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THE PHYSIOLOGICAL EFFECTS OF SOME STRESS INDICATORS IN RAINBOW TROUT RAISED IN DIFFERENT SYSTEMS

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Abstract

Regardless of the accuracy of the technology implementation, the final quality of the fish is also influenced by the conditions to which it is subjected after being extracted from the rearing pond. In the present case study, we proposed an analysis of the physiological reaction of the stock after extraction from the growth ponds. Among others, we were particularly interested in the level of the stress hormone, determined in the samples taken in the study under the conditions of the application of certain stressors. In contrast, comparison of the amount of glycogen led to obtaining very distinctly significant statistical fluctuations (the mean for specimens reared in semi-intensive system was 2.314±0.638 g/100 g and for those grown in intensive system 1.980±0.822 g/100 g). Growth hormone varied between 0.504±0.46 ng/ml in the case of semi-intensive system and 0.694±0.22 ng/ml for intensive system. The values obtained for cortisol showed a significant influence of stress factors on the studied trout. The stress hormone level rises in correlation with both the intensity and type of stressors, starting from the moment the fish are removed from the ponds.

Key words: *growth system, hormones, salmonids, stressors.*

INTRODUCTION

Worldwide, the highest colonization rate for commercial exploitation is observed in rainbow and brown trout. However, the two species have different responses in terms of adaptation capacity and ecological impact (McGlade et al., 2022).

Many environmental factors specific to salmonid growth (temperature, pH, turbidity, toxic substances, diseases, food etc.) are stress generators when their levels exceed normal physiological limits (Schreck & Tort, 2016).

Biodiversity of animal species is crucial for various reasons, and its importance extends across ecological, economic, scientific, cultural, and ethical dimensions (Davidescu et al, 2023).

When stressors act for short periods, the adaptive response of fish is fast, and they have the ability to restore homeostasis. Diametrically opposite, their long-term maintenance (chronic stress) leads to the appearance of negative effects on the immune system, productivity and health of the livestock (Sneddon & Wolfenden, 2016; Simeanu et al., 2022; Popa et al., 2023).

Rising water temperatures (e.g. due to global warming) affect all species, including rainbow trout, which is a cold-water fish. Biochemical studies carried out on specimens grown in warm waters (20-24°C) revealed significant changes in liver metabolites (aminotransferase, lysozyme, total bilirubin, alkaline phosphatase, superoxide dismutase, glutathione peroxidase and malondialdehyde, which did not return to normal values even after passing into water with normal temperature (14°C) (Li et al., 2022).

Different solutions have been tested to prevent the negative effects of heat stress on rainbow trout. For example, a supplement of 5 mg/kg nanoselenium introduced into the food of rainbow trout subjected to heat stress (24°C) significantly increased the activity of liver glutathione peroxidase. Thus, the levels of alanine aminotransferase, aspartate aminotransferase, superoxide dismutase and malondialdehyde were reduced and, on the

other hand, lipid accumulations in the liver decreased and its tissue structure improved (Marin et al., 2020; Li et al., 2022; Simeanu et al., 2022; Surmeli et al., 2023).
In rainbow trout raised under conditions of thermal stress (+24°C), through high-throughput sequencing of the kidney tissue, microRNAs involved in the response of some target genes to thermal stress, including the transformation of proteins in the endoplasmic reticulum, were identified (Ma et al., 2019).
Also in this sense, the idea was launched that long noncoding RNAs (lncRNAs) can be used in the selection of genetic variants of heat-resistant trout, given their essential role in the regulation of heat stress by association with genes involved in immune regulation, apoptosis and signalling pathways of metabolic activity (Zhou et al., 2022).
A common problem in intensive fish farming is poor water quality (especially dissolved oxygen, turbidity, and total dissolved solids), which significantly affects the growth performance of rainbow trout and greatly increases stress indicators (Welker et al., 2018; Usturoi et al., 2023).
Another stressor is the pH value of the water (acid stress), an indicator influenced by acid rain, acid pollutants, acid wastewater, and the application of excessively high densities. Interestingly, the studies highlighted that exposure of rainbow trout to acidic water (pH-5.2) for 4 days led to increases in the activity of glycoproteins, lysozymes, and myeloperoxidase only in diploid specimens, and non-specific immune functions were not affected in triploid fish (Yilmaz et al., 2017).
Evaluation of the effect of some stressful factors (water temperature, handling, and low water level in the ponds on some antioxidant enzymes in rainbow trout revealed a significant increase in glutathione peroxidase and catalase in all analysed situations, glucose 6-phosphate dehydrogenase only at high water temperature, and glutathione reductase in specimens stressed by handling and low water (Oezmen et al., 2007).
The stress generated by exposure to air (3 min) caused an acute response for 24 h post-exposure, which resulted in significant increases in cortisol, lactate, and plasma glucose as an expression of the reactivity of liver microRNAs (Ikert et al., 2021).
In the case of juvenile rainbow trout reared in small volume tanks, the application of isolation stress resulted in increases in plasma cortisol, glucose, and lactate after one hour of treatment. In the same specimens, after two hours of isolation stress, significant increases in the values of the three indicators were observed; however, during this interval, food consumption was also drastically reduced (Pankhurst et al., 2008).
The duration of the pre-sacrifice period is another stressor that correlates with the frequency of the feeding schedule. Studies have shown that feeding every other day and fasting for two days prior to slaughter results in lower cortisol levels and higher triglycerides and liver glycogen levels than daily/4-day feeding followed by fasting for 9 days, an aspect that indicates a reduction in the response to food stress (Bermejo-Poza et al., 2016; Usturoi et al., 2023).
Although salmonids react well to various stress factors, repeated and chronic exposure to such conditions alters physiological processes and metabolism, with effects on growth and development, reproductive function, and immune response (Rousseau & Dufour, 2017). Most studies have shown that plasma cortisol is the best indicator of acute stress in fishes. In parallel, the researchers believe that it is essential to identify other molecular, biochemical or hormonal markers, which reflect more accurately the state of stress, in order to improve the productivity and quality of the meat obtained in aquaculture (Vijayan et al., 2010).
The current trend in the Romanian population, also observed at the global level, is to eat in the healthiest possible way. In this context, consumers purchase products obtained in the simplest possible way, as close as possible to what many call "natural".
In general, we mention the fact that the area where the trout farms under study are located is one of real and major tourist interest. This shows that the area is frequently visited by a large number of tourists who, in addition to tourist attractions, are also directly interested in the culinary typology of the area.

