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BLOOD PARAMETERS OF RAINBOW TROUT *Oncorhynchus mykiss* (Walbaum, 1792) FED DIETS SUPPLEMENTED WITH NATURAL PHYTOADDITIVES DURING THE COLD SEASON

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Abstract

The use of phytoadditives in animal feeds has been gaining more attention due to their positive effects on growth and health of animals. This study aimed to determine the effects of adding natural phytoadditives to the diet of rainbow trout on its blood parameters during the cold season. One control and three experimental groups consisting of 50 adult rainbow trout each were fed a standard feed and three experimental diets consisting of the standard feed with 2% carrot, tomato, and spinach powders, respectively, for 90 days. Blood parameters were determined. Most parameters changed significantly ($p<0.05$) from the start to the end of the experiment. There were few significant changes among the control and experimental groups at the end of the experiment. Cholesterol and triglycerides increased in the control and experimental group with 2% carrot powder. Some minerals showed different ($p<0.05$) values in the experimental groups (Na, K, and Ca). The study shows that the incorporation of phytoadditives in rainbow trout feed does not produce negative effects on the blood parameters, with some advantages being present, such as a generally better mineral profile of the plasma and the stabilization of hematological parameters.

Key words*: carrot, hematology, plasma biochemistry, salmonids.*

INTRODUCTION

Natural phytoadditives have gained more and more interest for aquaculture due to their potential benefits for fish health, growth performance, and disease resistance (Ivan et al., 2022; Kuebutornye et al., 2023; Ivan et al., 2024a; Zhelyazkov & Stoev, 2024). Flavonoids, carotenoids, saponins, and other compounds found in different plants have antimicrobial, antioxidant, and immune stimulatory properties (Samtiya et al., 2021; Rahimi et al., 2022; Hu et al., 2024). Thus, phytoadditives can serve as alternatives to synthetic antibiotics, reducing the risk of antibiotic resistance, minimizing environmental contamination and presenting a safer product for consumers (Păpuc et al., 2024). There are many studies demonstrating that dietary supplementation with plant extracts improves growth performance and immunity in cultured species (Gherescu et al., 2023).

Consumer awareness regarding the welfare of farmed animals, including fish, has been continuously increasing, especially based on access to transparent information about farming practices. Certification programs and labelling schemes have been developed to address ethical concerns about aquaculture (Hammarlund et al., 2024). Farmers must consider the nature of the feed used, as natural ingredients in fish feed may prove more attractive and safer for consumers concerned with the welfare of the fish and with the sustainability of the practice. Thus, the supplementation of fish feeds with

phytoadditives could not only provide benefits for the fish, but also for the farmers, in the form of a distinct selling point.

Classic intensive salmonid farms need a careful management of environmental parameters. Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) is more resistant to fluctuations in these factors than other salmonids. However, they still directly impact growth rates and overall health (Uiuiu et al., 2021). Climate variability and seasonal changes pose significant challenges to rearing conditions (Tekeli & Bildik, 2023). Rainbow trout experience seasonal variations in growth, with stagnation or reduction in winter due to lower metabolic activity (Tidwell et al., 1991; Taylor et al., 2006). Water temperature is a primary determinant of growth, as metabolic processes are reduced in colder temperatures, lowering feed intake and weight gain, energy conservation becoming a priority. Consequently, aquaculture systems must adapt feeding strategies to accommodate these physiological changes during the winter.

Many traditional trout farms adopt a winter fasting strategy, drastically decreasing feed administration, or even ceasing it during the coldest months. This practice aligns with the reduced metabolic rate of trout, helping prevent feed waste. Winter fasting has some advantages, such as the reduction of operational costs or minimization of uneaten feed accumulation, but also some disadvantages, like the possibility of excessive weight loss or fish health deterioration (Weatherup et al., 1997). Therefore, the decision to implement fasting should consider these factors. On the other hand, some farmers prefer to adopt different strategies, such as feeding to satiation or the administration of sufficient feed to maintain trout weight during winter (Taylor et al., 2006). However, the effects of such feeding strategies during winter on rainbow trout health are less studied.

