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Research article

Intra-variability of some biochemical parameters and serum electrolytes in rainbow trout (Walbaum, 1792) bred using a flow-through system

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ABSTRACT

This study aims to evaluate some biochemical parameters and serum electrolytes in cultured rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) to evaluate a potential correlation with biometric parameters (weight and length). For this purpose, 100 cultured trout (300–700 g weight range, 25–38 cm length range) bred on a fish-farm were used for the study. Physico-chemical characteristics of water were measured on the farm. Blood samples were collected from each fish to analyze the following parameters: glucose, triglycerides, cholesterol, aspartate aminotransferase (AST); alanine aminotransferase (ALT), calcium (Ca^{2+}), chlorine (Cl^{-}), iron (Fe^{2+}), phosphorus (P), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}) and urea. Statistical data analysis showed a significant correlation between size and glucose and cholesterol. No correlation was found between size and other parameters studied. These results represent a contribution to the study of fish size leading to better understanding some biochemical parameters and serum electrolyte profiles in cultured rainbow trout. This research contributes to understanding the intra-individual variability of some blood parameters in cultured rainbow trout *O. mykiss* offering reliable information on chronic stress status, metabolic disorders and deficiencies in relation to different sizes. These could help in improve the health monitoring in trout fish farms.

1. Introduction

The rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is amongst the most important fish cultured in North America, Europe, Japan, Chile, Turkey and Australia [1], along with Italy, from a financial point of view. Rainbow trout is a species of high commercial value and represents one of the main economic products of Italian aquaculture. *O. mykiss* does not naturally reproduce in Italy, consequently, its presence depends on artificial breeding activities. This is possible thanks to a high level of adaptation to fresh water farm systems, elevated growth rate and high compatibility to different environmental conditions [2]). Blood studies can aid the diagnosis of fish diseases and stress; biochemical parameters measured in blood greatly assist monitoring the health and condition of both cultured and wild fish [3, 4, 5, 6, 7], as along with assisting monitoring aquatic ecosystems, indirectly [4, 8]). Blood glucose levels can be used as a stress indicator in teleost. In fact, it has been highlighted that glucose levels in blood is directly related to environmental stressors that can induce hyperglycemia and subsequent biochemical alteration in fish physiology [9, 10]. Triglycerides are useful tools in evaluating the nutritional status and lipid metabolism of freshwater fishes. Their levels alterations can indicate the insurgence of some pathological conditions, as nephritic syndrome or glycogen storage disease, or also the stress induced by toxic compounds in the water [11, 12]. Monitoring of cholesterol levels, being an essential structural component of cell membranes and the precursor of all steroid hormones, is of fundamental importance to determine the healthy status of teleost [12]. Aspartate aminotransferase (AST); alanine aminotransferase (ALT) are two major aminotransferases being the most significant enzymes involved in protein and amino acid metabolism [13, 14] These, with other parameters, are commonly used to detect health of aquatic animals [15, 16, 17].

The concentration of serum electrolytes offers significant information regarding the impact of stress on fish and health status of diseases [18, 19]. Serum electrolytes are a part of the biochemical composition of blood and values change in some conditions, such as stress, environmental condition, bacterial infection, nutrition and season [20]. The evaluation of some electrolytes such as calcium (Ca^{2+}), chlorine (Cl^{-}),

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iron (Fe^{2+}), phosphorus (P), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}) is also useful to assess the possible influence of size on osmoregulation in *O. mykiss*. Blood and body fluids contain several electrolytes. As the concentration of electrolytes in the body fluid of freshwater fish is much higher than the surrounding water, osmoregulation is essential to maintain correct fluid-electrolyte balance in the body fluids of fish. The study of blood characteristics and size is valuable instrument for the detection of health status in cultured fish species [21, 22]. Various studies have demonstrated a relationship between biometric indices and blood parameters in cultured fish [23, 24]. In trout, some authors demonstrated that fish size was an essential factor that helped understand intra-variability to contribute to accurate interpretation of hematological parameters [25]. Although previous studies have reported the reference range values for rainbow trout [26, 27, 28, 29], there are difficulties in establishing the "normal" ranges of blood parameters in cultured fish and finding a possible correlation between biometric indices and blood parameters. Therefore, further studies are necessary.

The objective of the present research was to study some biochemical parameters and serum electrolytes in cultured rainbow trout *O. mykiss* to contribute to knowledge of intra-variability response and evaluate their possible relationship with size (weight and total length). This could lead to improve the health monitoring in trout fish farms.

