EFFECT OF STARVATION AND RE-FEEDING WITH DIFFERENT DIETARY PROTEIN LEVEL ON SOME HEMATOLOGICAL PARAMETERS OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM, 1792)

Mirela Creţu[1*], Lorena Dediu[1], V. Cristea[1], Angelica Docan[1], Raluca Cristina Andrei[1], Anca Nicoleta Cordeli[1]

[1]*"Dunărea de Jos" University of Galaţi, Romania*

Abstract

The aim of this experiment was to analyze the effect of applying of cyclical short periods of starvation (2 days and 4 days) on the hematological profile of rainbow trout. This experiment lasted for 46 days and was carried out in the facility of the University "Dunărea de Jos" from Galaţi. Six treatments with duplicate were assigned, as follows: two control groups, feed daily, ad libitum, with commercial pellets containing 41% crude protein (D41) and 50% crude protein (D50); two groups starved for 2 days (D2) and then fed with commercial pellets with 41% crude protein (D2/41), respectively 50% crude protein (D2/50) and two groups starved for 4 days (D4) and then fed with commercial pellets with 41% crude protein (D4/41), respectively 50% crude protein (D4/50). Starvation and re-feeding with different dietary protein level had no significant ($p>0.05$) effect on some hematological parameters including hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, while hemoglobin, Red blood cell counts and mean corpuscular hemoglobin concentration, registered significant differences ($p<0.05$). Significantly higher ($p<0.05$) concentration of hemoglobin was observed in the case of fish fed with higher protein content, while the increasing of the starvation period led to a significant decrease of the hemoglobin concentration. Furthermore, starvation and subsequent feeding led to a significant decrease of the erythrocyte number with the increasing of the starvation period.

Key words: starvation, protein level, hematological profile, rainbow trout

INTRODUCTION

Feeding is one of the most important factors which can affect growth, welfare and physiological performance of fish. In the natural environment, fish can be subjected to starvation periods when, due to a variety of reasons, such as water quality issues, variation of temperature, occurrence of diseases, the feeding is stopped [2], [20]. In aquaculture, starvation of fish is not frequent, but could occur by an inadequate diet or feeding protocols [11], but feed restriction could also be intentionally performed to evaluate specific effects of starvation on the digestive process in fish [10], or as a management tool to reduce the feeding costs [6]. In this context the potential of fish to compensate growth after the exposure of unfavorable conditions it can be the best technological solution.

The term "compensatory growth" refers to the ability of an organism to exhibit accelerated growth after a period of dietary restriction, imposed by adverse environmental conditions or drug treatment [1].Generally, after restoring the feeding conditions, the compensatory growth response is influenced by the environmental factors, for example, water temperature [13], [15], water quality [15], hierarchical dominance behavior [9], but also by nutritional factors, such as protein and energy content of the feeds administered [5].

The measurement of hematological and biochemical blood parameters are reliable diagnostic tools to determine the health

*Corresponding author: mirela.cretu@ugal.ro
The manuscript was received: 01.10.2019
Accepted for publication: 17.04.2020

condition of fish. Since the assessment of blood parameters during periods of fasting and refeeding could be a valuable tool in evaluating the health status of fish, the present study examined the effects of short-term fasting and subsequent refeeding on the alteration of hematological of rainbow trout.

MATERIALS AND METHODS

Experimental design. The rainbow trout, *Oncorhynchus mykiss* (initial weight 111.93±15.76 g; initial length 21.35±1.04 cm), used in this experiment were obtained from a private fish farm (Prejmer, Braşov City, România). Fish were stocked into a circular tank and acclimated to the experimental conditions for two weeks. During this period, fish were fed ad libitum, twice a day. After acclimation, 19 fish were randomly stocked in twelve glass aquaria of 132 L volume, 19 fish in each (n = 19).

Six treatments with duplicate were assigned, as follows: two control groups, feed daily, *ad libitum*, with commercial pellets containing 41% crude protein (D41) and 50% crude protein (D50); two groups starved for 2 days (D2) and then fed with commercial pellets with 41% crude protein (D2/41), respectively 50% crude protein (D2/50) and two groups starved for 4 days (D4) and then fed with commercial pellets with 41% crude protein (D4/41), respectively 50% crude protein (D4/50) (table 1).

Table 1 Biochemical composition of experimental diets

Ingredients	Composition	Diet 41	Diet 50
Crude protein	%	41	50
Crude fats	%	12	20
Crude fiber	%	3	0.7
Crude ash	%	6.5	8
Phosphorus	%	0.9	1.2
Vitamin A	UI	10000	6000
Vitamin D3	UI	1250	1200
Digestible energy	MJ/kg	14.2	19.7
Ingredients: Fish meal, fish oil, hemoglobin, full-fat soybean, soybean oil, wheat gluten, sunflower flour, wheat, and wheat products.			

