzoloAcreide (Siracusa, Italy) and Fethiye (Mugla, Turkey). They were divided into two equal groups on the basis of the site of collection. Forty fish (32.59±0.25 cm total length, 397.40±6.49 g weight) were caught in PalazzoloAcreide, (Italy), where they were subjected to natural day/night cycle (11L/13D) and 40 fish (33.00±0.24 cm total length, 385.70±3.50 g weight) were caught in Fethiye (Turkey) and subjected to natural day/night cycle (13L/11D). Fish of both farms were fed the same commercial dry food (Crude Protein 46%; Crude Fat 20%; Ash 10%; Fiber 1.5%) distributed twice daily 7 days a week. All fish samples were collected in November 2015. All fish were considered healthy on the basis of an external examination for any signs of abnormalities or infestation.

In both farms, physical and chemical characteristics of water have been detected. Temperature, salinity, pH and dissolved oxygen (DO) were measured using a handheld multiparameter instrument (model YSI 556 MPS - Ohio, USA) and these values are shown in Table 1.

On the same day, all fish, in the respective farms, were randomly captured by the same tank to evaluate the biometric, haematological and biochemical parameters. After capture the fish of both groups were anaesthetized prior to blood sampling using MS222 at the concentration of 0,7 g/L. Immediately after anaesthetization the fish were individually weighted using a balance (Kern 440-49 N, Germany) and their total length was recorded using an ictiometer (Scubla SNC, 600 mm, Italy).

The Condition Factor (K) was also calculated as $W \times 100/L^3$ where W is the weight of the fish in grams (g), L is the length of the fish in centimeters (cm). For salmonids, K values usually fall in the range 0.8 to 2.0 (Davis and Lebourdais, 2007).

Blood samples were collected between 8:00 a.m. and 10:00.a.m. and were obtained from the puncture of caudal vein using a 18 G × 1 ½ syringe and transferred into 2 different tubes, one (Miniplast 0.6 mL; LP Italiana Spa, Milano) containing ethylenediaminetetraacetic acid (EDTA) (ratio 1.26 mg/0.6 mL) as anticoagulant agent for the assessment of hematological profile and the other (Terumo Corporation, Japan) without anticoagulant agent for the assessment of biochemical profile.

The analytical procedure was performed in order to determine the following blood parameters: red blood count (RBC), hemoglobin concentration (Hgb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). RBC, Hct and Hgb were determined by using the method of Blaxhall and

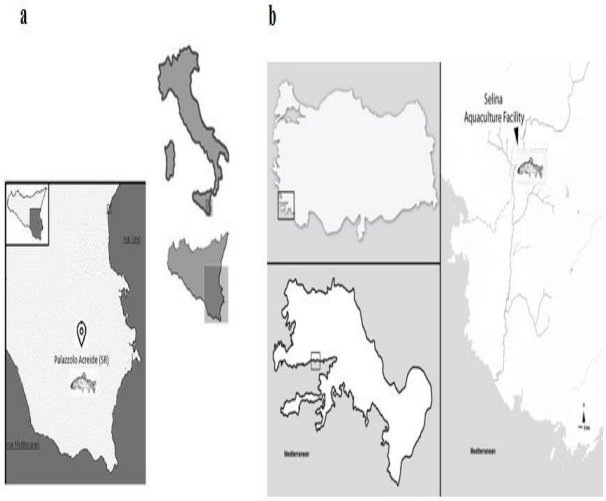

Figure 1. Map of the study areas. A, location of Italian farm; location of Turkish farm.

Table 1. Water quality values (Mean±SEM) for the two farms assessed in the month of November 2015 and biometric data of Italian and Turkish Rainbow trout (*Oncorhynchusmykiss*). Means without the same alphabetical characters within the same parameters represent statistical differences (P < 0.05)

Water Parameters	Italian Farm	Turkish Farm
Temperature (°C)	16.60±0.26[a]	14.13±0.09[b]
Water salinity (ppt)	0.30±0.06[a]	0.22±0.01[a]
Dissolved Oxygen (mg/L)	7.33±0.09[a]	8.56±0.10[b]
pH	8.23±0.09[a]	8.21±0.05[a]
Biometric Parameters		
Weight (g)	397.40±6.49[a]	385.70±3.50[a]
Total length (cm)	32.59±0.25[a]	33.00±0.24[a]
Condition factor K	1.17±0.04[a]	1.09±0.02[a]

Daisley (1973). RBC and WBC were counted with a Neubauerhaemocytometer (Shah and Altindag, 2005) with the usage of Dacie's diluting fluid. Hct was determined by using a capillary hematocrit tube. Hgb concentration was determined with spectrophotometry (540 nm) by using the cyanomethahemoglobin method. MCV, MCH and MCHC were calculated indirectly by the direct parameters values using following formula (Bain *et al.,* 2006):

MCV = Hct × 10/RBC, MCH= Hbg × 10/RBC, MCHC= Hgb × 100/Hct

For biochemical analysis sera were obtained by centrifugation (10 min at 3000 rpm at 4 °C) of blood samples and stored at -20 °C until they were used. Measured biochemical parameters include glucose, triglycerides, cholesterol, total protein and serum albumin that were determined using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) and a Shimadzu spectrophotometer (PG Instruments, UK). All haematological and biochemical analysis were performed in triplicate by the same operatorwith a same instrument under the same conditions, and in a short period of time.