Trout is the producer of high-quality meat that is appreciated from both a taste and nutritional point of view. In the area from which the trout comes, there are a multitude of such holdings, most of which are semi-intensive and intensive. Starting from the previously mentioned premises, through the present work, the authors proposed to carry out a case study in which to observe how rainbow trout react, from the point of view of the state of stress, to different growth conditions.

MATERIALS AND METHODS

The present work represents a case study that aims to follow the way rainbow trout react, from the point of view of stress factors, to different growth conditions.
The objectives taken into account were the monitoring of some water quality parameters and the observation of the physiological responses of the trout (glucose, glycogen, growth hormone, cortisol) under the conditions provided by the two growth systems.
The fish were captured randomly from the breeding ponds, with the limitation of stressing them as much as possible. Later, to apply the stress factors, the trout were kept in special ponds.
Water quality was monitored for a week to exclude the existence of significant fluctuations that would disturb the state of stress in the trout. Samples were collected and the following indicators were determined: temperature, dissolved oxygen, and pH value. The research took place at the end of September-2022, because it is an interval in which moderate temperatures and precipitation are recorded, which leads to constant water quality.
The samples were collected by authorized personnel in characteristic containers and were subjected to analysis.
The dosage of the stress hormone was determined under different circumstances for both trout as follows:

- unstressed: taking the samples immediately after extraction from the pond, without applying a stress beforehand;
- stressed "0 h" - sampling after applying stress factors (lack of oxygen, handling, and higher water temperature);
- stressed "1 h" - sampling after applying stress factors (lack of oxygen, handling, higher water temperature) and keeping them on ice (additional stress factor) for a period of one hour.

Biological material
The biological material was rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), aged 2 years and a summer. The samples were taken from 36 specimens directly from the aforementioned trout farms (18 specimens/farm).

Location of farms
The trout farms studied are located in a mountainous area, in the central-eastern part of Romania, at a close distance from each other, subject to similar pedoclimatic conditions.
This area is characterized by high temperatures in the warm season (25-30°C) and low temperatures during the cold season (up to -25°C). The area is highlighted by the existence of abundant precipitation, especially during the cold season (the snow layer is maintained for up to 120 days in the valley area and up to 160 days on the slopes).
Both holdings were designed outdoors, as there were no closed farms in the area. This results in a potentially major influence of environmental factors on everything that means "development" of livestock.

The growth and nutrition system
The farms, one of which operates in a semi-intensive exploitation regime and the other in an intensive system, are symbolically marked P-si, and P-i.
The semi-intensive farm (P-si) has a production capacity of approximately 3 tons/year and earthen basins (with a gravel hearth).
The water depth is between 0.8 m (at the inlet) and 1.8 m (at the monk).
The ponds were fed with water from a nearby stream, ensuring a constant flow of approximately 60 L/s.
For consumption of trout, granulated combined feeds with 42% P.B. and 4100 kcal/kg E.M. were administered in a single daily portion, representing 0.9% of the fish's mass.

The intensive rainbow trout farm (P-i) was designed for a production of 20 tons/year and consists of concrete basins (including the hearth), with depths between 1.3 m (at the inlet) and 1.9 m (to monk).
The water source is a natural mountain stream that springs from approximately 1.5 km from the holding, which can ensure a flow of approximately 200 L/s.
Feeding was carried out with extruded granulated feed (44% B.W. and 4200 kcal/kg E.M.) administered in two sessions/day, in a daily amount that represented 1.1% of the fish mass.