Fish were fed manually at 9^{00} and 18^{00} and *ad libitum* feeding was done until the fish have not shown interest in administered fed (over an hour).

Blood hematological parameters. At the end of the experiment, about 1 mL of blood was taken by caudal venous puncture, from 5 fish/on each experimental unit. After sampling, blood samples were divided into two parts. One of which was added to heparinized tube and another part for the determination of serum proteins and glucose was centrifuged immediately for the determination of total proteins serum and glucose.

The values of basic haematological parameters were evaluated according to standard methods described by [19]. Briefly, the erythrocytes (RBC) were counted immediately after blood collection in hemocytometer, the hematocrit value (Ht) was determined in heparinized capillary tubes after being centrifuged in a standard micro hematocrit centrifuge at 12,000 rotation for 5 min. The blood hemoglobin concentration (Hb) was measured using spectrophotometric cyan methemoglobin method. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH) mass and mean corpuscular hemoglobin concentration (MCHC) were calculated using Ht, RBC and Hb values, according to [7]. Serum total proteins were determined by biuret method at the wave length 540 nm and serum glucose was determined using o-toluidine and spectrophtometrically dosed at a wavelength λ=635 nm.

*Statistical analysis.*The hematological parameters of the experimental groups were expressed by mean and standard deviation (Mean±SD) and were analyzed with SPSS 20. Statistical differences between variables were tested using ANOVA (α =0.05) and post hoc comparisons between sample means were made By Turkey`s test.

RESULTS AND DISCUSSIONS

In this study, we investigated the effects of nutritional restriction and subsequent re-feeding on physiological responses of rainbow trout. In table 2 are presented the values of the hematological parameters at the end of the experimental period.

Table 2 Values of hematological parameters measured at the end of the experimental period

Experimental variants	Hematological parameter (Mean±SD)					
	Ht (%)		Hb (g/dl)		RBC x10^6/µl	
	min-max.	Mean±SD	min-max.	Mean±SD	min-max.	Mean±SD
D_{M41}	37-39	38.00±0.63	9.20-9.71	9.48±0.22	1.00-1.09	1.06±0.04
$D_{2/41}$	38-39	38.17±0.41	8.42-9.52	8.74±0.42	0.88-1.01	0.98±0.05
$D_{4/41}$	36-40	38.33±1.37	8.00-8.84	8.35±0.29	0.86-0.99	0.90±0.05
D_{M50}	36-38	37.17±0.75	8.91-9.95	9.53±0.36	1.05-1.12	1.09±0.02
$D_{2/50}$	37-38	37.67±0.52	9.00-9.67	9.35±0.23	0.95-1.06	0.99±0.04
$D_{4/50}$	37-38	37.83±0.41	9.02-9.64	9.23±0.25	0.74-1.04	0.88±0.11

Hematocrit values were not significantly influenced by the protein content of the feed or by the starvation period ($p>0.05$). However, it can be observed that slightly higher values were obtained in the case of fish fed a protein content of 41%, compared to those fed with a feed with 50% protein content. Regarding the influence of feeding regimes on the dynamics of the hematocrit, we can observe an insignificant increase ($p>0.05$) with the increase of the starvation period. Increasing of the hematocrit levels during the starvation period were also reported by other authors in the case of the Atlantic code (*Gadus morhua*) or in the case of [17] while in the case of starvation for 31 days, of the European bass (*Dicentrarchus labrax*) and sea bream (*Pagellus bogaraveo*) no significant changes were recorded [2].

The hemoglobin values at the end of the experimental period, show significant differences ($p<0.05$) between the experimental variants being influenced by both the protein content of the feeds and the starvation period. So, it was observed an increase of hemoglobin concentration at fish that were fed with higher protein content (D50, D2/50 and D4/50) than those fed with a lower protein content (D41, D2/41 and D4/41). Regarding the influence of starvation period on the hemoglobin concentration, a significant decrease ($p<0.05$) can be observed with the increase of the starvation period.

The number of erythrocytes was not significantly influenced ($p>0.05$) by the protein content from the feed. Regarding the influence of the feeding regimes on the number of red blood cells, a significant decrease ($p<0.05$) can be observed with the increase of the starvation period. Thus, in the case of starved fish a decrease in the number of red blood cells is observed near the lower range specified in the literature (0.80 x10^6 /µl) [8]. Generally, the decreasing of the number of red blood during starvation periods is associated with the reduction of oxygen utilization through hematopoiesis control and implicitly with the decree of the metabolic activity [16].

With the help of hematological indices, the erythrocyte constants (MCV, MCH, MCHC) were calculated and presented in table 3.

