SUMMARY

The effects of phytoadditive administration on bioproductive performance and physiological status in rainbow trout (*Oncorhynchus mykiss*)

PhD Student **Tudor-Andrei Păpuc**

Scientific coordinator **Prof. Univ. Dr. Vioara Mireșan**

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is the main freshwater salmonid species farmed worldwide due to its adaptability to environmental conditions, rapid growth rate, and well-established rearing technologies (Samuel-Fitwi et al., 2013). In 2019, global trout production reached approximately 939000 tonnes, of which rainbow trout accounted for 97%. Compared to previous years, production has increased (EC, 2021). In Romania, in 2021, a little over 3200 tons of rainbow trout were produced (approx. 28% of the total fish production).

Rainbow trout farming technologies have evolved in leaps and bounds over time (Hinshaw et al., 2004). Currently, high-performance technologies are used, the nutritional requirements of trout are known, the feed used is high-performance, the optimal values of the water parameters are known, the breeding systems achieve adequate bioproductive performances. However, new challenges have also emerged, generally regarding fish welfare, alternative protein sources, environmental protection, use of natural ingredients in feed, etc.

Adequate feeding with a feed that meets the nutritional requirements of rainbow trout is important for normal development to achieve high survival and low feed conversion rate (FCR) (Hilton & Slinger, 1981). In farms, rainbow trout often rely only on the given feed as a food source, so a balanced diet is very important. Since feed ingredients are sometimes limited in terms of nutrient balance, various supplements are often used. Supplements have multiple functions, becoming widely used ingredients in aquaculture. Rainbow trout need essential nutrients for growth, health, reproduction, etc. There are essential amino acid supplements, premixes (minerals and vitamins), etc. (Rodehutscord et al., 1997; Ringø et al., 2014). Natural supplements are obtained from natural sources and can have positive effects on fish growth and health. They have positive effects on their well-being, and they have low negative effects. In general, natural supplements are sustainable, especially when they have renewable sources (plants, algae). They have a wide range of bioactive compounds (Gherescu et al., 2023; Barad et al., 2024). Consumers prefer fish fed with feed supplemented with natural additives.

Through this PhD thesis, we wanted to contribute to overcoming some challenges in rainbow trout aquaculture, by trying to achieve adequate growth during the winter in a classic, intensive farm, as well as by evaluating the effects of some phytoadditives on the physiological status and meat quality of rainbow trout.

PURPOSE AND OBJECTIVES OF THE RESEARCH

THE PURPOSE of the doctoral thesis entitled "The effects of phytoadditive administration on bioproductive performance and physiological status in rainbow trout (*Oncorhynchus mykiss*)" was to determine the influence of some natural phytoadditives added to rainbow trout feed on growth, on some hematological and plasma biochemistry parameters, and on some elements of meat quality, compared to a standard feed. The objectives of the thesis are the following:

1. Synthesis of current knowledge regarding rainbow trout breeding;
2. Obtaining and physico-chemical characterization of phytoadditives, standard feed and experimental feed;
3. Organization of the experimental device in a salmon farm with an intensive system;
4. Determining the bioproductive performance of rainbow trout fed with phytoadditive supplemented feed;
5. Characterization of the physiological status of rainbow trout based on blood parameters;
6. Analyzing, interpreting and disseminating the results obtained.

STRUCTURE OF THE DOCTORAL THESIS

The doctoral thesis entitled "The effects of phytoadditive administration on bioproductive performance and physiological status in rainbow trout (*Oncorhynchus mykiss*)" comprises a total of 137 pages and is structured in two parts, namely part I, "Current state of knowledge" and part II "Personal contribution". The doctoral thesis contains 13 tables and 52 figures.

1. The current state of knowledge comprises four chapters.
2. The personal contribution is comprised of five chapters.

PART I - Current state of knowledge

Part I comprises 4 chapters and represents a synthesis of the current knowledge related to rainbow trout, both regarding the biology of the species and aspects regarding production elements, growth, feed and supplements used, especially carotenoids.

Chapter I is entitled "General considerations on rainbow trout (*Oncorhynchus mykiss*)" and contains 3 subchapters: "Rainbow trout distribution", "Morphological description of rainbow trout" and "Rainbow trout biology, ecology and ethology".