Blood indices provide essential information about the physiological and health status of rainbow trout. Hemoglobin concentration, erythrocyte count, hematocrit levels, plasma glucose, and enzymatic activity serve as indicators of stress, nutritional status, and immune function (Cocan et al., 2019). Multiple factors simultaneously influence blood parameters in rainbow trout, including water temperature, stocking density, handling stress, age, sex of the fish, and many others (Barnhart, 1969; Martinez et al., 1994; Yarahmadi et al., 2014; Jiang et al., 2021; Manera et al., 2021). Seasonal changes, particularly winter conditions, can alter hematological and biochemical profiles due to metabolic adaptations (Lahnsteiner & Dünser, 2024). Monitoring blood parameters allows the assessment of the diet, environmental parameters, and disease impacts on fish physiological status. Understanding these interactions is crucial for optimizing trout farming technologies and improving fish welfare in aquaculture systems.

The present study aims to assess the blood parameters of rainbow trout fed with phytoadditive-supplemented feeds, in a classic, intensive trout farm, in the cold season.

MATERIALS AND METHODS

The present study was conducted at a salmonid farm, near Cluj-Napoca, Romania, from November 2023 to February 2024, for 90 days. From an initial group (Gi) of 200 fish, the control and the three experimental groups were formed, each with 50 fish, consisting of clinically healthy adult rainbow trout. Each group was assigned to a race-way type concrete pond, with a volume of 2.5 m^3 (5 x 1 x 0.5 m), being stocked at a density of 20 ind m^{-3}.

A standard feed in the form of 6 mm pellets was used as the control feed, for the control group (G1). The experimental feeds consisted of the standard feed with an addition of 2% meal from different natural carotenoid sources: carrots (Nantes), tomatoes (giant tomato) and spinach (Nores). The vegetal materials were previously farmed in a nearby household, harvested, dried in an aerated oven at 39.5°C, and blended to obtain a powder from each type of material used. Three types of experimental feed were prepared, each with a 2% addition of one of the vegetal powders, obtaining an experimental feed with 2% carrot powder (EFC) fed to group G2, an experimental feed with 2% tomato powder (EFT) fed to group G3, and an experimental feed with 2% spinach powder (EFS) fed to group G4. The phytoadditive was added after mixing with water, simply by coating the pelleted feed and

drying in a ventilated oven at 39.5ºC. The feed was administered once per day, until satiation.

The chemical composition of the powders and of the experimental feeds is presented in Table 1. The proximate composition of the feeds and powders was determined using the FT-NIR Tango from Bruker (Billerica, MA, USA) (Masithoh et al., 2020). Five samples were selected from each studied material, and each sample suffered triplicate measurements.

Table 1. Chemical composition of phytoadditives and experimental feed

Phytoadditives and feeds	DM (%)	Protein (%)	Fat (%)	Ash (%)	Fibers (%)
Carrot powder	81.96±0.84	8.48±0.1	1.64±0.09	1.32±0.05	36.46±0.92
Tomato powder	84.3±0.5	15.69±0.43	1.96±0.18	2.85±0.07	22.3±0.82
Spinach powder	75.54±1.12	13.73±0.12	2.69±0.12	3.12±0.06	32.31±0.79
Standard feed	91.53±0.57	37.82±0.47	13.76±1.29	5.27±0.03	5.88±1.29
EFC	91.53±0.2	36.67±0.47	13.49±1.28	5.19±0.04	6.21±0.49
EFT	91.54±0.29	36.85±0.46	13.56±1.24	5.21±0.02	5.93±0.48
EFS	91.44±0.16	36.59±0.51	13.53±1.24	5.22±0.02	5.99±1.21

EFC - experimental feed with 2% addition of carrot powder; EFT - experimental feed with 2% tomato powder addition; EFS - experimental diet with 2% spinach powder addition; DM - dry matter; values are presented as means ± SD (n = 5).

Water parameters were specific to an intensive salmonid farm, with outside ponds, in a temperate climate region, during the cold season. At the start of the experiment, the temperatures had the highest values, decreasing until the end of the experiment. The water source was the same for each pond, a drinking water supply reservoir, situated above the farm. The water flow was maintained at 3 L s^{-1} during the experimental period. The water parameters determined were: temperature, dissolved oxygen (DO), pH, electrical conductivity, total dissolved solids (TDS), and turbidity. The water parameters were monitored daily, before the first feeding, with a Hanna HI 9829 Multiparameter, as water quality is an essential factor in the success of a trout farm (Ivan et al., 2024b). The water having the same source, its parameters did not suffer differences among ponds. The water parameters are presented in Table 2.