2. Materials and methods

2.1. Experimental design

One hundred rainbow trout *O. mykiss* (511.10 ± 108.30 g weight, 32.59 ± 2.14 cm total length) from a commercial fish-farm in Sicily, Italy, were investigated in this research. Data are given as mean ± standard deviation.

Fish were bred using a flow-through system under a photoperiod of 11L:13D. Water requirement at each tank was provided using untreated well water. Chemical and physical characteristics of water (dissolved oxygen, salinity, temperature, and pH) were detected with a handheld multi-parameter instrument (model YSI 556 MPS-Ohio, USA).

The fish were fed with a commercial dry food (Crude Protein 46%; Crude Fat 20%; Ash 10%; Fiber 1.5%) twice a day.

Fish samples in the farm were randomly captured on the same day from the same tank to assess biochemical and biometric parameters (electrolytes and non-electrolytes) and an external examination for indications of infestation or abnormalities was the basis for evaluating health status.

After capture, the fish were anesthetized before blood sampling using MS222 at a concentration of 0.7 g L^{-1} [7]. Fish were individually weighed directly following anesthetization using a balance (Kern 440-49 N, Germany) and an ictiometer (Scubla SNC, 600 mm, Italy) was used to record total length.

Condition factor (K) was also calculated as $W \times 100/L^3$, where W is the weight of the fish in grams (g), and L is the length of the fish in centimeters (cm). K values are generally found in the range 0.8–2.0 [30] for salmonids.

Once the necessary measurements were performed, sampled fish were released into a continuous aerated system before reintroduction into breeding tanks.

2.2. Blood sampling

Blood samples were taken between 08:00 a.m. and 10:00 a.m. and by puncturing the caudal vein using an 18 G × 1 ½ syringe and transferred into microtubes (Miniplast 0.6 mL; LP Italiana Spa, Milano) without anticoagulant agent for the assessment of biochemical parameters (electrolytes and non-electrolytes).

Time between capture and blood sampling was less than 3 min. Sera were acquired for biochemical analysis by centrifugation (10 min at 3000 rpm at 4 °C) of blood samples and stored at -20 °C until analysed (no more than 30 days). Measured biochemical parameters include: cholesterol, triglycerides, glucose, AST, ALT, iron, urea, K^{+}, Na^{+}, Ca^{2+}, Cl^{-}, P and Mg^{2+} and were evaluated by means of commercial kits (SEAC, Florence, Italy) with an automated analyser UV Spectrophotometer (SEAC, Slim, Florence, Italy).

2.3. Statistical analysis

Analytical data represented as mean ± standard deviation (SD) are the averages of three analyses carried out by the same operator.

Overall intra-assay coefficient of variation (CV%) of blood samples was on average less than 5%, with similarly low intra-assay variations. Before statistical analysis, the Kolmogorov–Smirnov test was used to test obtained data for normality. The relationship between blood parameters and biometric indices (weight and total length) was assessed using linear regression analysis (Pearson's correlation coefficient). Level of significance was set at <0.05. All results are expressed as mean ± standard deviation. Data were analysed using statistical software prism v.5.00 (Graphpad Software Ltd., USA, 2003).

Animal husbandry and experimentation protocols were examined and approved in accordance with the recommended standards of the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments.

3. Results

The mean values of pH, dissolved oxygen, temperature, conductivity, and water flow rate were 7.58 ± 0.31, 6.72 ± 0.35 mg/L, 16.4 ± 0.48 °C, 112.13 ± 11.2 μS/cm, and 8.42 ± 2.12 L/min respectively. These values were within the acceptable range for cultured *O. mykiss*.

Statistical results for some biochemical parameters and serum electrolytes together with size value (weight and length) in cultured rainbow trout *O. mykiss* are reported in Table 1. Regression analysis demonstrated a linear relationship between size and glucose and cholesterol in *O. mykiss*. In particular, fish length was positively related to glucose, whereas fish weight was positively related to glucose and cholesterol levels (Figure 1).

No statistically significant correlation with size (length and weight) was seen from other evaluated parameters (triglycerides, AST; ALT, iron, urea, Na+, K+, Cl^{-}, Ca2+, Mg2+, P).

4. Discussion

Our results showed a remarkable variability of the biochemical parameters and serum electrolytes studied in cultured trout, with commercial size ranging from 300 to 700 g.