Water quality determination

It was highlighted by monitoring three main parameters, known to be potential generators of stress in salmonids, namely: temperature, amount of dissolved oxygen and pH value (Păsărin, 2007). The determinations were made three times a day, at 24^{00}, 8^{00}, and 16^{00}, for seven days, and the average values for the two holdings were then calculated. Probing depth was established in the comfort zone of the fish, approximately 90 cm from the pond earth.
Water characteristics may change rapidly as a result of chemical, physical or biological processes in the water sample. For this reason, whenever applicable, it is advisable to measure the parameters value immediately at the sampling point. The water sample is placed in a 100-150 ml chemical beaker. The electrodes are inserted into it in such a way that they are completely inserted into the water. After 0.5-1 min, the values are read.
The temperature was recorded with the help of a professional digital thermometer used in aquaculture-type TFA-Dostmann, produced by the Roth company.
The determination of dissolved oxygen was carried out with the help of TMT-DO-5512SD.
The pH value was determined using the WTW ProfiLine pH 3310 portable pH meter, produced by the Toth company.

Biochemical and immunological determinations

They were performed in an accredited laboratory by personnel authorized to perform these tests. Biochemical analyses were performed using an ILab Taurus analyzer and laboratory instrumentation. All determinations were performed in accordance with the methodology and legislation. Venous blood was collected in an amount of approximately 0.5 mL in collection containers (vacutainer without anticoagulant with separating gel). Afterwards, the serum was separated by centrifugation, the sample being stable for 7 days at 2-8°C.

Blood glucose measurement

Determination of glucose in the serum was performed using the GOD-PAP method at a wavelength of 510 nm on the ILab Tautus automatic biochemistry analyzer. The measurement range was between -35,000 ÷ +35,000 mAbs/10. To check the performance parameters, the "ready to use" the quantILab glucose (oxidase) mono reagent was used, kept in stable conditions (2-8°C), and within the validity period. The reagent was chosen because there is an adaptable work protocol provided by the manufacturer for the equipment used in this study. The reference material (calibrator) used was Randox calibration serum (code CAL2351). The traceability certificate of the calibrator specifies the traceability of the glucose value to the certified reference material and the total measurement uncertainty (Strath et al., 2013; World Health Organization, 2002).
The reagents used to determine the amount of glucose, code GL3815, lot I0822263, are dedicated to the equipment used and are within their validity period according to the quality certificate. The reagents were stable at 2-8°C until the expiration date was recorded on the bottle and 28 days onboard the analyzer. ILab Assayed Multiser control sera (with the matrix and measured values) were used. In medical laboratories for analytical biochemical determinations, only two concentration levels are generally used for control sera that are relevant in clinical decisions.
Way of working. The GOD/PAP method was developed for quantitative determination of glucose and other biological liquids. The GOD/PAP method has the following measurement principle: the glucose in the sample to be analysed is hydrolysed in the presence of glucose oxidase (GOD, component enzyme in reagent R1) with the formation of

gluconic acid and hydrogen peroxide (World Health Organization - WHO, 2002). This, in the presence of phenol and aminophenazone and under the action of peroxidase (POD, the 2nd enzyme in reagent R1) forms a red colored compound (quinoneimine) (Burtis et al., 2005; Bartiam & Trinder, 1972). The optical density of the colored compound formed is directly proportional to the amount of glucose in the sample and is measured at 510 nm. The GOD/PAP method does not require special environmental conditions. The reactions during the measurement take place on automatic equipment equipped with a thermoregulation system. The ambient working conditions are: temperature of 21±2°C and humidity 40-60% (Bartiam & Trinder, 1972).

Quantitative determination of glycogen

Glycogens are the main storage form of carbohydrates in the animal body. Glycogen is present in all animal organisms at different stages of development. In higher animals, the liver is the organ richest in glycogen. In smaller quantities, this polyglucoid is also found in muscles, the brain, etc. (World Health Organization - WHO, 2002).

Animal tissue undergoes heat desmolysis in a strongly alkaline solution. The glycogen contained in the desmolyzate, in the presence of concentrated sulphuric acid, is split into glucose, which is dehydrated with the formation of hydroxymethyl-5-furfural (Burtis et al., 2005).

Hydroxymethyl-5-furfural condenses with anthrone (9,10-dihydro-9-oxoanthracene), resulting in a blue-green coloured compound, the intensity of which was directly proportional to the glucose concentration obtained following the hydrolysis of the glycogen contained in the analysed tissue. Condensation is carried out between the aldehyde group of 5-hydroxymethylfurfural and the active methylene group from the anthrone molecule (Burtis et al., 2005).