Table 2. Water parameters during the study

Descriptive statistics parameters	Parameters					
(n = 90)	Temp.	DO	pH	Electrical conductivity	TDS	Turb. [FNU]
Mean	5.6972	14.0084	6.823	78.6111	38.9222	1.0633
SD	2.368	0.6045	0.1231	3.4502	1.8512	0.6138
Min.	2.74	12.18	6.6	72	36	0.2
Max.	9.91	14.98	7.12	86	43	2.6

SD - standard deviation; Temp - temperature; DO - dissolved oxygen; TDS - total dissolved solids; Turb. - turbidity.

Blood samples were randomly collected from 10 rainbow trout at the start of the experiment, from the initial group Gi, of 200 fish. Five samples were used for hematological analyses and five others for plasma biochemical analyses, as the volume did not allow to perform all the analyses from a single sample. At the end of the experiment, samples were collected similarly from ten trout, this time from each of the four groups. Blood was collected by puncture of the caudal vein and transferred to vacutainers with LiHeparin for analyses. The fish were priorly anesthetized with clove oil at a dose of 30 mg L^{-1} (Javahery et al., 2012). As anaesthesia can influence blood parameters, care was taken to keep the anaesthesia time to a minimum. The blood samples were transported in refrigerated conditions (2-4°C) to the Hematology and Biochemistry Laboratory of UASVM Cluj-Napoca, for further analyses (Cocan et al., 2018). After the blood samples were collected, the sampled fish were transferred to well-oxygenated water to facilitate recovery.

The hematological profile was characterized by determining hemoglobin (Hb), erythrocytes (Ery), and hematocrit (Hct) from the blood samples. The Hb concentration was determined within a maximum of 6 h after harvesting by the END-POINT method, employing a colorimetric reaction, with VIS reading at 546 nm and 37°C. Erythrocyte determinations were conducted using Gowers reagent through a turbidimetric

reaction in the visible range at a wavelength of 546 nm and at 37°C. The Hct was determined by centrifugation in capillary tubes at 12000 rotations per min for 3 min. The Ery indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to standard formulas (Sarma, 1990).

All analyses were performed per the instructions of commercial test kits available for each parameter with the UV-VIS Screen Master Touch spectrophotometer in the 340-620 nm wavelength range.

The protein profile parameters determined were the following: total protein (TP) concentration, albumins (ALB), gamma globulins (GG), urea (UR), and creatinine (CRN). TP was determined at 546 nm in the VIS domain and ALB at 630 nm by the END-POINT type method. The GG concentration was determined turbidimetrically at 546 nm in the VIS domain and UR at 340 nm with the kinetic enzymatic method. CRN was determined by a fixed-time colorimetric method (FXT) with VIS reading at 510 nm.

To analyse the lipid profile, the concentrations of triglycerides (TG) and cholesterol (CHOL) were determined through END-POINT type colorimetric reaction at 546 nm (TG) and 510 nm (CHOL).

The following enzymes were determined for the enzymatic profile: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma-glutamyltransferase (GGT), and lactate dehydrogenase (LDH). ALAT, ASAT, and LDH were determined at 340 nm by the kinetic method. GGT was determined at 405 nm.

The following parameters were analysed for the mineral profile: Ca^{2+}, PO_4^{3-}, Fe^{3+}, Na^{+}, Mg^{2+} and K^{+}. The Ca^{2+}, PO_4^{3-}, Mg^{2+} and Fe^{3+} determinations were made using an END-POINT-type colorimetric reaction at 650 nm (Ca^{2+}), 340 nm (PO_4^{3-}), 405 nm (Fe^{3+}), and 510 nm (Mg^{2+}). Na^{+} was made using the colorimetric enzyme type method. K^{+} was determined by the kinetic method at 380 nm.

Glucose (GLC) was determined through the END-POINT enzymatic colorimetric assay (λ=500 nm). Total bilirubin (TB) was also determined, at 540 nm.

Data analysis was performed using GraphPad Prism 9 software. The Shapiro-Wilk test was used to assess the normality of data distribution. When data followed a normal distribution, differences among groups were analysed using one-way ANOVA, followed by Tukey's post-hoc multiple comparison test. For data that did not meet the assumption of normality, the Kruskal-Wallis test was applied, followed by Dunn's multiple comparison test. The significance level was set at $\alpha=0.05$.

RESULTS AND DISCUSSIONS

The hematological profile presented no significant differences ($p>0.05$) for the studied parameters among G1 and the experimental groups. However, there were significant differences ($p<0.05$) for some parameters between the start and the end of the experiment (Table 3).