Table 3 Value of erythrocyte constant at the end of the experimental period

Exp. variants	Hematological parameter (Mean±SD)					
	MCV (µm^3)		MCH (pg)		MCHC (g/dl)	
	min-max.	Mean±SD	min-max.	MeanX±SD	min-max.	Mean±SD
V_{M41}	341.01-381.91	358.84±14.63	84.79-97.59	89.60±4.93	24.50-25.55	24.96±0.39
$V_{2/41}$	375.12-434.29	392.13±21.55	84.18-96.23	89.72±4.81	22.08-25.06	22.90±1.16
$V_{4/41}$	393.94-446.93	425.92±18.50	82.62-96.26	92.83±5.17	20.97-22.86	21.80±0.83
V_{M50}	331.84-361.22	342.63±10.25	79.95-91.80	87.85±4.37	24.09-26.89	25.64±1.02
$V_{2/50}$	358.49-393.78	380.19±12.33	89.05-98.17	94.41±3.15	23.68-26.13	24.84±0.79
$V_{4/50}$	365.38-517.01	436.82±15.48	89.69-13.16	106.76±15.62	23.74-25.37	24.40±0.62

MCV was not significantly influenced by the protein content of the two commercial feeds ($p>0.05$). However, a slight increase was observed in the case of feed with 41%

crude protein, an increase that correlates with the insignificant growth of the hematocrit, respectively with the insignificant growth of the number of red blood cells. Regarding the influence of feeding regimes on MCV, a significant increase ($p<0.05$) can be observed with the increase of starvation period.

MCH was not significantly influenced ($p>0.05$) by the protein content of the two commercial feeds. Regarding the influence of feeding regimes on MCH, the statistical analysis did not reveal significant differences ($p>0.05$) between control and starved variants for 2 days, while between control group and groups starved for 4 days it was observed a significant increase ($p<0.05$) of MCV values.

The significant increase in hemoglobin values in fish fed with higher protein content implicitly led to a significant increase in MCHC values. Regarding the influence of feeding regimes on MCHC, the statistical analysis showed a significant decrease ($p<0.05$) in the case of fish starved for 4 days, while between the control groups and the fish starved for 2 days, not significant differences were noted ($p>0.05$).

Analyzing the values obtained for serum proteins, it was observed that they were not significantly influenced by the protein content of the two commercial feeds or by the feeding regimes ($p>0.05$). The average values of the total serum protein were between 3.57 ± 0.22 g / dl and 4.42 ± 0.27 g / dl, observing slightly lower values in the fish fed with feed of 41% PB, respectively for fish starved for 2 days (D2 /50-3.97 ± 0.93 g / dl; D2 /41- 3.93 ± 0,27 g / dl) and 4 days (D4/50-3.83 ± 0.42 g / dl; D4/41- 3.57 ± 0.22 g/dl) (Figure 1).

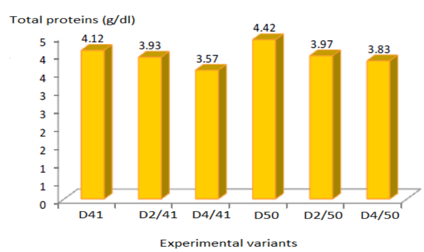

Fig. 1 The values of serum total proteins recorded at the end of the experimental period

The lowest value of serum proteins was observed in the case of experimental variant D4/41, an explainable aspect, both due to the lower protein content from the feed and the starvation period applied to the fish. However, the values obtained fall within the range recommended by the literature (3,5 ÷ 5,5 g / dl) [18].Some authors observe that in the case of the rainbow trout, a decrease in serum protein after 110 days of starvation, at a water temperature of 15-17°C, to about 30% of the initial values [12].

From the statistical analysis of blood glucose values, insignificant differences were observed between the two types of commercial feed ($p>0.05$), slightly higher values being recorded in the case of feed with 50% crude protein. Regarding the influence of the feeding regimes on the blood glucose, no significant differences were observed ($p>0.05$) between the control and fish starved for 2 days, while significant differences ($p<0.05$) were registered between the control groups and the fish starved for 4 days. Thus, a decrease in blood glucose was observed with the increase of starvation period, the lowest values being obtained in the starved groups in 4-day cycles (D4/50-65.77± 7.53 mg / dl; D4/41- 63.49 ± 8.86 mg/dl)

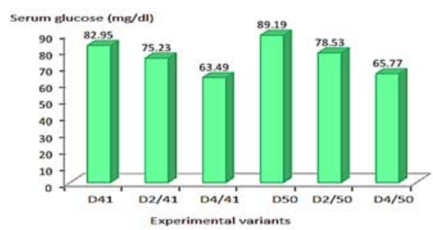

Fig. 2 The values of serum glucose recorded at the end of the experimental period

Decreasing of blood glucose levels were also reported by other authors during starvation periods. [3], [4] observed, in the case of the rainbow trout, a decrease of glucose levels only after 5 days after starvation. At fish, blood sugar levels are quite large depending on a number of factors, such as diet, photoperiod, water temperature and others. According to [14], for culture species, glucose values vary within the range 40 ÷ 90 mg/dl.