Reagents used were: 30% potassium hydroxide solution; 95% sulphuric acid solution. It was added 100 ml of concentrated sulphuric acid to 5 ml of distilled water and cool. For obtaining 0.2% anthrone solution in 95% sulphuric acid it was dissolved 0.2 g of anthrone in 100 ml of 95% sulphuric acid. The reagent is extremely unstable and therefore it will be prepared no more than 1 hour before the determination, using it only on that day (Young & Friedman, 2001).

The work protocol starts with introductions of 3 ml of potassium hydroxide solution (reagent 1) in a 20 mm ×150 mm heat-resistant glass test tube and closing it with a rubber stopper. A quantity of 0.1-0.5 g of tissue to be analysed (liver, muscle, brain, etc.) cut into small pieces is then weighed on the analytical balance and introduced into the potassium hydroxide solution in the test tube. The stopper was removed and the test tube was placed in a boiling water bath for 20 min. After cooling with tap water, the contents of the test tube were transferred to a 50-100 ml volumetric flask, washing several times with 4-5 ml of distilled water; filled to the mark and shacked vigorously (Schumann et al., 2010).

From the glycogen solution thus obtained, it measured 0.5-2.5 ml in a 30 x 200 mm heat-resistant glass test tube and top up the volume to 2.5 ml with distilled water, if is necessary. Distilled water (2.5 ml) was used to control the reagents in another identical test tube. Both test tubes were placed in an ice-water bath. After cooling, 5 ml of anthrone solution in 95% sulphuric acid (reagent 3) was carefully measured from the burette into each test tube in a thin thread and continuously stirred. The test tubes were covered with a glass pear and placed in a boiling water bath for 10 min. They were then cooled in an ice water bath and immediately the extinction was read on a spectrophotometer at a wavelength, $\lambda = 620$ nm (Young & Friedman, 2001; Schumann et al., 2010).

After the extinction value obtained, the amount of glycogen (mg) in the volume of the solution used is found on the standard curve. The glycogen content, expressed in mg %, in the studied tissue is then calculated according to the formula:

$$X = \frac{a \cdot V_1 \cdot 100}{V_2 \cdot P} \text{ (mg \%)}, \qquad (1)$$

were:

a - the amount of glycogen, in mg, found on the calibration curve;

V1 - the volume (ml) to which the desmolyzate obtained after the treatment with potassium hydroxide was diluted;

V2 - the volume of the glycogen solution (ml) taken for the lane reaction;
P - the weight of the analysed tissue (g);
100 - conversion factor in percentage.
To construct the standard curve, a glycogen solution was used, which was prepared by dissolving 40 mg of glycogen in a 500 ml volumetric flask in hot distilled water. After cooling the solution to room temperature, it was made up to the mark with distilled water and shaken. This solution contains 0.08 mg of glycogen in one millilitre. From the obtained solution, prepare a series of samples for drawing the standard curve by pipetting the volumes of the solution.
It is recommended that the addition of 0.2% anthrone solution in 95% sulphuric acid be performed at certain intervals (e.g., 2 min) from one test tube to the next. This time interval will also be respected when reading extinctions after cooling the samples to room temperature. The extinction of the cooled samples was measured on a spectrophotometer at a wavelength λ of 620 nm. The standard curve is drawn by writing the glycogen concentration (mg) on the abscissa and the extinction values E on the ordinate (Schumann et al., 2010).

Cortisol dosage
The cortisol (antigen) in the sample competes with peroxidase (enzymatically labelled antigen) to bind a limited number of anti-cortisol antibodies in the microplates (solid phase). After incubation, bound/free separation was accomplished using a simple solid phase wash. The enzyme substrate (H2O2) and TMB substrate were added. After an appropriate time for maximum colour development, the enzymatic reaction was stopped and the absorbance was determined. Cortisol concentration in the samples was calculated based on a standard series. The intensity of the colour is inversely proportional to the concentration of cortisol in the sample (Gornall et al., 1949).
The kit determination included: micro titration plate with 96 wells; standard reference cortisol set, containing 0, 10, 50, 150, 500 ng/ml; control reagent; conjugated reagent, 13 ml; TMB (One-Step) reagent, 11 ml; stop solution (1N HCl), 11 ml.
To determine cortisol levels, the samples were processed in duplicate. It was prepared two wells for each of the five points of the standard curve (S0-S4), two for each sample and one for Blank (Toni et al., 2019).

