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HAEMATOLOGICAL PROFILE OF RAINBOW TROUT UNDER DIFFERENT FEEDING INTENSITIES

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Abstract

The aim of this study was to evaluate the haematological profile of rainbow trout after applying of different intensities of feeding (FL1-2.5% BW day^{-1}, FL2-3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5 % BW day^{-1} and, ad libitum-FL6). Fish were reared in a recirculating aquaculture system (RAS), provided with twelve rearing units (in duplicate) for 44 days. Blood samples were collected from twelve fish on each experimental variant, and were made before and after the experimental trial. Red blood cell counts (RBCc), haematocrit values (Hct), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total serum glucose (Glu) and total proteins (TP) were determined. Also, in order to determine the leukogram and the absolute number of leukocytes, blood samples were collected and used immediately to make smears. At the end of the experiment, ANOVA test revealed no significant changes ($p>0.05$) in the RBCc, Hct, Hb, MCV, MCH, MCHC and glucose concentration (Glu), while the total proteins (TP) from blood serum increased significantly ($p<0.05$) with the increasing of the feeding intensity, except for ad libitum feeding, were total serum protein decreased. Regarding the total number of leukocytes, in general, it has not been registered statistically significant changes ($p>0.05$). Lymphocytes (relative and absolute) have remained relatively constant, without significant variation ($p>0.05$). The main conclusion that emerges from this current study shows that in the case of rainbow trout reared under recirculating aquaculture system conditions, feeding intensity does not significantly influence the haematological profile.

Key words*: feeding intensity, haematological profile, rainbow trout, recirculating aquaculture system.*

INTRODUCTION

In the last years, global fish production has notably increased, intensive farming having a big contribution to the development of aquaculture.

In Romania, rainbow trout is a major farmed species, due to its adaptability at intensive farming conditions.

Between all the technological aspects from a fish farm, feeding is one of the most important factors which can affect fish growth performance, welfare, and physiological status. In salmonid aquaculture, feeding cost represents the highest cost from the farm operations, accounting for around 50% of the total cost of production (Rauw et al., 2016), that`s why it is crucial to determine the optimal feeding rate.

Choosing the optimal feeding intensity is very important in the case of recirculating production systems because non-consuming feeds represent the main factor of deterioration of water quality and implicit of fish welfare. Feeding intensity can be influenced by many factors, such as time of day, season, water temperature, dissolved oxygen levels, and other water quality variables (Craig and Helfrich, 2002). Generally, the optimal growth of fish and welfare is directly associated with feed and feeding conditions (Peres and Oliva, 2014). Because feed management can affect the blood cells, the examination of fish haematology may be helpful in the assessment of nutritional status and diagnosis of diseases (Gingerich et al., 2010).

In this context, the objective of the present study was to evaluate the haematological profile of rainbow trout after applying of different intensities of feeding (FL1-2.5% BW day^{-1}, FL2- 3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5 % BW day^{-1}, and *ad libitum*-FL6).

MATERIALS AND METHODS

Experimental design and feeding trial. The study was carried out at Faculty of Food Science and Engineering, University of Galați, Romania. Fish were procured from a fish farm from

Prejmer, Brașov County, România. The experimental fishes were first acclimatized to laboratory conditions for two weeks (14 days) and were fed with commercial fish feed (54% C.P) (Table 1).

Table 1. The composition of the experimental diet

Composition	U.M	Quantity
Crude protein	%	54
Crude lipids	%	18
Cellulose	%	1
Ash	%	10
Phosphorus	%	1.4
Digestible energy	Mj/kg	19.4
Vitamin A	UI	14000
Vitamin D3	UI	2300
Vitamin E	mg	250
Vitamin C	mg	500
Lysine	%	3.5
Methionine	%	1.5
Cystine	%	0.7
Ingredients: Fish meal, fish oil, hemoglobin, full-fat soy, soybean oil, wheat gluten, sunflower meal, wheat, and wheat products.		

After two weeks, 360 rainbow trout fingerlings (average weight of 34.17±0.11 g) were randomly distributed in a recirculating aquaculture system, composed of twelve rearing units, with a capacity of 132 L, each. The experimental design and data regarding the growth performance indicators were published in our previous paper (Crețu et al., 2019).

