

LIVER HISTOLOGY AND VARIATION OF HEPATOCYTES NUCLEAR AREA OF RAINBOW TROUT (*Oncorhynchus mykiss*) REARED IN CAGES

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HISTOLOGIJA JETRE I VARIRANJE POVRŠINE JEDARA HEPATOCITA PASTRMKE GAJENE U KAVEZNOM SISTEMU

Abstrakt

U hidroakumulacionom jezeru "Bočac" gajena je kalifornijska pastrmka (*Oncorhynchus mykiss*, Walbaum, 1792) u dva odvojena eksperimenta u trajanju od po 90 dana – jedan u periodu jesen – zima, a drugi u periodu proleće – leto. Pastrmke su hranjene sa šest različitih komercijalnih hraniva i ispitan je njihov uticaj na histološku gradnju jetre riba. U eksperimentu je preovladavala normalna histološka građa jetre, a malobrojne histopatološke promene koje su uočene se mogu pripisati periodu godine i sastavu hrane. Kvantifikacija rezultata je pokazala da se sa rastom temperature vode i količine hrane kojom su ribe hranjene, prosečna površina jedara hepatocita povećava, dok se sa opadanjem temperature i količine hrane prosečna površina jedara hepatocita povećava, nezavisno od tipa hrane koja je korišćena.

Ključne reči: kalifornijska pastrmka, kavezni system, histologija jetre, temperature, hrana

INTRODUCTION

Fish from aquaculture often have alterations in vital organ morphology. As function of food availability, season, species, and/or environmental conditions modifications in histological structure of digestive organs occur (Strussmann and Takahima, 1990; Takahima and Hibia, 1995; Carriquiriborde et al. 2007). Liver plays a key role in metabolism of nutrients absorbed in the digestive tract (Olsvik et al. 2007). Morphological effects on the liver depend on fish species. A number of studies

were carried out on nucleoli of eurythermal species showing increase of liver metabolism alterations with sudden change of temperature (Alvarez et al. 2006; Carriquibiriborde et al. 2007; Itoi et al. 2003, Sarmento et al. 2000). Eurythermal fish have evolved compensatory responses to cope for the naturally occurring seasonal changes which affect their habitat. Although temperature and photoperiod are the main physical factors that distinguishes summer from winter, food availability, among others also influence the adaptive regulation of fish (Alvarez et al. 2004). Studies monitoring nuclear area of hepatocytes in rainbow trout are mainly stereological studies of normal liver (Rocha et al. 1997), or studies concerning breeding cycle: variation mainly attributed to sex differences (Rocha et al. 2009), starvation experiments (Power et al. 2000), effects of different feed types (Polksic et al. 2006; Ostaszewsk et al. 2005), and ecotoxicological studies (Hofer et al. 1999).

In the previous 60 days experiment with different commercial feed used for trout reared in cages, a statistically significant decrease of hepatocytes nuclear area was found in the period spring - summer (Polksic et al. 2006). The present study was carried out in order to investigate the effect of temperature and food quantity on liver histology and hepatocytes nuclear area of trout cultured in cage system.

MATERIAL AND METHODS

Experiments were performed in two 90-days periods. Rainbow trout yearlings had an individual mass from 93.97 to 99.43 grams (Tab. 1). The first cycle was carried out in the period autumn – winter (A/W) (19/10/2005 – 24/01/2006), and a second in the period spring – summer (S/S) (02/04/2006 – 01/07/2006). Experimental design was the following: 6 (six) identical cages of the cage system "Tropik" in the hydro accumulation Bočac in Bosnia and Herzegovina were used. Cages dimensions were 5 x 5 x 7 m, useful production volume of 162.5 m³. Cages were marked as 1, 2, 3, 4, 5, and 6, as were six different commercial compound feeds. Each cage was stocked with 400 kg rainbow trout. Fish were fed six different commercial feed types; their composition is given in Tab. 2.

Table. 1. Average individual weight of rainbow trout per treatment at the beginning of the first and second period.

Cycle	1	2	3	4	5	6
I	96.28	94.75	97.95	94.29	94.56	93.97
II	94.08	97.32	95.04	99.43	96.33	94.39

Water quality was monitored during the experiment and was within the first class of quality. Water temperature and oxygen concentration were measured daily at 1, 2, and 3 m depth (1, 2 i 3 m) using the Oxi 330i/SET 2B20-0011 WTW, Germany.

Table. 2. Feed composition used in the experiment.

Food type	1	2	3	4	5	6
Crude proteins, %	44,0	48,0	42,0	42,0	44,0	42,0
Crude fat, %	14,0	26,0	22,0	23,0	26,0	18,0
Crude fiber, %	5,0	1,0	3,3	1,8	1,3	1,7
Ash, %	9,0	8,5	10,0	8,0	10,0	8,8
Phosphorus, %	1,2	0,9	1,3	1,1	1,5	1,2
Vitamine A (IU/kg)	6000	15000	6000	15000	6000	15000
Vitamine E(mg/kg)	200	200	200	200	200	200
Copper (mg/kg)	3,0	5,0	3,0	5,0	3,0	5,0
Bruto energy(MJ)	20,4	23,8	21,8	22,3	23,2	21,0
Digestible energy (MJ)	17,7	21,9	19,3	20,3	20,9	19,1
Metabolic energy (MJ)	15,7	19,6	17,4	18,3	18,9	17,2
Nitrogen-Free Extract, %	21,0	17,0	15,0	17,2	13,0	21,5

Samples for histological analysis were taken prior to experiment beginning and after 90 days. Three fish per cage were sacrificed, and samples of the liver fixed in 4 percent formaldehyde and embedded in paraffin. For histological analysis slides were prepared using a standard histological technique with hematoxiline/eosine staining of 5 μm sections. Slides were examined under the Leica DM LS light microscope, with a DC 300 camera. Morphometric parameters were measured using a Leica IM 1000 program. Average nuclear area of 30 hepatocytes per each liver section was determined. At least 5 sections per liver sample were analyzed.