Growth hormone dosage
Growth hormone (GH) is a polypeptide that is secreted by the anterior pituitary gland. It promotes protein conservation and is involved in a wide range of protein synthesis mechanisms. GH also improves glucose transport and facilitates glycogen storage.
The test system used a solid-phase anti-GH polyclonal antibody (microtiter wells) and a mouse anti-ferritin monoclonal antibody in an antibody-enzyme (horseradish peroxidase) conjugate solution. The sample was allowed to react simultaneously with the antibodies, resulting in the formation of GH molecules between the solid phase and the enzyme antibodies. After 60 min incubation at room temperature, the wells were washed with water to remove unbound labelled antibodies. TMB solution of TMB is added and incubated for 20 min, resulting in the appearance of a blue colour. Colour development was stopped by the addition of 2N HCl and the colour changed to yellow, which was measured spectrophotometrically at 450 nm. The GH concentration is directly proportional to the colour intensity of the test sample (Young, 2000; Toni et al., 2019).
Determinations were made with the HGH ELIZA kit, Lot 5811A, Ref. DKO 050, produced by DIA METRA, Italy.
The determination kit included: micro titration plate with 96 wells; standard reference set, containing 0, 2.5, 5, 10, 25, and 50 ng/ml of GH, ready for use; conjugated reagent, 13 ml; TMB (One-Step) reagent, 11 ml; stop solution (1N HCl), 11 ml.
The working protocol included the following steps:
- set the desired number of covered wells in the work support;
- pipette 50 µl of the standard and control solution into the appropriate wells;
- distribute 100 µl of conjugated reagent in each well;

- homogenize well for 30 seconds (complete homogenization is very important at this stage);
- incubate at room temperature (18-25°C) for 60 minutes;
- throw the incubation mixture contained in the plate into a waste container;
- rinse and shake the micro titration wells 5 times with distilled or deionized water;
- suddenly tap the work plate on a support of paper or absorbent towels to remove all residual water drops;
- distribute 100 µl of TMB reagent in each well. Shake gently for 10 seconds;
- incubate at room temperature for 20 minutes;
- stop the reaction by adding 100 µl of stop solution to each well;
- shake gently for 30 seconds (it is important to be sure that all blue wells turn completely yellow);
- the samples are read at the optical density of 450 nm, with a microtiter plate reader, in a maximum of 30 minutes.

Data processing

The experimental data were processed using the calculation algorithms in Microsoft Excel, and the statistical interpretation was based on the SPSS Statistics 21.

RESULTS AND DISCUSSIONS

Environmental factors

Rainbow trout is one of the less pretentious species in terms of the physicochemical characteristics of water compared to other salmonids. This species tolerates turbid waters quite easily, but only for short periods of time, also during the hottest seasons, but with high flows (approx. 1 L/min/kg fish) and relatively rich in dissolved oxygen (over 6 mg O_2/L) (Schreck et al., 2016; Sneddon & Wolfenden, 2016).

In order to accurately determine the influencing factors on the health status of the analysed populations, it was monitored a series of growth parameters in the two studied trout farms. Therefore, the technical parameters applied at the farm level, the water quality in the rearing tanks, and the characteristics of the combined feed administered for trout feeding were taken into consideration.

The technical parameters studied included water flow rate, population density, and trout weight.

It was observed that a significantly higher water flow rate was used in P-i, with a rate of 200 l/sec, compared to only 60 l/sec in P-si. This parameter was evidently influenced by precipitation; however, during the analysis period, it did not represent a significant factor.

A similar remark can be made in the case of the applied densities, which are 25 and 75 head/m^2 for P-si and P-i, respectively.

Differences also existed in the body weight at the time of the study. Thus, the average value obtained for P-si was 262.84±5.12 g, while for P-i 274.36±5.23 g (Table 1). Within the batches, the character was homogeneous, the coefficient of variation indicating very small values.

In the trout farms, the water flow was ensured to be 60 L/sec at P-si and 200 L/sec at P-i.

This fish feeds efficiently at water temperatures between 15°C and 19°C but stops feeding above 23°C. In deep water, it is a feared predator (Păsărin, 2007).

Studies have shown that rainbow trout adapt best to intensive growth in farms designed according to modern principles; in some specialized lines, reproduction can be induced in all seasons of the year (Pankhurst et al. 2008; Li et al., 2022).

Water temperature plays an important role in the growth of salmonids because it influences the feeding and body temperature of trout (McGlade et al., 2022). Optimal feeding temperatures and a high digestibility for rainbow trout are at 15-19ºC (Yilmaz et. al., 2017). The brown trout is particularly demanding of water temperature, which must not fall below 4°C or exceed 15°C (when feeding stops). Feeding activity is more intense between 12°C and 14°C (Păsărin, 2007).

Water quality was analysed using three indicators: temperature, pH, and dissolved oxygen.

Within the two rainbow trout farms it was found that the temperature values had variations, but insignificant, the average being 11.24±0.76 at P-si and 10.14±0.40 at P-i (Table 1). In both cases, there were no drastic

increases or decreases in parameter values, which could lead to trout stress.

The pH value in the water ponds has fluctuated around 7.5, with an average 7.28±0.14 of P-si and 7.65±0.22 at P-i. In this context, according to STAS 4706/1988, the water can be classified as II quality level. For salmonids, an optimal pH must be between 7.5 and 8.5 (Li et al., 2022).