For all parameters studied, only blood glucose and cholesterol showed a statistically significant correlation with fish size (Figure 1). In particular, fish length was positively related to glucose, whereas fish weight was positively related to glucose and cholesterol levels. Increased metabolic activity as a result of growth and swimming, impacts on blood glucose levels in fish, therefore, the increase of blood glucose in relation to the increase of weight and length is probably due to mobilization of glucose from storage sites. Glucose concentration varies according to fish habits and above all on locomotive capacity. Fish with omnivorous habits may frequently be exposed to natural food with varying quantities of carbohydrates [31], and must be capable of modifying glucose absorption according to the quantity found in the food. Cholesterol concentrations increase as fish size increased in trout [32]. The positive correlations observed between weight and glucose and cholesterol could indicate the metabolic strategy used by trout during growth. The use of non-protein energy sources (lipid and carbohydrate) may decrease protein utilization as energy for metabolism processes (protein sparing effect); in this way, as has been reported protein retention augments and ammonia excretion decreases [33].

Table 1. Statistical results for biochemical parameters and serum electrolytes together with size value (weight and length) in cultured rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792).

Rainbow trout (n = 100)	Mean ± SD	Min-Max	95% C.I.	2.5^{th}-7.5^{th} P.R.
SIZE				
Weight (g)	511.10 ± 108.30	300.00–700.00	483.10–539.10	440.00–317.50
Length (cm)	32.59 ± 2.14	25.00–38.00	32.04–33.14	32.00–33.75
BIOCHEMICAL PARAMETERS				
Glucose (mmol/l)	4.28 ± 1.43	1.66–7.60	3.91–4.65	3.34–5.38
Triglycerides (mmol/l)	3.67 ± 1.24	1.74–8.25	3.35–3.99	2.73–4.30
Cholesterol (mmol/l)	5.91 ± 1.62	2.85–10.13	5.49–6.33	4.90–6.70
AST (U/l)	276.10 ± 94.37	91.00–527.00	251.70–300.50	218.50–340.80
ALT (U/l)	2.78 ± 2.00	1.00–10.00	2.27–3.30	2.00–3.00
Iron (μmol/dL)	13.76 ± 6.24	3.76–31.86	12.15–15.37	9.22–17.81
Urea (mmol/L)	2.56 ± 1.24	1.07–6.75	2.24–2.88	1.71–3.28
SERUM ELECTROLYTES				
Sodium (mmol/L)	157.40 ± 5.15	139.00–168.00	156.10–158.80	154.00–161.00
Potassium (mmol/L)	4.50 ± 1.19	1.80–7.00	4.19–4.81	3.80–5.27
Calcium (mmol/L	3.16 ± 0.50	1.92–5.25	3.03–3.29	2.90–3.38
Phosphorus (mmol/L)	4.72 ± 1.07	2.71–8.20	4.45–4.10	4.01–5.02
Magnesium (mmol/L)	1.30 ± 0.18	0.90–1.64	1.25–1.35	1.15–1.44
Chlorine (mmol/L)	125.10 ± 6.42	107.90–162.20	123.50–126.80	122.50–128.00