The obtained Hb values are relatively normal considering the low average water temperature and high DO levels during the study. At higher temperatures, Martinez et al. (1994) obtained Hb values between 6.2 and 12.1 g dL^{-1}. Erythrocyte levels ranged from 2.66 to 3.41x106 mm^{-3}, a range considered normal by some authors (Řehulka et al., 2004; Nabi et al., 2022; Lahnsteiner & Dünser, 2024), but higher than normal by others (Mesalles et al., 2024).

Table 3. Hematological profile of rainbow trout

Group	Hb (g dL^{-1})	Ery (x 10^6 mm^{-3})	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Gi	5.88±1.16^{a}	2.66±0.3^{a}	30.4±7.6^{a}	112.85±15.57^{a}	21.99±2.68^{a}	19.65±2.72^{a}
G1	6.31±0.49^{a}	3.41±0.33^{a}	45.6±5.41^{b}	133.77±9.56^{ab}	18.58±1.22^{a}	13.95±1.39^{b}
G2	6.47±0.58^{a}	2.97±0.36^{a}	43.6±7.43^{ab}	145.83±11.75^{b}	21.82±1.3^{a}	15.07±1.82^{b}
G3	6.45±1.03^{a}	3.25±0.42^{a}	43.2±7.94^{ab}	132.22±10.31^{ab}	19.95±3.54^{a}	15.23±3.43^{b}
G4	6.49±1.29^{a}	3.08±0.56^{a}	44.2±9.03^{ab}	143.44±14.86^{b}	20.98±2.17^{a}	14.66±1.1^{b}

Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; Hb - hemoglobin; Ery - erythrocytes; Hct - hematocrit; MCV - mean cell volume; MCH - mean cell hemoglobin; MCHC - mean corpuscular hemoglobin concentration; values are presented as mean ± SD (n = 5); different superscripts in the same column show significant differences ($p<0.05$); Hb, Hct, MCV, MCH and MCHC data were analysed with one-way ANOVA, followed by Tukey's multiple comparisons test; Ery data was analysed with the Kruskal-Wallis test.

The Hct had values that can be considered upper normal (Wells & Weber, 1991). A clear increase was observed at the end of the experiment, with a peak of 45.6% in the control group. These slightly elevated values can be attributed to the lower temperatures of the water at the end of the experiment.

MCV values increased by the end of the experiment in all groups, with significant differences ($p<0.05$) between MCV levels in Gi, and G2 and G4. As the optimal MCV level is between 350 and 400 fL (FAO, 1991), the values obtained in this study can be considered low for rainbow trout, indicating small sizes for erythrocytes. There are instances with a lower MCV, for example, when rainbow trout is exposed to zinc or cobalt chloride (Atamanalp et al., 2011; Baladehi et al., 2017). Fazio et al. (2016) note that healthy rainbow trout in an Italian farm had an MCV of 190.8 fL, while healthy rainbow trout in a Turkish farm had a mean MCV of 85.66 fL. Ihuț et al. (2018) also note a decrease of MCV in winter for rainbow trout, with a value of 109.62 ± 3.17 fL. MCH values were situated between 18.58 and 21.99 pg. Although normal MCH values for rainbow trout are higher than the values obtained in the current study (Fazio et al., 2017a; Ihuț et al., 2018), in some cases, comparable values were obtained for healthy rainbow trout (Lone et al., 2012; Fazio et al., 2016). The MCHC values obtained in this study at the end of the experiment are within the normal range (8.8-18.2 g dL^{-1}) according to Mesalles et al. (2024), while the initial value slightly exceeds the normal range. Most studies find MCHC in healthy rainbow trout at higher values (Fazio et al., 2017b; Ihuț et al., 2018; Nabi et al., 2022), although there are some reports similar to the results of this study (Řehulka et al., 2004).

As the fish experienced normal conditions in the winter season in an intensive salmonid farm, without significant differences between the experimental groups and the control group regarding the hematological profile, the most likely explanation for values outside normal ranges may be cold-induced physiological adaptations.

Generally, the protein profile of the plasma remained the same throughout the experiment. There were two cases when significant differences occurred, namely: the TP concentration in G2 was significantly higher ($p<0.05$) than that of G4, and the CRN level in Gi was significantly higher ($p<0.05$) than that of fish from G1. The protein profile obtained is presented in Table 4.

TP levels in the plasma of rainbow trout obtained in this study were relatively normal. Řehulka et al. (2004b) found TP levels between 3.1-5.8 g dL^{-1}, with significant differences between males and females, while Manera & Britti (2006) suggest a normal level of TP in rainbow trout of 3.32-3.85 g dL^{-1}. TP generally shows the health status of the fish. Morro et al. (2020) identified 1822 proteins in the plasma of rainbow trout.