Fish were fed manually twice per day at 9 a.m. and 6 p.m. at different feeding levels: FL1-2.5% BW day^{-1}, FL2- 3% BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 % BW day^{-1}, FL5-4.5% BW day^{-1} and, *ad libitum*-FL6, with the same diet used during acclimatization. In the *ad libitum* feeding, fish were fed until the first two or three pellet remains to the bottom of the rearing units.

Collection and Determination of Experimental Fish Blood Samples. At the beginning of the experiment and at the end of the experiment, from each experimental variant, 7 fish were randomly sampled in order to evaluate the haematological profile. Fish were anesthetized and blood samples were taken from the caudal vein employing a heparinized syringe. After sampling, a part of the blood was placed in heparinized Eppendorf tubes and another part in un-heparinized tubes for determining the glucose and total protein. At the same time, for determining the leukogram and the absolute number of leukocytes blood smears were made, fixed and colored with May-Grünwald Giemsa panoptic method (MGG). The type of leukocytes was determined based on identification characters listed by Svobodová et al., 1991. The number of thrombocytes and the white blood cell count were determined in relation to 10000 erythrocytes and converted to unit blood volume.

The haemoglobin concentration (Hb, g dl^{-1}) was determined based on cyanmethemoglobin spectrophotometry method. In this context, 20µl of the blood sample was taken from the lithium heparinized tube with the aid of a pipette. Mixing was achieved by slow inversion of the tube, for about 20 times with 5 ml of Drabkin's solution. After that, the samples were read at SPECORD 210 Spectrophotometer Analytikjena, at the wavelength of 540 nm.

The red blood cell counts (RBCc, $\times 10^6$ µL^{-1}) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution.

The haematocrit (Ht, %) was determined by microcentrifugation of whole blood for 5 minutes at 12000 rpm in a microhematocrit centrifuge.

Using standard formulas (Ghergariu et al., 1985), the erythrocyte constants were calculated: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC).

Determination of glucose (Glu) and total protein (TP) from serum was performed spectrophotometric by SPECORD 210 Analytik Jena. For glucose determination we used the colorimetric method with o-toluidine, readings were made at 635 nm wavelength. Total proteins from serum were determined by Biuret method, the readings were done at a 546 nm wavelength.

Statistical analysis. Data obtained from the experiment was expressed in mean±SD and it was subjected to ANOVA test using SPSS 21 version. When the ANOVA reveals significant difference ($p<0.05$), Duncan multiple range test was used to compare differences among individual treatment means.

RESULTS AND DISCUSSIONS

The results of haematological analyses are presented in Table 2. The results of our study are

analysed in in conjunction with the results obtained regarding the performance of fish farming, results that were previously published by Crețu et al. (2019). At the end of the study fish individual weight increased with the increasing of feeding level, ranging from 87 g in FL1 to 125 g in FL6.

Analysing the values obtained for the haematological indices at the end of the experiment it is observed that are within the optimum range of the species, being observed some changes with feeding intensity.

At the end of the experimental period, the hematocrit (PCV, %) values showed an insignificant increase ($p>0.05$) compared to the initial moment for all the experimental variants. Also, comparing the hematocrit values between the six experimental variants, no significant differences were obtained ($p>0.05$), varying between 39.62% and 43%.

Table 2. The haematological parameters of rainbow trout at different feeding intensities