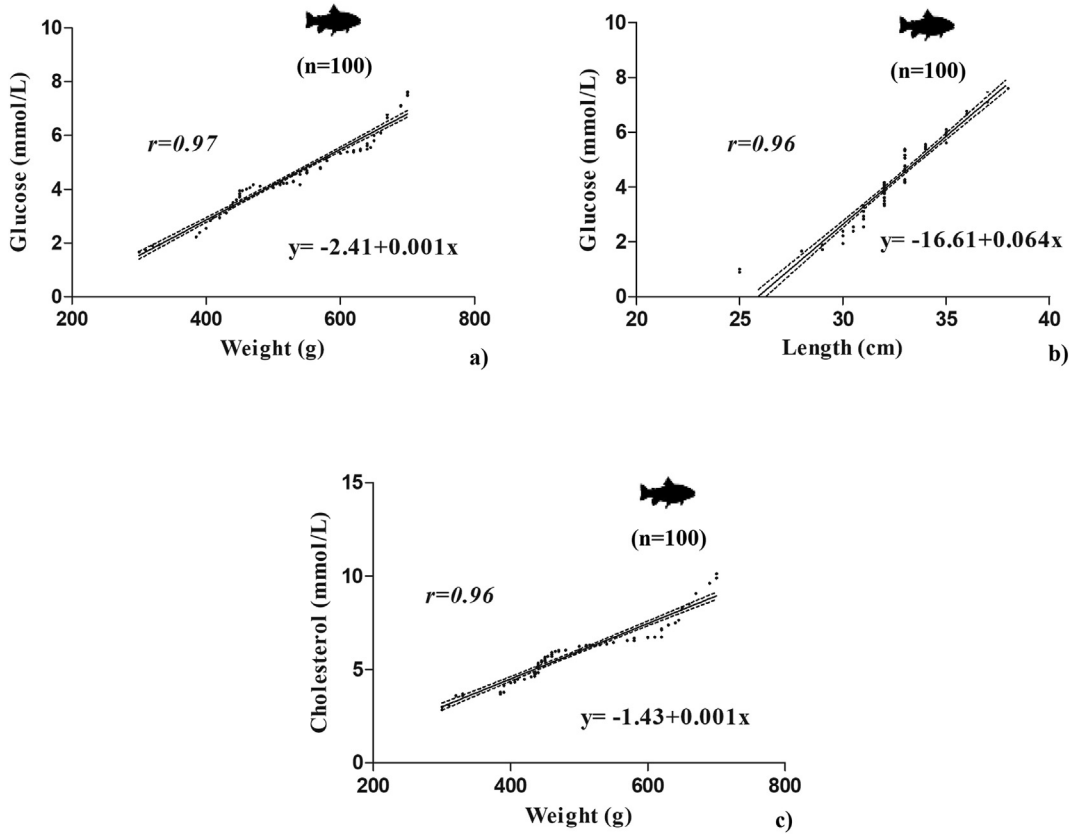

Figure 1. Positive correlation between weight and glucose (r = 0.97, P < 0.0001) (a), length and glucose (r = 0.96, P < 0.0001) (b), weight and cholesterol (r = 0.96, P < 0.0001) (c) in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792).

It is known that factors such as size can influence the profile of blood parameters in fish. Previous research [23] showed a positive correlation between hematological parameters and biometric indices in two fish species (*Sparus aurata* and *Dicentrarchus labrax)*. Serum biochemical data are fundamental in monitoring the changes of health status in cultured fish. Liver enzyme markers (ALT and AST) are generally used to indicate cellular damage both in fish and in mammals. Serum AST and ALT are generally higher in the presence of severe hepatic damage and the degree of organ damage depends on type of toxicant, mechanism of action and duration of exposure [34].

Fish blood contains urea, and the liver is the primary organ of production while the gills are the principal organ of excretion [35]. The increased level of blood urea nitrogen (BUN) might not be indicative of renal disease in fish [36]. Research has shown that the immune system

may be affected by excessive ammonia nitrogen in the environment may [37]. Furthermore, damage may occur to the liver, gill, kidney and other fish organs [38].

Electrolytes are good indicators of identifying fish health [39]. Electrolytes are fundamental substances that influence retention and distribution of body water. The osmoregulatory process is an important adaptation of freshwater fish to overcome changes in salinity in natural and bred conditions. The values of some biochemical parameters and serum electrolytes of *O. mykiss* collected from a commercial farm are presented in Table 1. Na^+, K^+ and Cl^- are the most important osmotically effective electrolytes that contribute to electrolyte homeostasis.

Other important electrolytes in fish include calcium and phosphorus which are essential for bone formation. It has been hypothesized that bone acts as a calcium reservoir for tissues and plasma, and it is commonly recognized that calcium has an essential role in reproduction, mitochondrial function and osmoregulation [18]. Phosphorus is involved in the growth process and bone mineralization and in lipid and carbohydrate metabolism. Magnesium stimulates nerve irritability (contraction) and muscle, is involved in the regulation of intracellular acid-base balance, and is critical in carbohydrate, protein and lipid metabolism.

Ours results did not show a significant correlation between serum electrolytes studied and size in *O. mykiss*.

Our results demonstrated high correlation between energetic metabolites (glucose and cholesterol) and biometric indices in trout, indicating evidence of use of these metabolites during growth. However, electrolytic balance does not appear to be influenced by biometric indices, probably due to water conditions which did not present any osmotic variations during the experiment.

Further studies are necessary to evaluate blood electrolyte changes in cultured *O. mykiss* in relation to changes in the saline environment.

The knowledge of intra-individual variability of some biochemical parameters is useful in identifying parasitism, health issues, erroneous feeding practices, subclinical disease conditions, and in evaluating the health of cultured fish. This research contributes to understanding the intra-individual variability of some blood parameters in cultured rainbow trout *O. mykiss* to provide reliable information on deficiencies, metabolic disorders, and chronic stress status in relation to different sizes. There is urgent need to improve the monitoring and management of aquaculture, and therefore, the more information on blood serum biochemical factors, in studying the health status of fish is a necessary prerequisite for successful management.

Declarations

Author contribution statement

Francesco Fazio: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Concetta Saoca: Performed the experiments.

Gioele Capillo, Carmelo Iaria, Michele Panzera: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Giuseppe Piccione, Nunziacarla Spanò: Conceived and designed the experiments.

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Data availability statement

The authors do not have permission to share data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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