CONCLUSIONS

From the overall analysis of the hematological parameters, it can be concluded that the change of their values appeared as a reaction of adaptation of the fish organism to the technological conditions. Thus, the significant reduction of the number of red blood cells and the concentration of hemoglobin associated with the starvation period, implicitly led to the significant increase of the average red blood cell volume (MCH), respectively the decrease of the concentration of mean red blood cell hemoglobin (MCHC), which means the appearance of hem concentration, which may be a consequence of water loss in the body during the starvation period.

ACKNOWLEDGMENTS

This work was supported by the project "EXPERT", financed by the Romanian Ministry of Research and Innovation, Contract no. 14PFE/17.10.2018. The authors are grateful for the technical support offered by MoRAS through the Grant POSCCE ID 1815, cod SMIS 48745 (www.moras.ugal.ro).

REFERENCES

[1] Ali M., A Nicieza & R J Wootton, 2003: Compensatory growth in fishes: a response to growth depression. Fish and fisheries, 4, p 147-190.
[2] Caruso G., Denaro M. G., Caruso R., Mancari F., Genovese L., Maricchiolo G., 2011: Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*), Marine Environmental Research, 72(1):p 46-52.
[3] De Silva, S.S., Anderson, T.A., 1995: Fish Nutrition in Aquaculture. Aquaculture Series, Chapman & Hall, London.
[4] Furné.M, García –Gallego, M. Hildago, M.C., Morales, A.E., Domezain, A., Domezain, J.Sanz A., 2009: Oxidative stress parameters during starvation and refeeding periods in Adriatic sturgeon (*Acipenser naccarii*) and rainbw trout (*Oncorhynchus mykiss*). Aquaculture Nutr.,15, p 587-595.
[5]Gaylord, T. G. and D. M. Gatlin, 2001: Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*), Aquaculture 194, p 337-348.
[6] Guzel S., Abdullah A. 2011: Effects of different feeding strategies on the growth of young rainbow trout (*Oncorhynchus mykiss*), African Journal of Biotechonolgy, Vol.10 (25) p 5048-5052.
[7] Ghergariu S., Pop A., Kadar, 1985: Guide-clinical veterinary laboratory, Bucureşti.
[8] Ghittino P., 1983: Technology and Pathology in Aquaculture, vol. 1.
[9] Hayward R.S., Wang N., Noltie, D.B. 2000: Group holding impedes compensatory growth of hybrid sunfish, Aquaculture183, p 299-305.
[10] Krogdhal A, Bakke-McKellep A. M, 2005: Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp Biochem Physiol 141, p 450–460
[11] López-Olmeda J.F., Noble, Sánchez-Vázquez C. F.J., 2012: Does feeding time affect fish welfare? Fish Physiology and Biochemistry, 38: p 143-152.
[12] Oprea Lucian, Rodica Geoergescu, 2000; Nutriţia și alimentaţia peștilor, Editura Tehnică, București.
[13] Pastoreaud, A., 1991: Influence of starvation at low temperature on utilization of energy reserves, appetite recovery and growth character in sea bass, *Diocentrarchus labrax*.Aquaculture 99, p 167–178.
[14] Patriche, T., 2008: Imunitatea la peşti. Ed. Didactică şi Pedagogică Bucureşti, p. 5-8; 18-36.
[15] Quinton J. C., Blake, R. W., 1990: The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. Journal of Fish Biology 37, p 33–41.
[16] Shan Xiujuan, Huang Wei, Cao Liang and Wu Yunfei, 2008: Advances in Studies of the Effects of Starvation on Growth and Development of Fish Larvae. J. Ocean Univ. Chin. (Oceanic and Coastal Sea Research), Vol.7, No.3, p 319-326.
[17] Shoemaker C.A., P.H. Klesius, C. Lim, M. Yildirim, 2003: Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to Flavobacterium columnare, J. Fish Dis. Vol. 25, p 558-561. [18] Siwicki, A.K. & Anderson, D.P. 1993: Nonspecific defense mechanism assay in fish I.Phagocytic index, adherence and activity test-Fish diseases diagnosis and prevention methods, p.95-104,FAO Project International Workshop and training course In Poland.
[19] Svobodova Z., 2001: Stress in fishes. A review, Bull VURH Vodnany 4, p 169-191.
[20] Wang, Y. Cui, Y. Yang, Y. & Cai, F., 2000: Compensatory growth in hybrid tilapia, *Oreochromis mossambicus*× *O. niloticus*, reared in seawater', Aquaculture, 189(1), p 101- 108.