Experimental variants	Average±SD					
	PCV (%)	Hb (g dl^{-1})	RBCc (x10^6 μl^{-1})	MCV (μm^3)	MCH (pg)	MCHC (g dl^{-1})
Initial	38.27±3.95	9.25±0.97	1.41± 0.16	274.46±53.42	66.26±11.69	24.45±4.00
FL1	42.00±3.55^{ac}	8.35±0.31^{ad}	0.90±0.12^{ad}	473.16±49.82^{ad}	94.63±11.97^{ad}	20.03±1.94^{ad}
FL2	41.25±1.03^{ac}	8.46±0.25^{ad}	0.95±0.17^{ad}	445.77±81.09^{ad}	91.80±17.48^{ad}	20.58±0.75^{ad}
FL3	41.25±4.68^{ac}	8.70±0.55^{ac}	0.96±0.18^{ad}	440.32±79.96^{ad}	94.02±21.70^{ad}	21.31±2.58^{ac}
FL4	39.62±2.62^{ac}	8.49±0.33^{ac}	0.91±0.15^{ad}	448.03±80.98^{ad}	95.62±13.74^{ad}	21.49±1.48^{ac}
FL5	40.12±4.19^{ac}	8.47±0.22^{ad}	0.89±0.16^{ad}	465.23±95.77^{ad}	98.31±19.44^{ad}	21.34±2.64^{ac}
FL6	43.00±3.93^{ac}	8.36±0.29^{ad}	1.01±0.16^{ad}	433.12±68.64^{ad}	84.20±11.51^{ad}	19.60±2.04^{ad}
Zorriehzahra et al., 2010	47	9.4	1.33	353	71	20
Ghittino, 1983	24 - 55	7.6 - 16	0.8 – 1.5	276 - 476	55 - 82	14 - 26

RBCc - red blood cell counts, PCV - haematocrit, Hb - haemoglobin concentration
MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration

a- No Significant differences between the experimental variants, $p>0.05$.
b- Significant differences between the experimental variants, $p<0.05$.
c- No significant differences between the initial moment of the experiment, $p>0.05$.
d- Significant differences between the initial moment of the experiment, $p<0.05$.

Comparing the haemoglobin concentration with the values from the initial moment it was observed a significant decrease ($p<0.05$) in the experimental variants FL1, FL2, FL4, FL5, FL6, respectively an insignificant decrease ($p>0.05$) in the variant FL3. Regarding the influence of feeding intensity on haemoglobin concentration, no significant differences ($p>0.05$) can be observed in all six experimental variants.

The red blood cells showed no significant differences ($p>0.05$) between the all experimental variants, but a significant decrease was observed ($p<0.05$) in comparison with the initial moment.

The erythrocyte constants were calculated after the determination of the hematological indices. The obtained results revealed some changes in the values, in comparison with the initial moment, and between the six experimental variants.

Comparing the MCV values between the six experimental variants, the ANOVA test showed no significant changes ($p>0.05$). However, as a compensatory reaction, the reduction of the number of red blood cells caused the increase of the MCV values, being observed slightly higher values in the case of FL1 and FL5 (FL1- 473.16±49.82 μm^3; FL5- 465.23±95.77 μm^3), while in the case of intensities of FL2, FL3 and FL4 similar values were recorded (FL2- 445.77±81.09 μm^3; FL3-440.32±79.96 μm^3; FL4- 448.03±80.98 μm^3.

Regarding the obtained values of mean corpuscular haemoglobin concentration (MCH) at the end of the experiment is observed a significantly increasing tendency ($p<0.05$) with the feeding intensity, but no significant differences ($p>0.05$) were recorded between the six experimental variants.

The mean corpuscular haemoglobin concentration (MCHC) it can be observed a significant decrease ($p<0.05$) in the variant FL1, FL2 and FL6 respectively, from 24.45±4 g dl^{-1} (initial) to 20.03±1.94 g dl^{-1}, 20.58±0.75, respectively to 19.60±2.04 g dl^{-1}. In the case of the experimental variants FL3, FL4 and FL5,

there was no significant increase ($p>0.05$) compared to the initial moment.

Comparing the obtained values of MCHC in the six experimental variants, no statistically significant differences ($p>0.05$) were recorded.

In order to determine the influence of the feeding intensity on immune system, another aim of this experiment was to analyse the reaction of the leukocyte`s system.

In Table 3 are given the relative (the leukogram) and the absolute number of the various types of cells that make the leukocytic complex.

On the examined blood smears, we observed a predominance of the lymphocytes, followed by the neutrophils and monocytes. Eosinophils and basophiles were not found. Analysing the variation of the number of different types of the leukocytes some modification was recorded both, in comparation with the initial moment and between the six experimental variants, presented below.

