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Article

The Physiological Effects of Some Stress Indicators in Rainbow Trout Raised in Different Systems

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Abstract: The general desire of the world's population is to eat as healthily as possible, an aspect that has a direct impact on the technological complex aimed at animals raising (proper breeding systems, microclimate factors with implications for the welfare of animals, etc.), as well as on the quality of the finished products that will enter the food supply. Therefore, the authors set out to study how the growth system influences the health status of rainbow trout raised in different systems. The observed experimental factors did not undergo significant changes. However, differences were generated by the applied growth system. For the amount of glucose in the blood, no statistically significant differences were identified between the groups. In contrast, comparison of the amount of glycogen led to obtaining very distinctly significant statistical fluctuations (the mean for P-si was 2.314±0.638 and for P-i 1.980±0.822). Growth hormone varied between 0.504±0.46 in the case of P-si and 0.694±0.22 ng/ml for P-i. The values obtained for cortisol showed a significant influence of stress factors on the studied trout. The general conclusion of the present study is that various technological factors influence the health status of livestock.

Keywords: rainbow trout; rearing system; stress factors; hormones

1. 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 [1].

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 [2].

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 [3,4].

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...+240C) 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^{0}C) [5].

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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^{0}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 [6,7].

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 [8].

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 signaling pathways of metabolic activity [9].

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 [10,11].

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 [12].

Evaluation of the effect of some stressful factors (water temperature, handling, and low water level in the pool) on some antioxidant enzymes in rainbow trout revealed a significant increase in glutathione peroxidase and catalase in all analyzed situations, glucose 6-phosphate dehydrogenase only at high water temperature, and glutathione reductase in specimens stressed by handling and low water [13].

The stress generated by exposure to air (3 min) caused an acute response for 24h post-exposure, which resulted in significant increases in cortisol, lactate, and plasma glucose as an expression of the reactivity of liver microRNAs [14].

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 [15].

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 [16,17].

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 [18]. 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 [19].

2. Materials and Methods

2.1. Animals and Housing

This study focused on the influence of rearing systems on some indicators that highlight the state of stress in cultured trout.

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The biological material was represented by rainbow trout (*Oncorhynchus Mykiss*), aged 2½ summers, from two farms, one of which operates in a semi-intensive exploitation regime (P-si) and the other in an intensive system (P-i).

It is very important that the two trout farms were located in a mountainous area, in the central-eastern part of Romania, at a close distance from each other; therefore, they were subject to similar pedoclimatic conditions. This area is characterized by high temperatures in the warm season (25-30^{0}C) and low temperatures during the cold season (up to -25^{0}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).

The semi-intensive salmon farm (P-si) has a production capacity of approx. 3 tons/year and has earth basins (with a gravel hearth), with depths between 0.8 m (at the inlet) and 1.8 m (at the monk). The pools were fed with water from a neighboring stream, ensuring a constant flow of approximately 60 L/s. For consumption of trout, granulated combined feeds with 41% B.P and 4100 kcal/kg M.E. were administered in a single daily portion, in 1.1% of the fish mass.

The intensive salmon 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). In this case, the source of water is a natural one, a mountain stream that springs approximately 1.5 km from the holding that can ensure a flow rate of approximately 200 L/s. Feeding was carried out with extruded granulated feed (43% B.P. and 4300 kcal/kg M. E.), administered in two sessions/day, in a daily amount that represented 1.2% of the fish mass.

The actual determinations were carried out at the end of September 2022, on samples taken from a number of 40 specimens of rainbow trout, aged 2½ years, purchased from the mentioned trout farms (18 specimens/farm); the capture of the fish was done randomly, limiting their stress to the greatest extent possible.

In the week preceding the collection of the biological material, water samples were taken, on which the following indicators were determined: temperature, dissolved oxygen, pH value.

2.2. Methods

Water quality 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 [20]. To benefit from the highest possible accuracy in the determinations, three distinct and metrologically approved professional devices were used instead of multifunctional devices.

The temperature was recorded using a professional digital thermometer used in aquaculture, type TFA-Dostmann, produced by Roth. It is metrologically approved, has an error margin of 0.1^{0}C, can probe up to a depth of 2.5 m and is compatible with a PC interface, an aspect that facilitates the storage of data for various control periods.