Chapter II is entitled "Rainbow trout (*Oncorhynchus mykiss*) in salmon farming". This chapter comprises 9 sub-chapters: "History of rainbow trout farming", "Rainbow trout farming systems", "Nutrition and feeding in rainbow trout farming", "Artificial reproduction of rainbow trout", "Rainbow trout prevention, diseases and treatments", "Rainbow trout harvesting and processing", "Economic and marketing aspects in Rainbow Trout Breeding', 'Rainbow Trout Production in Figures' and 'Rainbow Trout Meat Quality'.

Chapter III is entitled "Current and Future Trends in Rainbow Trout (*Oncorhynchus mykiss*) Farming" and includes 2 subchapters: "Current Trends in Rainbow Trout Farming" and "Emerging Trends in Rainbow Trout Farming".

Chapter IV is entitled "Food supplements used in raising rainbow trout" and includes 3 subchapters: "Artificial supplements", "Natural supplements" and "Carotenoids".

PART II - Personal Contribution

Part II comprises five chapters, including the personal contribution. The purpose and objectives of the research, the biological material used, the methods used, the results obtained and the discussions regarding the results obtained, the conclusions and recommendations, as well as aspects of originality and innovative contributions of the thesis are presented.

Chapter V is called "The purpose and objectives of the research", presenting the six previously mentioned objectives and the experimental plan.

Chapter VI is called "Materials and methods" and includes four subchapters: "Obtaining vegetable flours", "Preparation of experimental feed", "Experimental device" and "Measurements, determinations and analyzes carried out during the research", each detailing the procedures, material and methods used.

Chapter VII is entitled "Results and discussions", and contains 5 sub-chapters: "Values of water parameters", "Physico-chemical characterization of meals and feeds", "Bioproductive growth performance of rainbow trout", "Hematology and plasma biochemistry", and "Chemical composition and meat quality".

Chapter VIII is entitled "Conclusions and recommendations" and includes 2 subchapters: "Conclusions" and "Recommendations.

Chapter IX is entitled "Originality and innovative contributions of the thesis", presenting elements of originality, as well as contributions to the scientific environment, and not only, of the thesis.

MATERIAL AND METHODS

For this research on the effects of some phytoadditives on growth performance and physiological status in rainbow trout, three plant species were selected: carrot (*Daucus carota*), tomato (*Solanum lycopersicum*) and spinach (*Spinacia oleracea*). After obtaining the meals from the plants, the experimental feed was prepared. The meals, the standard feed and the experimental feed were characterized physicochemically. The actual experiments took place at Gilău trout farm, Cluj county, for 90 days, from November 2023 to February 2024. The biological material was rainbow trout with commercial body weight. 200 specimens of rainbow trout, of approximately equal body weight, without clinical signs of disease, were selected for testing the diets with phytoadditives. Thus, 50 specimens were assigned to each group: G1 – the control group, fed with the standard feed; G2 – the group fed with an addition of 2% carrot meal to the standard feed; G3 - the group fed with an addition of 2% tomato meal to the standard feed; and G4 - the group fed with an addition of 2% spinach meal to the standard feed. At the beginning of the experiment, morphometric determinations were performed and blood samples were collected. At the end, the same determinations were repeated. Furthermore, muscle tissue samples were collected for meat analysis. Feeding was carried out according to a feeding schedule established according to water temperature, biological biomass, amounts previously consumed and the manufacturer's recommendations. Water parameters were measured daily. The activities and procedures carried out on fish in order to achieve the objectives were approved by the Decision of the Bioethics Commission of USAMV Cluj-Napoca with no. 499, for application form no. 486 of 03.02.2023.

RESEARCH RESULTS

Result regarding water parameters

The determined parameters were specific of a salmon farm in Romania, during the cold period of the year (Table 1). Some parameters had optimal values, while others were outside the optimal ranges, possibly causing stress to the fish. Temperatures were decreasing, for most of the duration of the experiment being below the optimal range for rainbow trout, sometimes reaching below 3°C. Turbidity, dissolved oxygen and pH were optimal for rainbow trout. Conductivity and total dissolved solids were slightly below optimal for rainbow trout, also due to time and space. Thus, some water parameters did not have optimal values for rainbow trout, but were within tolerable values for them. These values are a reality in salmon farms in Romania, which is why rainbow trout growth slows down, stagnates or is negative during the winter.