ALB levels were relatively normal, in line with the findings of many different studies (Vigiani et al., 2005; Manera & Britti, 2006; Pastorino et al., 2022). Monitoring albumin levels may help indicate the nutritional status and overall health of rainbow trout.

Table 4. Protein profile of rainbow trout plasma

Group	Total protein concentration (g dL^{-1})	Albumin concentration (g dL^{-1})	Gamma globulin concentration (g dL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Gi	4.58 ± 1.62^{ab}	1.44 ± 0.50^{a}	0.63 ± 0.35^{a}	14.94 ± 1.53^{a}	0.65 ± 0.12^{a}
G1	4.63 ± 0.36^{ab}	1.30 ± 0.08^{a}	0.38 ± 0.09^{a}	15.64 ± 2.80^{a}	0.43 ± 0.05^{b}
G2	5.17 ± 0.42^{a}	1.57 ± 0.18^{a}	0.44 ± 0.10^{a}	14.84 ± 0.99^{a}	0.54 ± 0.08^{ab}
G3	4.31 ± 0.13^{ab}	1.29 ± 0.14^{a}	0.27 ± 0.11^{a}	16.18 ± 3.00^{a}	0.47 ± 0.02^{ab}
G4	4.02 ± 0.36^{b}	1.30 ± 0.17^{a}	0.38 ± 0.13^{a}	14.54 ± 1.59^{a}	0.47 ± 0.15^{ab}

Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; values are presented as mean ± SD (n = 5); different superscripts in the same column show significant differences ($p<0.05$); total protein concentration data was analysed with the Kruskal-Wallis test, followed by Dunn's multiple comparisons test; albumin, gamma globulin concentration, urea and creatinine data were analysed with one-way ANOVA followed by Tukey's multiple comparisons test.

GG levels varied from 0.27 to 0.63 g dL^{-1}. GG in rainbow trout are a key indicator of immune health and disease resistance. Olesen & Jørgensen (1986) determined serum immunoglobulin M (IgM) levels in rainbow trout at 0.33 g dL^{-1}, with a minimum of 0.11 and a maximum of 1.06 g dL^{-1}. Aghili et al. (2024) determined globulin levels from 1.9 to 2.92 g dL^{-1}, with increases when phytoadditives (curcuma and black pepper) were added to the feed of rainbow trout. The values obtained in this study are similar to those obtained by Aghili et al. (2024). GGs are also dependent on environmental parameters. Sano (1960) observed a decrease of GG in rainbow trout when temperatures decrease, an observation later confirmed by Le Morvan et al. (1998) for most teleost fish.

Usually, UR levels in rainbow trout are under 1 mg dL^{-1} (Wedemeyer & Chatterton, 1970). UR levels were much higher than normal in the current study. Other studies also determined UR levels in rainbow trout up to 7.21 mg dL^{-1}, in some pathological cases (Řehulka, 1998), or under different environmental conditions (Řehulka, 2000; Kopp et al., 2011). A peak of 25 mg dL^{-1} was found in the case of trout fed a UR supplement (Kaushuk et al., 1983). The high values in this study may have occurred due to metabolic stress because of extremely cold temperatures.

CRN levels can provide insights into renal health and overall physiological status (Banaee et al., 2023). The values obtained in this study are just above normal levels (0.09-0.45 mg dL^{-1}) (Wedemeyer & Chatterton, 1970). It is not clear if temperature directly influences creatinine levels. However, it does impact metabolic processes, suggesting that temperature may indirectly affect metabolic waste products (Kieffer et al., 1994).

The lipid profile of the plasma changed significantly during the experiment. In Gi, the CHOL level was 280.72±95.71 mg dL^{-1}, significantly lower ($p<0.01$) than the value in G1 (555.66±92.65 mg dL^{-1}), and that of G2 (625.16±183.01 mg dL^{-1}; $p<0.001$). Fish from G2 had a CHOL level significantly higher than that of G3 ($p<0.05$) and G4 ($p<0.01$), with 381.28±63.93 and 361.74±63.08 mg dL^{-1}, respectively (Figure 1).

TG levels increased significantly ($p<0.01$) during the experiment in G1 (876.1± 318.63 mg dL^{-1}) compared to Gi (299.78±94.75 mg dL^{-1}). Similarly, the TG levels in G2 (920.02±261.33 mg dL^{-1}) were also significantly higher ($p<0.001$) that those obtained from fish in Gi (Figure 2).