Table 3. The relative and absolute modification of white blood cells (Average±SD)

WBCc	SI units	Average±SD						
		Initial	FL1	FL2	FL3	FL4	FL5	FL6
LK	$\times10^3$ cel μl^{-1}	63.59±14.88	50.79±11.52^{ad}	51.23±10.22^{ad}	54.20±14.26^{ad}	55.18±10.23^{ad}	55.55±14.03^{ad}	55.75±16.23^{ad}
T	$\times10^3$ cel μl^{-1}	2.89±1.20	13.39±7.36^{ad}	10.12±6.36^{ad}	10.04±5.62^{ad}	10.88±7.75^{ad}	10.55±6.45^{ad}	10.29±7.42^{ad}
Ly	$\times10^3$ cel μl^{-1}	58.83±5.97	45.69±10.11^{ad}	46.77±12.02^{ad}	50.41±10.24^{ad}	52.00±11.25^{ad}	52.08±10.65^{ad}	52.53±10.24^{ad}
	%	92.50±1.48	90.00±2.83^{ac}	91.25±1.60^{ac}	93.00±1.12^{ac}	94.25±1.35^{ac}	93.75±1.06^{ac}	94.25±1.31^{ac}
M	$\times10^3$ cel μl^{-1}	1.74±0.34	1.15±0.16^{ad}	0.89±0.12^{ad}	0.82±0.10^{ad}	0.55±0.09^{ad}	0.97±0.08^{ad}	0.56±0.09^{ad}
	%	2.75±0.07	2.25±0.35^{ac}	1.75±1.06^{ac}	1.50±0.71^{ac}	1.00±0.12^{ac}	1.75±0.15^{ac}	1.00±0.10^{ac}
N	$\times10^3$ cel μl^{-1}	3.01±1.07	3.96±1.1^{ac}	3.57±1.02^{ac}	2.97±1.09^{ac}	2.63±1.12^{ac}	2.50±1.16^{ac}	2.66±1.01^{ac}
	%	4.75±0.34	7.75±1.47^{ac}	7.00±1.54^{ac}	5.50±1.71^{ac}	4.75±1.35^{ac}	4.50±1.71^{ac}	4.75±1.35^{ac}

LK- Leukocytes; T- Trombocytes; Ly- Lymphocytes; M- Monocytes; N- Neutrophiles

a- No Significant differences between the experimental variants, $p>0.05$.

b- Significant differences between the experimental variants, $p<0.05$.

c- No significant differences between the initial moment of the experiment, $p>0.05$.

d- Significant differences between the initial moment of the experiment, $p<0.05$.

The results regarding the absolute number of leukocytes ($x10^3$ cells μl^{-1}), show a significant decrease in the circulating blood, compared to the initial moment in all the six experimental variants ($p<0.05$). The absolute number of leukocytes in the six experimental variants, showed an insignificant increase ($p>0.05$) with the increase of the feeding intensity.

The absolute number of thrombocytes revealed a significant increase in comparation with the initial moment of the experiment ($p<0.05$), while no significant differences ($p>0.05$) were recorded between the six experimental variants.

At the beginning of the experiment, the absolute number of lymphocytes recorded an average of $58.83\pm5.97\times10^3$ cells μl^{-1} of blood, showing a significant decrease in all six experimental variants ($p<0.05$). Comparing the absolute number of lymphocytes from the six experimental variants, it can be seen that the intensity of feeding did not lead to significant changes ($p>0.05$).

In comparation with the initial moment, the average of relative numbers of lymphocytes showed a slight increase, statistically insignificant ($p>0.05$) in the experimental variants FL3, FL4, FL5 and FL6, while in the case of variants FL1 and FL2 the percentage of lymphocytes shows an insignificant decrease ($p>0.05$). Also, comparing the values of the average percentage of lymphocytes between the six experimental variants, no significant differences ($p>0.05$) were registered.

The absolute number of monocytes did not show significant differences ($p>0.05$) between the experimental variants, but in comparison with the initial moment a significant decrease ($p<0.05$) was observed in all variants. Slightly higher values of the relative number of monocytes were recorded in variants FL1, FL2 and FL5. In comparison with the initial moment, the relative number of monocytes decreased slightly ($p>0.05$). The relative number of monocytes did not reveal significant differences between the six experimental variants ($p>0.05$).