The amount of dissolved oxygen determined in the water of studied farms was 8.46±0.22 mg/L P-si and 9.82±0.18 mg/L at P-i, and no significant fluctuations were recorded. Water who has a content of dissolved oxygen over 9 mg/L, and temperatures of 18-19°C, is considered appropriate for trout rearing. Dissolved oxygen concentration has been identified as a critical factor for the survival of salmonids in all phases of development, from the fry to the reproductive stage. The concentration of dissolved oxygen in water is inversely proportional to water temperature (Păsărin, 2007). Salmonids can live in water with an oxygen content of 9-10 mg/L (Schreck & Tort, 2016).

The administered combined feeds were evaluated in terms of protein content, metabolizable energy, number of taints, and amount administered.

The protein level of the recipe administered at P-si was 41%, whereas that at P-i was 43%. Differences also existed in the case of metabolizable energy, for which the values were 4100 kcal/kg (P-si) and 4300 kcal/kg (P-i).

In both fish farms, three daily feedings were administered, the difference being that in P-si, the amount of combined feed represented 1.1% of the weight of the fish, while in P-i, it represented 1.2% of its weight (Table 1).

Table 1. Experimental factors applied to 2.5 years old rainbow trout

Specification		Semi-intensive system (P-si)	Intensiv (P-i)
The technical parameters	Water flow rate (L/s)	60 L/sec	200 L/sec
	Density	25 heads/m^2	75 heads /m^2
	Rainbow trout weight (g) (average values)	262.84±5.12	274.36±5.23
Water quality (average values)	Water temperature (°C) (average values)	11.24±0.76	10.14±0.40
	Dissolved oxygen (mg/L - average values)	8.46±0.22	9.82±0.18
	pH value (average values)	7.28±0.14	7.65±0.22
Combined feeds administered	Protein content (%)	41	43
	Metabolizable energy (kcal/kg)	4100	4300
	Daily feed intake	3	3
	Proportion of combined feed (% of fish weight)	1.1	1.2

Blood glucose level

Because the concentration of glucose in the blood is an indicator that can be correlated with the stress hormone, it was dosed in the case of the samples taken in this study. In the case of the batch related in semi-intensive P-si system, average values of the parameter of 20.78±1.44 mg/dL were obtained. The minimum value was 19.36 mg/dL, while the maximum value was 22.28 mg/dL. For the lot raised in the intensive system, P-i, the minimum value determined was 18.82 mg/dL and the maximum value was 23.40 mg/dL. The calculation of the mean value indicated a result of 21.10±2.24 mg/dL, which was within the confidence interval of the mean. No statistically significant differences were found between the averages of the two batches (Table 2).

In fish, glucose provides most of the energy consumed during swimming. Normal blood glucose values in salmonids are between 28.41 - 64.00 mg/dL (Barton, 2000). Some factors can indirectly alter blood glucose levels. Some studies suggest that "*growth history, including nutritional status, may affect stress response and glucose release*" (Krasnov et al., 2001). This statement is also supported by other authors who found that blood glucose results must be interpreted considering extrinsic factors because they can affect the glycogen reserve in the liver. This category includes diet, age, time since last feeding, season, and so on (Shanghavi & Weber, 1999).

Nutrition is also an important factor that influences blood glucose levels. Thus, the concentration varies between species and

depends on the developmental stage (Jentoft et al., 2005). The intake of diets with different lipid and protein contents results in distinct blood glucose levels (Cheng, 2006).

Under conditions of stress, fish quickly consume glucose because the main function of the central nervous system is to maintain homeostasis; thus, no significant change in blood glucose is observed. However, it is possible that fish exposed to chronic stress may deplete the substrate, leading to a decrease in blood glucose levels (Ruibal et al., 2002).

The research in this paper highlighted average values of the parameter, of 20.78±1.44 mg/dL for P-si and 21.10±2.24 mg/dL in the case of P-i, lower than those stated in the specialized literature (Gilmour et al., 2012).

According to other studies, this phenomenon may be due to the poor quality of the water (in both holdings it fell to II quality), stressful factors (the capture of the studied specimens was done in the cold season), the quality of the feed administered, and the density practiced (Marcos et al., 2014).

The individual values obtained from the biochemical analyses showed variations in blood sugar levels from one individual to another. This aspect is due to the fact that glucose shows variations depending on sex, diet, stress conditions, etc. (Krasnov et al., 2001; Jentoft et al., 2005).

Table 2. Results on glucose analysis in 2.5 years old rainbow trout

Growth system	N	Average values $\overline{X} \pm s_{\overline{x}}$ (mg/dL)	The confidence interval of the mean (95%)		Min. (mg/dL)	Max. (mg/dL)
			Lower limit	Upper limit		
P-si	18	20.78±1.44	19.30	23.10	19.36	22.28
P-i	18	21.10±2.24	19.10	22.90	18.82	23.40
The statistical significance			P-si vs. P-i = n.s.; F=0.013, p=0.911, p> 0.05			

*significant differences between means for 0.01 < p < 0.05.
**distinguished significant differences between means for 0.001 < p < 0.01.
***highly significant differences between means for p < 0.001.