Table 1

Water parameter values during the study

Descriptive statistics	*Temp (°C)*	*DO (mg/L)*	*pH*	*Conductivitate (μS/cm)*	*TDS (mg/L)*	*Turb [UNF]*
Mean	5.70	14.01	6.82	78.61	38.92	1.06
SD	2.37	0.60	0.12	3.45	1.85	0.61
Min	2.74	12.18	6.6	72	36	0.2
Max	9.91	14.98	7.12	86	43	2.6
Median	5.28	14.04	6.81	78.5	38	1
CV	0.42	0.04	0.02	0.04	0.05	0.61

Note: SD – standard deviation; CV – coefficient of variability; Temp – temperature; DO – dissolved oxygen; TDS – total dissolved solids; Turb – turbidity.

Results regardin the physicochemical characterization of the meals and feeds

The chemical composition of the standard feed did not undergo changes by supplementing with 2% phytoadditives (Table 2). Total polyphenols increased in all experimental feeds, but not significantly (p>0.05). Total antioxidant capacity increased significantly in all experimental feeds (p<0.05). Total carotenoids increased significantly (p<0.05) in the experimental feed with carrot meal and in the one with spinach meal (Table 2).

Table 2

Total polyphenols, antioxidant capacity and total carotenoids for vegetable meals and feeds (mean±SD)

Phytoadditives and feeds	Total polyphenols (mg/kg GAE)	Total antioxidant capacity (μg/mg AAE)	Total carotenoids (mg/g BCE)
Carrot meal	$923±19.15^{a}$	$2.16±0.17^{a}$	$5.94±0.18^{a}$
Tomato meal	$2570±240.59^{b}$	$7.33±0.4^{b}$	$5.03±0.04^{b}$
Spinach meal	$1088.8±80.06^{c}$	$2.58±0.2^{a}$	$9.09±0.02^{c}$
Standard feed	$285.8±79.25^{a}$	$0.65±0.03^{a}$	$3.31±0.09^{a}$
Standard feed +2% carrot meal	$304.33±77.83^{a}$	$0.7±0.17^{b}$	$3.42±0.02^{bc}$
Standard feed +2% tomato meal	$337.97±78.41^{a}$	$0.8±0.18^{c}$	$3.35±0.02^{ab}$
Standard feed +2% spinach meal	$308.69±74.53^{a}$	$0.7±0.17^{b}$	$3.46±0.02^{c}$

Note: different superscripts in the same column (for the 2 categories of samples – meals and feeds) show significant differences (p<0.05).

Results on the bioproductive growth performance of rainbow trout

The growth indices had good values for the winter period, confirming the possibility of feeding rainbow trout at low temperatures. Fish in G2, fed a diet supplemented with 2% carrot meal, had the highest values for total growth gain (4,639 kg), daily gain (51.54 g/day), average daily gain (1.03 g/day), specific growth rate (24.71% for 90 days and 0.274%/day), and protein efficiency ratio (1.96), with the best feed conversion ratio (1.39). The condition factor with the best value was observed in G2 (1.23). Poorer results were obtained in G3 and G4, but there were also adequate growth index values here. The less intensive feeding in G3 and G4 can be attributed to the acidity produced by the tomatoes and to the modification of the palatability of the diet by the spinach, respectively. Kn, RGI, and WLR had the highest values in G2. In most cases, the values in G2 were not significantly better ($p>0.05$) compared to those in the control group.

Results regarding the blood hematology and plasma biochemistry

The values of hematological parameters were better for fish in the experimental groups, although the differences were significant in few cases (Table 3). The hematocrit values of the fish in the experimental groups were lower compared to the value in G1, which slightly exceeds the normal limits. The mean corpuscular volume value is closer to normal in G2 and G4 than in G1. Compared to the baseline population, changes were observed. The most likely explanation is the decrease in water temperature during the winter period. The experimental diets helped to maintain between relatively normal ranges the values of hematological parameters, with better values in most cases compared to those obtained in the control group.

The plasma protein profile underwent significant differences ($p<0.05$) only in the case of total protein and creatinine (Table 4). For total protein, the differences were between the slightly high value in G2 versus the slightly low value in G4, and for creatinine the difference was between the control group and the baseline population. Creatinine values were slightly above the limits proposed by the specialized literature.

Cholesterol and triglycerides increased significantly ($p<0.05$) in G1 and G2 compared to the baseline population, probably due to higher feed consumption. In the case of cholesterol, the value in G2 was significantly higher ($p<0.05$) than the values in G3 and G4. Another finding that can argue for the high levels of cholesterol and triglycerides, in addition to the consumption of feed in conditions of slowed metabolism, is the gonadal maturation, observed at the time of slaughter in some individuals, a maturation that involves the accumulation of lipids.