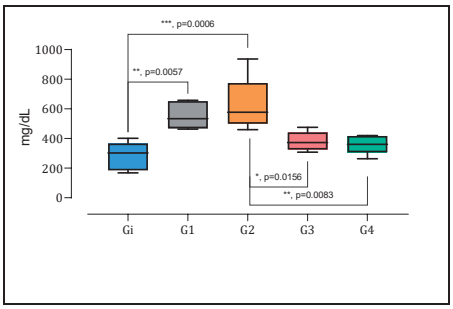

Figure 1. Cholesterol levels of rainbow trout plasma; Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; data was analysed with one-way ANOVA, followed by Tukey's multiple comparisons test

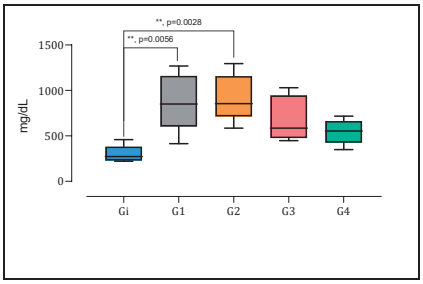

Figure 2. Triglyceride levels of rainbow trout plasma; Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; data was analysed with one-way ANOVA, followed by Tukey's multiple comparisons test

CHOL levels increased in all treatments and in the control, exceeding normal levels of 161-365 mg dL^{-1} (Wedemeyer & Chatterton, 1970) in G1 and G2. TG levels had a similar trend, with levels in G1 and G2 greatly increased.

A plausible explanation for the high levels of lipid indices, and for some plasma protein indices, can be constructed from the following factors: low water temperature, slowing

metabolism activity, and gonadal maturation (unpublished data from the current experiment). Bhat et al. (2022) noticed increased protein and lipid levels during the gonadal maturation in rainbow trout. Other authors also noticed increased lipid and protein levels in salmonid plasma during gonadal maturation (Johnston et al., 1987; Bon et al., 1997; Jenkins et al., 2019). Hoseini et al. (2013) noticed a different factor influencing TG level, namely fasting before blood sampling. In the present study, the increase in photoperiod and a slight increase in water temperature by the end of the experiment could have triggered gonadal development.

The values of the enzymatic profile parameters of the plasma did not differ significantly among the initial results, control and treatments. The values are presented in Table 5.

Table 5. Enzymatic profile of rainbow trout plasma

Group	Alanine aminotransferase (U L^{-1})	Aspartate aminotransferase (U L^{-1})	Gamma-glutamyltransferase (U L^{-1})	Lactate dehydrogenase (U L^{-1})
Gi	6.06±3.84^{a}	130.52±67.24^{a}	5.51±2.47^{a}	967.78±330.28^{a}
G1	3.48±1.55^{a}	97.8±26.8^{a}	5.98±3.15^{a}	326.46±147.29^{a}
G2	11.62±2.34^{a}	96.42±16.08^{a}	7.42±4^{a}	553.32±259.15^{a}
G3	11.08±5.24^{a}	83.66±37.66^{a}	8.8±4.69^{a}	248.24±78.42^{a}
G4	9.06±6.74^{a}	82.84±29.01^{a}	4.55±2.63^{a}	436.68±307.94^{a}

Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; values are presented as mean ± SD (n = 5); different superscripts in the same column show significant differences ($p<0.05$); alanine aminotransferase, and lactate dehydrogenase data were analysed with the Kruskal-Wallis test; gamma-glutamyltransferase, and aspartate aminotransferase data were analysed with one-way ANOVA.

ALAT serves as a useful biomarker for assessing liver health and general metabolic stress in fish. It varies under different conditions, being dependent on feed, environmental conditions, and other factors. In this study, ALAT levels ranged from 3.48 to 11.62 U L^{-1}. Considering the slow lipid metabolism due to low water temperature, the levels can be considered normal. Cao et al. (2023) obtained comparable levels of ALAT in rainbow trout farmed in net cages, ranging from 3 to 8.67 U L^{-1}, while Rozas-Serri et al. (2022) obtained values between 7.44-15.87 U L^{-1} for rainbow trout, depending on life stage, with a mean of 10.33±4.95 U L^{-1} for adults.

ASAT is commonly used as a biomarker for liver and muscle health in fish. As ALAT, ASAT varies based on many factors. Lower levels than those obtained in the current study were reported by Pastorino et al. (2020) for rainbow trout under normal conditions, while a range between 290.74 and 362.46 U L^{-1} was reported by Rozas-Serri et al. (2022), with a mean of 316.66±129.73 U L^{-1} for adult rainbow trout.