The determination of dissolved oxygen was carried out with the help of TMT-DO-5512SD, a device specially designed for high-precision measurement and recording of the parameters, with automatic temperature compensation (ATC) in the range of 0-50°C. The digital oxygen meter was equipped with a polarographic probe and an integrated temperature sensor. The sampling intervals can range from 1 s to 9 h, with the device having special functions of interest: maximum, minimum, and average values. The oximeter has an RS-232 serial interface required for data export for farm registers [21].

The pH was determined using a WTW ProfiLine pH 3310 portable pH meter manufactured by Toth. The device was equipped with a 5-point calibration system and an automatic temperature compensation function. From a technical point of view, the WTW ProfiLine is a top-quality model with a measuring range of -2/20 pH, resolution of 0.001 pH, and accuracy of ±0.005 pH. The PC interface also facilitates the transfer of data obtained during monitoring. [22].

Biochemical and immunological analyses were performed in an accredited laboratory by personnel authorized to perform these tests, with the application of approved procedures [23,24]. Biochemical analyses were performed using an ILab Tautus analyzer and laboratory instrumentation, with the application of standardized work methods [25,26]. The hormone dosage was determined

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using the ELISA method [27,28]. All determinations were performed in accordance with the methodology and legislation in force [29–31].

2.3. 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.

3. Results

3.1. Results Regarding the Experimental Factors

In order to accurately determine the influencing factors on the health status of the analyzed populations, the authors of this study monitored a series of growth parameters within the two trout farms under analysis. Therefore, the technical parameters applied at the farm level, the water quality in the rearing tanks used, 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/m2 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. Within the batches, the character was homogeneous, the coefficient of variation indicating very small values.

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

In the week preceding the determinations, average water temperature values of 11.24±0.76°C were obtained for P-si and approximately 1 °C lower, of 10.14±0.40°C for P-i. In both cases, there were no drastic increases or decreases in parameter values, which could lead to trout stress.

The amount of dissolved oxygen was maintained at an average level of 8.46±0.22 mg/l in the case of P-si and 9.82±0.18 mg/l in P-i. At the same time, no significant fluctuations were recorded.

The pH value of the water in the growth pools had very similar average values between the two groups, more precisely 7.28±0.14 for P-si and 7.65±0.22 for P-i. This parameter fell within the allowed limits and did not represent a disruptive factor for fish health.

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 (P-si) and 4300 (P-i).

In both fish holdings, 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. Experimental factors applied to 2½-year-old trout.

	Specification	Semi-intensive system (P-si)	Intensiv (P-i)
The technical parameters	Water flow rate (liters/second)	60 l/sec	200 l/sec
	Density	25 heads/m2	75 heads /m2
	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

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Combined feeds administered	Protein content (%)	41	43
	Metabolizable energy (kcal/kg)	4100	4300
	Daily feed intake	3	3
	Amount of combined feed (% of fish weight)	1.1	1.2

3.2. Results Regarding the Blood Glucose Level

Because the amount 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 the study.

Thus, in the case of the batch related to the semi-intensive P-si system, average values of the parameter of 20.78±1.44 mg/dl were obtained. The minimum determined 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. Results on glucose analysis in rainbow trout, at 2½ years old.

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.

3.3. The Results Regarding Hepatic Glycogen

Liver 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/100g, the value being higher than that determined for P-i, which was 1.980±0.822 g/100g. These values generated significant statistical differences among the studied lots.

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.

Table 3. Results regarding hepatic glycogen in 2½-year-old rainbow trout.

Growth system		n	Average values $\overline{X} \pm s_{\overline{x}}$ (g/100g)	The confidence interval of the mean (95%)		Min. (g/100g)	Max. (g/100g)
				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 0.01 < p < 0.05. **distinguished significant differences between means for 0.001 < p < 0.01. ***highly significant differences between means for p < 0.001.

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3.4. Results Regarding Growth Hormone hGH

In the case of fish, hGH 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 hGH 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. However, no statistically significant differences were observed between the two batches studied were highlighted.