Statistical Analysis

Analytical data, expressed as mean (M) ± standard error of the mean (SEM), are the averages of three analyses carried out by the same operator. Samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation was < 9%. Data obtained for haematological and biochemical parameters were tested for normality using the Kolmogorov-Smirnov test. P<0.05 was considered statistically significant. An unpaired *t*-test was applied in order to evaluate statistically significant existing differences between haematological and biochemical parameters of the two groups of fish (P< 0.05).

Data were analyzed using statistical software Prism v.5.00 (Graphpad Software Ltd., USA, 2003).

Results

In Table 1 the mean values (M) ± standard error of the means (SEM) of biometric data recorded in the two groups of fish studied and water quality values for the two farms are listed. No significant differences

were found in biometric data (total length, weight and condition factor) between the two groups.

The temperature of Italian farm was higher than Turkish with a statistical P value of (P<0.0001) while dissolved oxygen was higher in Turkish farm than Italian with P value (P<0.0001). No significant differences were found in water salinity and pH between the two farm.

Statistical results for the evaluated haematological and biochemical parameters are respectively showed in Tables 2 and 3.

Unpaired *t*-test showed statistically significant differences between the two groups of fish on RBC (*P*<0.0001), Hct (P<0.0001) MCV (P<0.0001), MCH (P<0.0001), MCHC (P<0.0001), glucose (P<0.001), triglycerides (P<0.0001), cholesterol (P<0.0001), total protein (P<0.0001) and serum albumin (P<0.001). In particular, MCV, MCH, MCHC, glucose triglycerides and serum albumin were higher in Italian farmed rainbow trout with respect to Turkish farmed trout. On the contrary, RBC, Hct, cholesterol and total protein resulted higher in Turkish farmed trout

No significant differences were found in weight, length, condition factor, WBC and Hgb in the two groups of fish.

Discussion

In this study some haematological and biochemical parameters of Italian and Turkish farmed rainbow trout *O. mykiss*were measured. Several parameters have revealed statistically significant differences (P<0.05) between the two groups of fish in unpaired *t*-test.

Many biotic and abiotic factors may affect blood parameters in fish. Previous researches showed that physico-chemical differences in the habitats influence the haematological parameters and serum

Table 2. Statistical results for the evaluated haematological parameters in farmed Rainbow trout (*Oncorhynchusmykiss*) in Italian and Turkish farms. Means without the same alphabetical characters within the same parameters represent statistical differences (P<0.05)

Parameters	Farms	Mean ± SEM	Median	95% confidence interval Mean±1.96 σ	2.5^{th}-7.5^{th} percentile range
RBC (x 10^6/µL)	Italy	1.55±0.02[a]	1.55	1.51±1.60	1.47±1.64
	Turkey	4.45±0.09[b]	4.55	4.25±4.64	3.93±5.07
WBC (x 10^3/µL)	Italy	20.24±0.13[a]	20.11	19.96±20.51	19.70±20.58
	Turkey	20.42±0.21[a]	20.36	19.99±20.85	19.07±21.47
Hgb (g/dL)	Italy	10.59±0.26[a]	10.55	10.06±11.12	9.50±11.90
	Turkey	10.07±0.20[a]	10.14	9.65±10.48	9.25±11.11
Hct (%)	Italy	29.67±0.48[a]	29.25	28.70±30.64	28.06±32.24
	Turkey	37.40±0.63[b]	37.00	36.13±38.67	33.25±41.75
MCV (fL)	Italy	190.80±1.04[a]	190.20	188.70±192.90	185.60±194.90
	Turkey	85.66±2.37[b]	84.12	80.86±90.45	75.47±94.28
MCH (pg/cel)	Italy	68.06±1.35[a]	67.86	65.34±70.79	63.36±71.13
	Turkey	23.16±076[b]	22.78	21.61±24.70	18.66±26.79
MCHC (%)	Italy	35.70±0.72[a]	34.87	34.24±37.17	32.81±37.36
	Turkey	27.22±0.71[b]	27.22	25.77±28.66	24.58±30.16

*RBC (red blood cells); WBC (white blood cells), Hgb (haemoglobin concentration); Hct (haematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration).