Hepatic glycogen

Hepatic glycogen is a parameter that is significantly influenced by the effort made by the trout and can be correlated with stress hormones. In the case of the P-si group, the determined amount of muscle glycogen was at an average level of 2.314±0.638 g/100 g, the value being higher than that determined for P-i, which was 1.980±0.822 g/100 g.

For P-si, the minimum recorded value of the indicator was 1.345 g/100 g, whereas the maximum was 3.796 g/100 g. In comparison, the minimum value determined for P-i was 1.098 g/100 g, with a maximum of 3.248 g/100 g. These values generated significant statistical differences among the studied lots (Table 3).

When interpreting the data, the conditions under which the trout studied were kept were taken into account, as glycogen content can reflect biochemical adaptations to any environmental stress (Figueroa et al., 2000). Of these, pH, oxygen and salinity levels, as well as prolonged physical activity, directly affect glycogen reserves (Winter et al., 2005; Usturoi et al., 2009).

Referring to other bibliographic sources, the amount of liver glycogen is quite low, which is explained by intense physical activity from the moment of capture (Lopez-Patino et al., 2014). Under hypoxic conditions, liver glycogen is mobilized to support the white muscle, suggesting that this is a biochemical strategy used as a response to such stress (Daskalova, 2019).

Table 3. Results regarding hepatic glycogen in 2.5 years old rainbow trout

Growth system		N	Average values $\overline{X} \pm s_{\overline{x}}$ (g/100 g)	The confidence interval of the mean (95%)		Min. (g/100 g)	Max. (g/100 g)
				Lower limit	Upper limit		
Hepatic glycogen	P-si	18	2.314±0.638	1.864	2.878	1.345	3.796
	P-i	18	1.980±0.822	1.534	2.444	1.098	3.248
The statistical significance				P-si vs. P-i=***.; F=48.006, p=0.001, p < 0.05			

*significant differences between means for 0.001 < p < 0.01.
**distinguished significant differences between means for 0.001 < p < 0.01.
***highly significant differences between means for p < 0.001.

Determination of hepatic glycogen content is of particular importance for the investigation of the physiological and pathological states of the animal body as well as for the investigation of the influence of certain factors on carbohydrate metabolism (Viant et al., 2003).

The fluctuation of the results indicates that the main causative factors are different growing conditions. A discrepancy can be observed regarding the liver glycogen reserve in trout specimens raised in a semi-intensive system compared to those raised in an intensive system. From a nutritional perspective, elevated blood glucose levels in trout may be attributed to their energy needs, given that trout are predatory creatures expending significant energy in pursuit of prey (Zhou et al., 2022).

Growth hormone

In the case of fish, growth hormone participates in almost all important physiological processes in the body. Specifically, it is involved in the metabolism of proteins and carbohydrates, growth of the skeleton and soft tissues, reproduction, and functioning of the immune system.

The average level of growth hormone in the specimens raised in the semi-intensive system had a value of 0.504±0.46 ng/ml, slightly lower than the 0.694±0.22 ng/ml, as recorded in the specimens raised in the intensive system. The minimum values recorded for P-si and P-i were 0.06 and 0.35 ng/ml, respectively, while the maximum values were at the level of 0.35 ng/ml for P-si and 0.98 ng/ml in the case of P-i (Table 4). However, no statistically significant differences were observed between the two batches studied, the data being similar to from the specialized literature (Jentoft et al., 2005; Ge et al., 2021)

The growth hormone is a pluripotent hormone produced by the pituitary gland and is secreted in response to exercise, stress, deep sleep, hypoglycemia, and insulin. If growth hormone is secreted deficiently or excessively in the first stages of growth, dwarfism and gigantism will appear respectively. Over the past two decades, many aspects of growth hormone physiology have been the subject of intense research in fish, particularly in salmonids, cyprinids, and sparids (Uiuiu et al., 2021; Morro et al., 2021).

Recent studies have shown that growth hormone affects several aspects of behaviour such as appetite, foraging, aggression and predator avoidance, with the finality of these changes having ecological consequences (Remo et al., 2017; Breves et al., 2020).

Table 4. Results of the analysis of growth hormone in 2.5 years old rainbow trout

Growth system	N	Average values $\overline{X} \pm s_{\overline{x}}$ (ng/ml)	The confidence interval of the mean (95%)		Min. (ng/ml)	Max. (ng/ml)
			Lower limit	Upper limit		
P-si	18	0.504±0.46	0.34	0.75	0.06	1.04
P-i	18	0.694±0.22	0.59	0.80	0.35	0.98
The statistical significance			P-si vs. P-i = n.s.; F=2.859, p=0.100, p > 0.05			

*significant differences between means for 0.01 < p < 0.05.
**distinguished significant differences between means for 0.001 < p < 0.01.
***highly significant differences between means for p < 0.001.

Cortisol

Cortisol is the most active and abundant corticosteroid in fish blood, and its structure has been highly conserved in all vertebrate species in which it is found (Sloman et al., 2001).