GGT is an enzyme involved in the metabolism of glutathione and the transfer of amino acids across cell membranes. Pastorino et al. (2020) determined GG levels in rain-bow trout similar to those obtained in the present study. Usually, GGT levels are low in the plasma of rainbow trout (Bauermeister et al., 1983).

LDH is an enzyme involved in energy production. LDH decreased from the initial values in this study, possibly due to the low water temperature. The LDH values at the end of the experiment were lower than those obtained by Rozas-Serri et al. (2022), who established an interval between 696.93 and 1215.77 U L^{-1} for rainbow trout under normal conditions, with a mean for adults of 882.25±308.17 U L^{-1}. Manera & Britti (2006) obtained a much higher mean value, of 2628.18 U L^{-1}, considering normal ranges of 462.04-4794.31 U L^{-1}.

PO_4^{3-}, Fe^{3+}, and Mg^{2+} did not suffer significant changes ($p>0.05$) during the experiment. The highest value of Ca^{2+} was observed in G2 (13.16±0.82 mg dL^{-1}), while the lowest was observed for G4 (8.56±0.56 mg dL^{-1}). The highest Na^{+} value was obtained in G3 (165.54±4.95 mmol L^{-1}), while the lowest was observed in Gi (141.38±8.08 mmol L^{-1}). The highest K^{+} level was observed in G4 (3.33±0.50 mmol L^{-1}), significantly different ($p<0.05$) from the value observed in G1 (2.33±0.14 mmol L^{-1}). The mineral profile of rainbow trout plasma is presented in Table 6.

Table 6. Mineral profile of rainbow trout plasma

Group	Ca^{2+} (mg dL^{-1})	PO_4^{3-} (mg dL^{-1})	Fe^{3+} (µg dL^{-1})	Na^{+} (mmol L^{-1})	K^{+} (mmol L^{-1})	Mg^{2+} (mg dL^{-1})
Gi	11.55±1.52^{abc}	10.47±3.09^{a}	133.06±19.01^{a}	141.38±8.08^{a}	2.73±0.30^{ab}	3.20±0.59^{a}
G1	11.09±0.72^{ab}	10.38±0.67^{a}	160.44±55.03^{a}	158.04±2.40^{ab}	2.33±0.14^{a}	3.53±0.10^{a}
G2	13.16±0.82^{c}	11.27±1.30^{a}	154.96±24.99^{a}	155.28±10.55^{ab}	2.48±0.38^{ab}	3.74±0.68^{a}
G3	10.92±0.82^{b}	11.71±2.47^{a}	114.24±18.55^{a}	165.54±4.95^{b}	2.77±0.20^{ab}	3.58±0.66^{a}
G4	8.56±0.56^{d}	11.84±0.82^{a}	93.16±34.08^{a}	156.00±2.05^{ab}	3.33±0.50^{b}	3.46±0.31^{a}

Gi - initial group; G1 - control group; G2 - group fed with 2% addition of carrot powder in the feed; G3 - group fed with 2% addition of tomato powder in the feed; G4 - group fed with 2% addition of spinach powder in the feed; values are presented as mean ± SD (n = 5); different superscripts in the same column show significant differences ($p<0.05$); Ca^{2+}, Fe^{3+}, and Mg^{2+} data were analysed with one-way ANOVA, followed by Tukey's multiple comparisons test; PO_4^{3-}, Na^{+} and K^{+} data were analysed with the Kruskal-Wallis test, followed by Dunn's multiple comparisons test.

Calcium is vital for the overall health and proper physiological functioning of rainbow trout. It has roles in bone health, muscle function, nerve signalling and reproductive health. The calcium results from the present study are within the range pro-posed by Řehulka & Minařík (2008), between 10 and 16 mg dL^{-1}, with the exception of the Ca level in G4, which was slightly lower. Rozas-Serri et al. (2022) obtained higher values, with a mean of 53.45±7.86 mg dL^{-1} Ca in the plasma of adult rainbow trout.

In rainbow trout, phosphate is involved in various biochemical processes, including the synthesis of nucleic acids and proteins, and the formation of ATP. Proper phosphate levels are vital for growth, health, and overall physiological functions (Coloso et al., 2003). Usually, phosphate levels in rainbow trout plasma ranges above the values obtained in this study (Řehulka & Minařík, 2008; Kopp et al., 2011; Prabhu et al., 2014; Rozas-Serri et al., 2022). However, it is important to note that phosphate uptake in cold water is impeded, and that energy requirements during the winter season are also lower for rainbow trout, low temperature affecting phosphate metabolism (Kurnianingtyas & Syahidah, 2024). Sugiura (2015) obtained similar levels to those obtained in the present study.