Table 3

Haematological profile of rainbow trout (*Oncorhynchus mykiss*)

Group	Hb (g dL^{-1})	RBC (x10^6 mm^{-3})	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
I	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

Note: I – initial population; G1 – control group fed with the standard feed; G2 – experimental group fed with the standard feed supplemented with 2% carrot meal; G3 – experimental group fed with the standard feed supplemented with 2% tomato meal; G4 – experimental group fed with the standard feed supplemented with 2% spinach meal; Hb – hemoglobin concentration; RBC – total number of red blood cells; Hct – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; different letters in the same column indicate significant differences ($p<0.05$); values are presented as mean ± standard deviation (n=5); Hb, Hct, MCV, MCH and MCGC were analyzed with ANOVA and Tukey's test; RBC was analyzed with the Kruskal-Wallis test.

Table 4

Protein profile of rainbow trout (*Oncorhynchus mykiss*) plasma

Group	Total protein (g/dL)	Albumin (g/dL)	Gamma globulin (g/dL)	Urea (mg/dL)	Creatinin (mg/dL)
I	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}

Note: I – initial population; G1 – control group fed with the standard feed; G2 – experimental group fed with the standard feed supplemented with 2% carrot flour; G3 – experimental group fed with the standard feed supplemented with 2% tomato flour; G4 – experimental group fed with the standard feed supplemented with 2% spinach flour; different letters in the same column indicate significant differences ($p<0.05$); values are presented as mean ± standard deviation (n=5); total protein values were analyzed with the Kruskal-Wallis test, followed by Dunn's test; albumin, gamma globulin, urea, and creatinine values were analyzed by ANOVA followed by Tukey's test.

The enzymatic profile did not undergo significant changes ($p>0.05$), the results being summarized in Table 5.

The plasma mineral profile varied according to the phytoadditives supplemented (Table 6). Thus, the level of Ca^{2+} increased significantly ($p<0.05$) in G2, but decreased significantly ($p<0.05$) in G4. The level of PO_4^{3-} increased in all experimental groups, but not significantly ($p>0.05$). The level of Fe^{3+} remained relatively constant. Na^+ increased in all groups, but significantly ($p<0.05$) only in G3. K^+ increased significantly in G4 ($p<0.05$). The level of Mg^{2+} remained constant ($p>0.05$).

Table 5

Plasma enzymatic profile of rainbow trout (*Oncorhynchus mykiss*)

Group	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Gamma-glutamyl transferase (U/L)	Lactate dehydrogenase (U/L)
I	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]

Table 6

Plasma mineral profile of rainbow trout (*Oncorhynchus mykiss*)

Grup	Ca^{2+} (mg/dL)	PO_4^{3-} (mg/dL)	Fe^{3+} (µg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)	Mg^{2+} (mg/dL)
I	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]

Note: I – initial population; G1 – control group fed with the standard feed; G2 – experimental group fed with the standard feed supplemented with 2% carrot flour; G3 – experimental group fed with the standard feed supplemented with 2% tomato flour; G4 – experimental group fed with the standard feed supplemented with 2% spinach flour; different letters in the same column indicate significant differences ($p<0.05$); different letters in the same column indicate significant differences ($p<0.05$); values are presented as mean ± standard deviation (n=5); PO43-, Na+ and K+ values were analyzed with the Kruskal-Wallis test, followed by Dunn's test; Ca 2+ , Fe 3+ , and Mg 2+ values were analyzed by ANOVA followed by Tukey's test.

Glucose values did not change.

Total bilirubin decreased compared to the initial value, being lower in the experimental groups including compared to the control group, but the differences were not significant ($p>0.05$).

Thus, the values of the hematological parameters as well as the plasma parameters indicated a slight state of stress at the end of the experiment. This can be attributed, on the one hand, to low water temperatures, an element of medial stress, and on the other hand, to feeding during these low temperatures, when trout metabolism is slowed.

Deviations from the optimal ranges are negligible, except for the lipid profile, where the changes are, at least partially, due to other factors (gonadal maturation).

In general, the addition of phytoadditives to the diet of rainbow trout either had positive effects or no effects on hematological and blood plasma parameters, except for the calcium level in G4, which decreased significantly ($p<0.05$), and the lipid profile, where other factors appear that can produce major changes.