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 3. Results of the analysis of growth hormone hGH to rainbow trout, at the age of 2½ years old.

Growth system	n	Average values $\bar{X} \pm s_{\bar{x}}$ (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.

3.5. Results Regarding Cortisol (Stress Hormone) Measurement

Cortisol was dosed in three different situations: lack of application of stress factors immediately after fishing and after one hour of transport.

The results obtained for the first studied situation (lack of stress factors), at P-si, highlighted the achievement of an average value for the studied parameter of 86.56±7.34 µg/dl, under the conditions of highlighting a minimum value of 75.26 µg/dl and one maximum of 95.48 µg/dl.

In the herd raised in the intensive system (P-i), the average was clearly lower, of 80.28±3.14 µg/dl, with oscillation limits between 77.46 µg/dl and 87.98 µg/dl.

The large differences between the two batches led to the emergence of significant statistical differences.

The 2nd control period, marked symbolically "0h" was characterized by obtaining average values of 163.62±17.96 µg/dl for P-si, respectively 159.87±14.36 µg/dl for P-i. In this situation, the comparison of batches did not highlight the presence of statistical differences between them. The minimum values obtained for P-si and P-i were 129.82 and 133.68 µg/dl, while the maximum values were 187.30 and 180.42 µg/dl, respectively.

After applying the "I h" factor, I observed significant increases in the values for this parameter, compared to the previous situations. However, no statistically significant differences were observed between the results obtained for trout grown in the two systems. Thus, the average values obtained for the indicator were 295.62±14.34 µg/dl for P-si and 298.12±8.18 µg/dl in the case of P-i. The minimum values fluctuated from 264.98 µg/dl in the case of P-si to 286.04 µg/dl in P-i. The maxima reached 312.66 µg/dl at P-si, respectively 314.98 µg/dl at P-i.

Table 4. Results regarding cortisol analysis in 2½-year-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

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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 $p < 0.001$.

4. Discussion

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/minute/kg fish) and relatively rich in dissolved oxygen (over 6 mg O_2/ it) [2,3]. 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 [20].

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 [5,15].

Water temperature plays an important role in the growth of salmonids because it influences the body temperature of trout [1]. Optimal feeding temperatures and high digestibility are 15-19°C for rainbow trout [12]. 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 was more intense between 12°C and 14°C [20].

Within the two salmon farms it was found that the temperature values showed variations, but insignificant, the average being 11.24±0.76 at P-si and 10.14±0.40 at P-i.

The pH value of the water in the salmon ponds where the research was carried out 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 quality II. For salmonids, an optimal pH must be between 7.5 and 8.5 [5].

Water in which the dissolved oxygen content does not fall below 9 mg/l, at water temperatures of 18-19°C, is considered good for trout. 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 [20]. Salmonids can live in water with an oxygen content of 9-10 mg/l [2]. The amount of dissolved oxygen determined in the farms studied was 8.46±0.22 mg/l P-si and 9.82±0.18 mg/l at P-i.

4.1. Blood Glucose

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 [32]. Some factors can indirectly alter blood glucose levels. Some studies suggest that *"growth history, including nutritional status, may affect stress response and glucose release"* [33]. 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 [34].

Nutrition is also an important factor that influences blood glucose levels. Thus, the amount varies between species and depends on the developmental stage [35]. The intake of diets with different lipid and protein contents results in distinct blood glucose levels [36].

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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 [37].

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 [38].

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 [39].

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. [33,35].

4.2. Liver Glycogen

Following the analyses of trout specimens raised in the semi-intensive system for liver glycogen, the average values obtained were 2.314±0.638 g/100 g and 1.980 ± 0.822 g/100 g for the intensive system. Statistically point of view, the differences between the two groups were very distinct. These results were slightly lower than those reported by other authors.

When interpreting the data, we first considered the conditions from which the studied trout benefited, since glycogen content can reflect biochemical adaptations to any kind of stress from the environment [40]. Of these, pH, oxygen and salinity levels, as well as prolonged physical activity, directly affect glycogen reserves [41,42].

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 [43]. 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 [44].

Determination of 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 [45].