The main targets of action of cortisol are the gills, intestine, and liver; they reflect the main adaptive functions of cortisol identified thus far: osmoregularity and maintaining a balanced energy metabolism (Overli et al., 2002).

Consistent with other studies (Best & Glimour, 2022), basal cortisol levels in unstressed salmonids ranged from to 0-5 ng/ml, but acute stress (handling or one hour of confinement) caused a temporary increase in cortisol levels, ng/mL, in the range of 4-20 ng/ml, with a return to basal level in 24-48 hours.

It is recommended that repeated measurements be made during or after acute exposure of the animal, and during chronic experiments, sampling should not be very frequent, as their handling may affect future measurements (Culbert & Glimour, 2016).

The degree of increase in cortisol levels in response to acute stress is also related to the trout species studied. Chronic stress (prolonged labor or crowding) results in an increase in

cortisol levels of approximately 10 ng/ml^{-1}, and blood cortisol levels remain elevated for up to 4 weeks before acclimation (Lequin, 2005; Pfalzgraff et al., 2021).

In specialized literature, pre- and post-stress cortisol variations are presented in two rainbow species of interest, *Oncorhynchus mykiss* and *Salvelinus fontinalis*. After manipulation and isolation, rainbow trout recorded pre-stress values of 77 nmol/l and post-stress values of 698 nmol/l, which was 19 nmol/l in the initial phase but increased to 242 nmol/l after the action of the stress factor (Sepahi et al., 2013; Mota et al., 2017).

In the case of trout studied, cortisol dosing was performed at three distinct intervals:

- unstressed: taking the samples immediately after extraction from the pond, without applying a stress beforehand;
- stressed "0 h" - sampling after applying stress factors (lack of oxygen, handling, and higher water temperature);
- stressed "1 h" - sampling after applying stress factors (lack of oxygen, handling, higher water temperature) and keeping them on ice (additional stress factor) for a period of one hour.

In individuals from the unstressed trout group, an average cortisol value of 86.56±7.34 µg/dL was recorded in the case of P-si, and in those from P-i of 80.28±3.14 µg/dL. The quantitative differences between the two fish batches were statistically significant.

Following the action of the stress factors, the analysed blood samples recorded much higher cortisol values, both in the "0 h" stressed individuals (163.62 ± 17.96 µg/dL in P-si, and 159.87 ± 14.36 µg/dL in P-i), as well as chosen for the "1 h" stressed samples (295.62 ± 14.34 µg/dl in P-si, and 298.12 ± 8.18 µg/dl in P-i). It is mentioned that, for both types of applied stress, the responses of the two species of trout were remarkably similar, the statement being confirmed by the lack of statistical differences (Table 5).

Table 5. Results regarding cortisol analysis in 2.5 years old rainbow trout

Parameter/ Growth system		N	Average values $\bar{X} \pm s_{\bar{x}}$ (µg/dl)	The confidence interval of the mean (95%)		Min. (µg/dl)	Max. (µg/dl)
				Lower limit	Upper limit		
Cortisol in rainbow trout non-stressed	P-si	18	86.56±7.34	82.84	91.64	75.26	95.48
	P-i	18	80.28±3.14	78.96	82.58	77.46	87.98
The statistical significance	P-si vs. P-i= ***; F=10.836, p=0.002, p< 0.05						
Cortisol in stressed rainbow trout at "0h""	P-si	18	163.62±17.96	154.70	174.24	129.82	187.30
	P-i	18	159.87±14.36	153.45	167.80	133.68	180.42
The statistical significance	P-si vs. Pi= n.s.; F=0.469, p=0.498, p> 0.05						
Cortisol in stressed rainbow trout at "1h"	P-si	18	295.62±14.34	288.04	302.26	264.98	312.66
	P-i	18	298.12±8.18	294.02	302.68	286.04	314.98
The statistical significance	P-si vs. P-i = n.s.; F=0.396, p=0.533, p> 0.05						

*significant differences between means for $0.01 < p < 0.05$.
**distinguished significant differences between means for $0.001 < p < 0.01$.
***highly significant differences between means for $p < 0.001$.

CONCLUSIONS

Water flows and densities were within specific norms for both intensive and semi intensive systems.

Water quality parameters were similar across both systems, with values within acceptable limits.

Differences in administered feed, including protein and metabolizable energy levels, were noted but supported technologically in both variants.

Blood glucose and liver glycogen levels showed slight variations between rainbow trouts rearing in intensive and semi intensive systems.

Growth hormone levels were higher in intensive system, but still within acceptable ranges for both systems.

Cortisol levels fluctuated, indicating stress responses, with slightly higher values in semi intensive system.

Overall, no significant differences were observed compared to specialized literature, highlighting the existence of major stress factors post-fishing.

Growth system variations did not significantly influence studied parameters.

Better adaptability of specimens in the intensive system was noted, likely due to additional feeding and handling practices.

The study suggests that exploiting salmonids in systems closer to natural conditions may not guarantee superior results compared to intensive systems.

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