Iron has roles primarily in oxygen transport, enzyme function, and overall metabolism. Kwong et al. (2013) reported levels of 55.9 to 223.4 µg dL^{-1} Fe in rainbow trout fed with a control feed and a feed supplemented with iron, a range including the values obtained in this study. Rainbow trout maintains iron homeostasis through dietary regulation and efficient storage mechanisms (Carriquiriborde et al., 2004). Iron metabolism is also influenced by temperature, decreased temperatures lowering metabolic rates, reducing the need for oxygen transport and potentially decreasing the synthesis of iron-dependent proteins (Kochhann et al., 2024).

Sodium is an essential electrolyte in rainbow trout plasma, playing a crucial role in osmoregulation, acid-base balance, nerve function, and overall metabolic processes. Cold temperatures (2-4°C) can reduce sodium uptake efficiency due to slower gill ion exchange and metabolism. The values obtained in this study are similar to those ob-tained by Hrubec & Smith (1999) in rainbow trout plasma, namely 152 mmol L^{-1}. Sodium levels are influenced by temperature, decreasing in colder waters (Brown et al., 1999).

Magnesium is also an essential electrolyte in rainbow trout plasma, playing an important role in enzyme function, nerve transmission, muscle contraction, osmoregulation, and metabolic balance. The values obtained in this study are on the upper end of the values obtained by Kopp et al. (2011), of 1.82-3.43 mg dL^{-1} Mg in rainbow trout farmed under intensive conditions.

Potassium plays roles in nerve function, muscle contraction, acid-base balance, and osmoregulation in rainbow trout. The values obtained in this study are situated between the values obtained by Holk & Lykkeboe (1998), namely 2.2 mmol L^{-1} in resting rainbow trout and 3.3 mmol L^{-1} when trout is exposed to higher waterflow.

Glucose levels in the plasma of rainbow trout did not differ significantly among groups ($p>0.05$; one-way ANOVA). G1 had the highest glucose level (129.98±70.01 mg dL^{-1}), followed by G2, G4, Gi, and G3, with 113.18±21.81, 109.1±51.07, 102.44±48.59 and 68.78±11.29 mg dL^{-1}, respectively. GLC is a critical energy source in rainbow trout plasma, supporting metabolism, stress response, and overall physiological function. Choi & Weber (2015)

note a blood glucose level of 225.2 mg dL^{-1}, in hyperglycemic rainbow trout. Normal glucose levels are around 108 mg dL^{-1} (Weber & Shanghavi, 2000; Polakof et al., 2012).

TB decreased by the end of the experiment, from 0.21±0.41 mg dL^{-1} in Gi, to 0.078±0.14, 0.006±0.008, 0.006±0.008 and 0.008± 0.011 mg dL^{-1} in G1, G2, G3 and G4, respectively. TB is low in the plasma of rainbow trout. TB in rainbow trout is an indicator of liver function, Ery breakdown, and overall metabolic health. The values obtained in our study are in line with values obtained by other authors, namely under 0.12 mg dL^{-1} (Sakai & Kawazu, 1978), and under 0.09 mg dL^{-1} (Manera & Britti, 2006). However, the changes were not statistically significant ($p>0.05$; Kruskal-Wallis test).

CONCLUSIONS

The hematological profile and plasma biochemistry of rainbow trout fed throughout the winter with diets supplemented with phytoadditives showed generally normal values for low water temperatures.

Notable differences in the hematological profile were observable in MCV, which increased in rainbow trout fed a 2% carrot meal supplemented feed and in the rainbow trout fed a 2% spinach meal feed, and MCHC, which decreased in all groups. CRN levels decreased in all groups, with a significant decrease in the conventionally fed rainbow trout.

CHOL and TG levels increased in all groups, possibly due to slowed metabolism and gonadal maturation.

The enzymatic profile did not suffer significant changes.

Some of the indices of the mineral profile suffered changes. Ca was highest in rainbow trout fed a 2% carrot meal supplemented feed, Na was highest in rainbow trout fed a 2% tomato meal supplemented feed, and K was highest in rainbow trout fed a 2% spinach meal supplemented feed.

This could be attributed to the higher mineral content of the phytoadditives, in corroboration with the slowed metabolism.

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