Although the trout belong to the same species, 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 point of view, a high level of blood glucose could be explained by the energy requirement, as trout are predatory species and considerable amounts of energy are spent catching prey [46].

4.3. Growth Hormone hGH

hGH is a pluripotent hormone produced by the pituitary gland and is secreted in response to exercise, stress, deep sleep, hypoglycemia, and insulin. If hGH is secreted deficiently or excessively in the first stages of growth, dwarfism and gigantism will appear respectively [40,47]. Over the past two decades, many aspects of hGH physiology have been the subject of intense research in fish, particularly in salmonids, cyprinids, and sparids [48,49].

Recent studies have shown that hGH affects several aspects of behavior such as appetite, foraging behavior, aggression and predator avoidance, with the finality of these changes having ecological consequences [50,51].

The data obtained after the hGH hormone dosage indicated an average value of 0.504±0.46 ng/ml for P-si and 0.694±0.22 ng/ml for P-i, without the difference between the growth systems having statistical significance, the data being similar to from the specialized literature [35,52].

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4.4. 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 [53].

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 [54].

Consistent with other studies [55], basal cortisol levels in unstressed salmonids ranged from to 0-5 ng/ml-1, 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 [56].

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 [57,58].

In specialized literature, pre- and post-stress cortisol variations are presented in two 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 [59,60].

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

- unstressed: taking the samples immediately after extraction from the pool,without applying a stress beforehand;
- stressed "0h" - sampling after applying stress factors (lack of oxygen, handling, and higher water temperature);
- stressed "1h" - 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 analyzed blood samples recorded much higher cortisol values, both in the "0h" stressed individuals (163.62±17.96µg/dl - P-si and 159.87±14.36 µg/dl P-i), as well as chosen for the "1h" stressed samples (295.62±14.34µg/dl at P-si and 298.12±8.18µg/dl at 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.

5. Conclusions

As a result of the present study, a series of conclusions related to the addressed topic resulted, the main ones being stated in the following.

Experimental factors. The water flow used was 200 l/sec for P-i and only 60 l/sec for P-si, owing to the applied densities (density was 25 head/m^2 for P-si and 75 head/m^2 for P-i).

The achieved weight recorded average values of 262.84±5.12 g for P-si, while for P-i they were 274.36±5.23 g.

The average values for water temperature were: 11.24±0.76 ^{0}C for P-si and 10.14±0.40^{0}C for P-i.

The amount of dissolved oxygen was 8.46±0.22 mg/l in the case of P-si and 9.82±0.18 mg/l in P-i.

The average water pH values were similar between the two batches, namely 7.28±0.14 P-si and 7.65±0.22 for P-i.

The protein level of the recipe administered at P-si was 41%, whereas that at P-i was 43%. Differences also existed in metabolizable energy: 4100 (P-si) and 4300 (P-i).

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In both trout farms, three daily feedings were administered, only that in P-si, the amount of combined feed represented 1.1% of the fish mass, while in P-i it was 1.2%.

Blood glucose level. For P-si, average values of 20.78±1.44 mg/dl were obtained, with a minimum value of 19.36 mg/dl and a maximum recorded value of 22.28 mg/dl. In the case of P-i, the minimum value was 18.82 mg/dl and the maximum 23.40 mg/dl, calculating the average generating the value of 21.10±2.24 mg/dl.

Liver glycogen. 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, higher than the 1.980±0.822 g/100 g valid for P-i. These values generated significant statistical differences among the studied lots.

Cortisol. In the absence of stress factors, at P-si, the average value was 86.56±7.34 μg/dl, and at lower P-i, 80.28±3.14 μg/dl.

The second "0h" period was characterized by obtaining average values of 163.62±17.96 μg/dl for P-si, respectively 159.87±14.36 μg/dl for P-i.

The application of the experimental factor "stressed 1 h" brought major increases in cortisol values (295.62±14.34 μg/dl in P-si and 298.12±8.18 μg/dl in the case of P-i).

The general conclusion of the study is that the intensive growing system brings more benefits (especially economic benefits) compared to the extensive system. As an extremely important measure, it is recommended to reduce the negative influence of stressors through technology.

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