Compared to the initial moment, the absolute number of monocytes registered a significant decrease ($p<0.05$) in all six experimental variants.

Comparing the absolute number of neutrophils between the six experimental variants the statistical analysis did not reveal significant differences ($p>0.05$). Also, no significant differences were notice in comparison with the initial moment ($p<0.05$).

Determining the value of the biochemical indicators represents an important aspect in defining the normal physiological state of the fish, because it reflects the possible pathological changes that may occur as a result of the organism's defence against stress conditions. Serum protein level is the most important indicator of the nutritional status of the fish, presenting, at the same time, wide quantitative and qualitative variations depending on the species, age, sex, sexual maturation stage, water temperature, quantity and feed quality (Patriche, 2008).

Determining the serum blood glucose is the most efficient and rapid method for assessing fish stress (Popescu et al., 1990, cited by Patriche, 2008). Stress in the environment can also be the cause of increased glucose levels in the serum (Martin et al., 1998, cited by Patriche, 2008). At the same time, nutritional status can have an important influence on the serum glucose levels.

Figures 1 and 2 present the values of total proteins and glucose.

Analyzing the values of the serum parameters, we can observe a significant increase of the serum proteins compared to the initial moment ($p<0.05$). Also, comparing the serum protein values between the six experimental variants, the statistical analysis revealed significant differences ($p<0.05$).

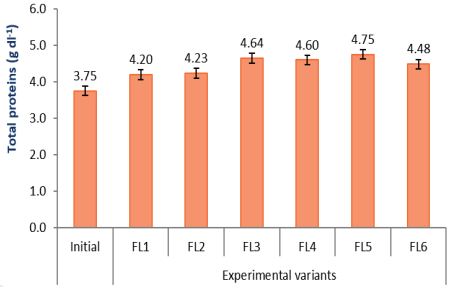

Figure 1. The values of serum total proteins recorded for rainbow trout

Thus, the significant increase of serum proteins was observed with the increase of the feeding level, while in the case of ad libitum feeding is observed their decrease.

Blood glucose content varies between 71.57± 4.43 mg dl^{-1} and 80.32±5.75 mg dl^{-1}. Slightly higher values were recorded in experimental versions FL1 (80.32±5.75 mg dl^{-1}), FL6 (77.26±5.68 mg dl^{-1}), FL2 (77.15±5.59 mg dl^{-1}), but the statistical analysis did not reveal significant differences between the six intensities of feeding ($p>0.05$). Compared to the initial time of the experiment, a significant increase ($p<0.05$) of the blood plasma glucose content was observed in all six experimental variants.

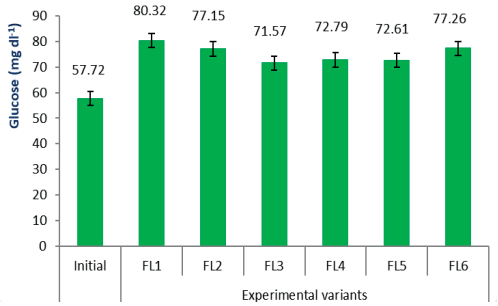

Figure 2. The values of serum glucose recorded for rainbow trout

CONCLUSIONS

Generally, the aim of the haematological studies is to contribute to an understanding of the relationship between blood characteristics and the adaptability of the species to the environment.

After the environmental factors, feeding affect the blood cells and the nutritional status of fish. Knowing that in a farm, feeding costs are very high, maximizing fish growth with lower costs, without affecting fish welfare represents a very important aspect from economical point of view. The results of our haematological study are corroborated with the results obtained regarding the growth performance and show that the feeding levels chosen do not influence the haematological and the immune response of the fish. In this context, we can say that the feeding level of 2.5% BW day^{-1} did not lead to significant changes of the hematological profile of rainbow trout, thus being indicated from the point of view of feeding costs.

The information obtained by the present study can be useful in developing some feeding strategies for rainbow trout in the intensive and semi-intensive culture

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