Table 3. Statistical results for the evaluated biochemical parameters in farmed Rainbow trout (*Oncorhynchusmykiss*) in Italian and Turkish farms. Means without the same alphabetical characters within the same parameters represent statistical differences (P<0.05).

Parameters	Farms	Mean ± SEM	Median	95% confidence interval Mean±1.96 σ	2.5^{th}-7.5^{th} percentile range
GLUCOSE	Italy	77.93±4.39[a]	74.00	69.04±86.81	59.25±98.75
(mg/dL)	Turkey	58.99±5.65[b]	49.83	47.56±70.43	36.07±67.15
TRIGLYCERIDES	Italy	331.70±19.49[a]	318.00	292.20±371.10	234.80±391.50
(mg/dL)	Turkey	133.40±9.99[b]	115.40	113.20±153.60	83.81±183.00
CHOLESTEROL	Italy	229.00±8.07[a]	229.50	212.70±245.30	200.80±252.00
(mg/dL)	Turkey	347.50±12.82[b]	345.80	321.60±373.50	288.00±392.20
TOTAL PROTEIN	Italy	3.10±0.05[a]	3.12	3.00±3.21	2.85±3.28
(g/dL)	Turkey	5.24±0.19[b]	5.17	4.86±5.61	4.50±5.94
SERUM ALBUMIN	Italy	0.84±0.02[a]	0.85	0.80±0.88	0.75±0.96
(g/dL)	Turkey	0.73±0.02[b]	0.74	0.68±0.78	0.64±0.81

biochemistry in fish suggesting that hematological parameters may be suitable for monitoring the effects of habitat changes on fish biology and fish culture practices (Fazio *et al*., 2012b, 2012c).

The influence of biometric data such as the weight on some haematological parameters like Hct, RBC and Hgb was previously shown in rainbow trout *O. mykiss* and in *Sparusauratab*y other authors (Lowe-Jinde and Niimi, 1983; García*et al*., 1992).

Environment, and in particular geographical variations, influences the biometric indices of fish (Rahman*et al*., 2013) but in this study the results showed no statistically significant differences of biometric indices and condition factor of rainbow trout sampled in Italy respect to the same species farmed in Turkey. On the contrary, statistically significant variations in some blood haematological and biochemical parameters between Italian and Turkish farmed *O. mykiss* were observed.

Regarding the haematological parameters, it was found RBC (1.55±0.02), WBC (20.24±0.13) and Hgb (10.59±0.26) values in accordance with those shown in a previous study (Fazio *et al*., 2015b).

In particular the results obtained showed that in Italian farmed rainbow trout RBC and Hct values were significantly lower than Turkish, instead MCV, MCH, MCHC shown statistically significant higher values. No statistically significant differences were found in WBC and Hgb.

In Italian farm we have recorded statistically significant higher values of water temperature and lower values of DO than Turkish farm; in fact it is well known that the first effect of increased temperature is an increased metabolic rates and consequently to enhance O_2 consumption. Therefore, to counterbalance this effect blood changes (with an increase of RBC) will occur that make up for any oxygen lost and provide a sufficient amount of oxygen to the tissues (Hazel and Prosser 1974; Haschemeyer, 1978).

However, our results disagree with this previous research and showed statistically significant lower values of RBC than Turkish farm, this can probably be due to the influence of photoperiod. Although the influence of photoperiod on haematological parameters are rather few in fish and responses observed are quite variable the results of this study are in accordance with what is shown in a previous work (Solomon Okomoda, 2012), in which it was found an increase of erythrocytes number in fish exposed of an increasing number of hours of light. In fact in our study the Italian farmed rainbow trout were subjected to a natural day/night cycle (11L/13D) with less hours of light than Turkish farmed fish (13L/11D).

Regarding the biochemical parameters the study showed significantly lower concentrations of total protein and cholesterol in Italian farmed rainbow trout than Turkish. Glucose, triglycerides and serum albumin whilst showed statistically significant higher values. These differences of the biochemical parameters in the two studied fish groups are a consequence of the influence of the different habitat conditions and in particular of the different environment conditions as proven by other researches (Jirasek*et al*., 1998; Hrubec*et al*., 2000; Chen *et al*., 2003; Mensinger*et al*., 2005; Aras *et al*.,2008.

Haematological and biochemical parameters have been acknowledged as valuable tools for monitoring fish health and their normal values are still undefined for the different species of cultured fish. The results of this preliminary study provide the knowledge of the characteristics of blood parameters of rainbow trout *O. mikiss*from two different countries and allow a better understanding of the environmental conditions influence on the haematological and biochemical parameters of rainbow trout farmed in two different areas. In the future researches it would be necessary to investigate other farmed species in these two locations increasing the number of the investigated parameters to expand the basic knowledge that contributes to the growth and improvement of European aquaculture.

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