Results regarding chemical composition and meat quality

The chemical composition of the meat did not change depending on the addition of phytoadditives to the feed (Table 7).

Table 7

Chemical composition of rainbow trout (*Oncorhynchus mykiss*) meat

Grup	Umiditate	Proteină brută	Grăsime brută
G1	67.5±2.37[a]	17.68±2.21[a]	7.8±2.91[a]
G2	67.92±3.9[a]	17.02±2.21[a]	6.54±2.41[a]
G3	64.48±0.9[a]	15.82±0.65[a]	6.52±0.83[a]
G4	65.44±1.51[a]	16.2±1.54[a]	6.18±1.84[a]

Note: G1 – control group fed with the standard feed; G2 – experimental group fed with the standard feed supplemented with 2% carrot flour; G3 – experimental group fed with the standard feed supplemented with 2% tomato flour; G4 – experimental group fed with the standard feed supplemented with 2% spinach flour; different letters in the same column indicate significant differences (p<0.05); different letters in the same column indicate significant differences (p<0.05); values are presented as mean ± standard deviation (n=5); values were analyzed with ANOVA.

Total carotenoids in the meat were drastically improved with the addition of phytoadditives, with the best results being in G4, followed by G3 and G2 (Figure 1).

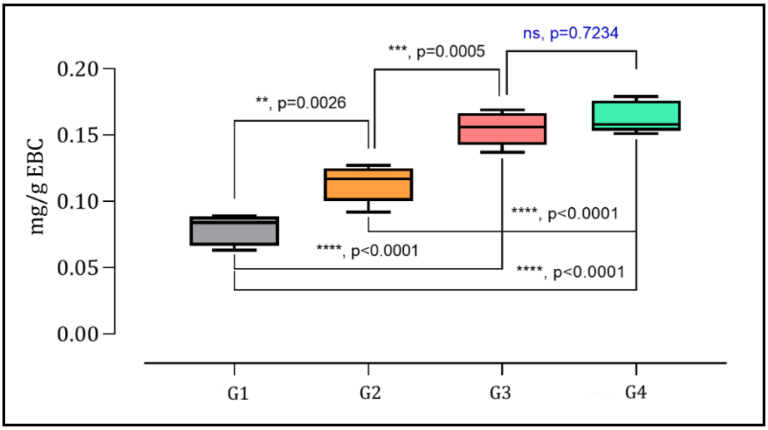

Figure 1. Total carotenoid content of rainbow trout (*Oncorhynchus mykiss*) meat; G1 – control group fed with the standard feed; G2 – experimental group fed with the standard feed supplemented with 2% carrot meal; G3 – experimental group fed with the standard feed supplemented with 2% tomato meal; G4 – experimental group fed with the standard feed supplemented with 2% spinach meal.

The addition of natural phytoadditives to rainbow trout feed improves meat quality, creating a product with health benefits for consumers.

The color of the meat did not undergo any noticeable changes. The intense coloring did not occur possibly because of the low consumption of feed, and, implicitly, of carotenoids, but also of the nature of the carotenoids in the phytoadditives, because the natural carotenoids existing in the phytoadditives used, β-carotene, lycopene and lutein, accumulate with more difficulty compared to synthetic astaxanthin, and are partly metabolized more easily. However, a digital analysis detected some color changes that are difficult to see with the naked eye. The meat color of the trout in G2 is closer to red than the colors in the other groups, and the meat color of the trout in G4 is closer to orange.

Conclusions

In the present study, the possibility of feeding rainbow trout during the winter, with good bioproductive performance, was demonstrated. The feed with added carrot meal produced the best growth results, followed by the standard feed. Experimental feeds with tomato meal and spinach produced mediocre growth results.

Hematological and plasma biochemistry parameters were either improved or unchanged in fish from the experimental groups. In general, the parameters fell within the limits proposed by the specialized literature.

The chemical composition of rainbow trout meat did not change. The carotenoid content of the meat was significantly improved by the phytoadditives in the feed, thus making it a healthier product for human consumption.

Recommendations

It is recommended to use a 2% addition of carrot meal in rainbow trout feed to improve growth performance and physiological status.

It is recommended to feed rainbow trout during the winter in intensive, classic salmon farms. Feeding should be done slowly, with a small amount of feed.

For meat coloring, the use of phytoadditives used in the conditions of this experiment is not recommended.

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