Statistical analysis was done using a Microsoft Office Excel 2003 program: Statistical Analysis Tools (ANOVA: Single Factor I t-Test: Two-Sample Assuming Equal Variances).

RESULTS AND DISCUSSION

Temperature varied from 4.8 °C (in the last 15 days) to 11.96 °C (in the first 15 days of the experiment) during the autumn – winter (A/W) period (Fig.1), while in the spring - summer (S/S) period variations were from 8.61 (in the first 15 days) till 19.37 °C (in the last 15 days) (Fig.2). In accordance with seasonal changes average values of temperature in the first period (A/W) have shown gradual decrease, and in the second period (S/S) gradual increase.

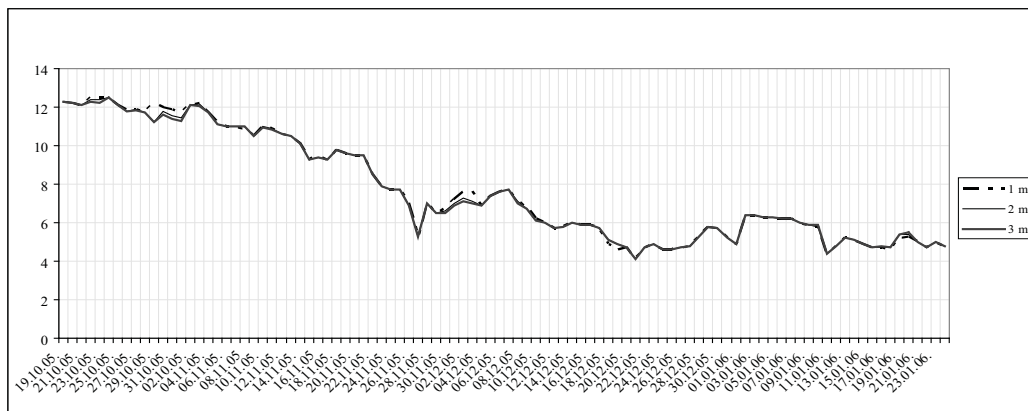


Figure 1. Temperature variation during the autumn – winter period (A/W).

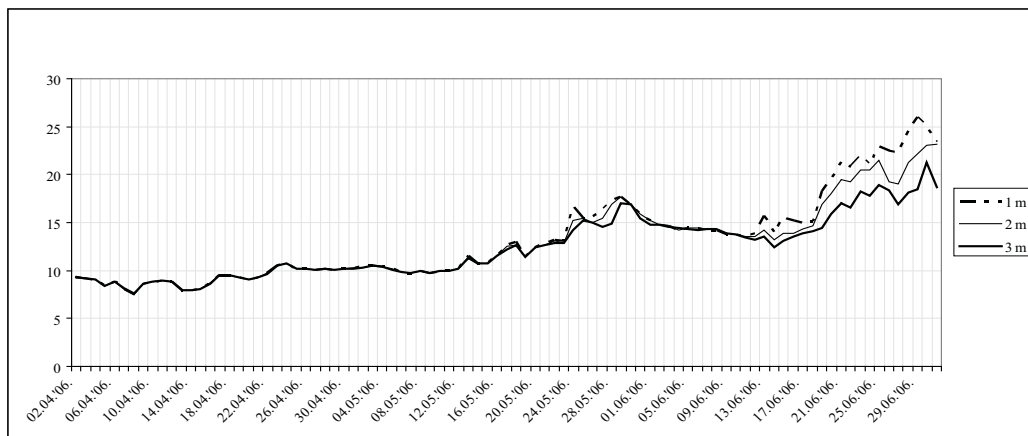


Figure 2. Temperature variation during the spring – summer period (S/S).

Liver histology

Normal histological structure of the liver was dominant on most of the samples examined. Changes such as hepatocytes vacuolization, occasionally fatty changes, congestion of sinusoids and larger blood vessels were found in both A/W and S/S experiments (Fig. 3 and 4). Vacuolated hepatocytes and other signs of fatty liver degeneration were found on livers of fish fed feed 2 and 5 in A/W period. Liver of fish fed feed type 4 had, in addition to the mentioned symptoms, signs of fibrosis. Similar alterations were noticed in the S/S experiment on livers of trout fed feed 2 and 5 when fish consumed greater quantity of food. In addition to these changes livers of trout fed feed 2 and 5 had some picnotic nuclei of hepatocytes. Picnotic nuclei were found on livers of fish fed feed number 1 and 6 as well (Savić et al. 2008).

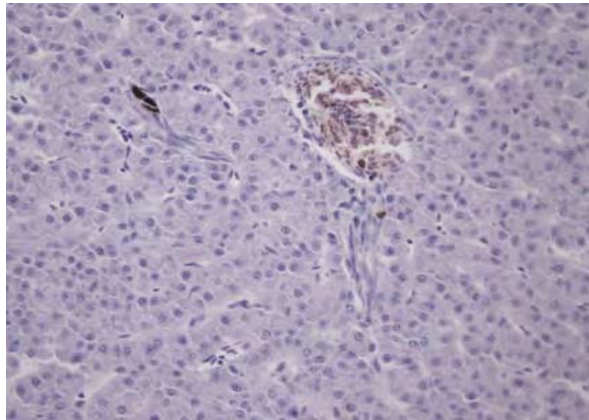


Figure 3. Congestion of blood vessels

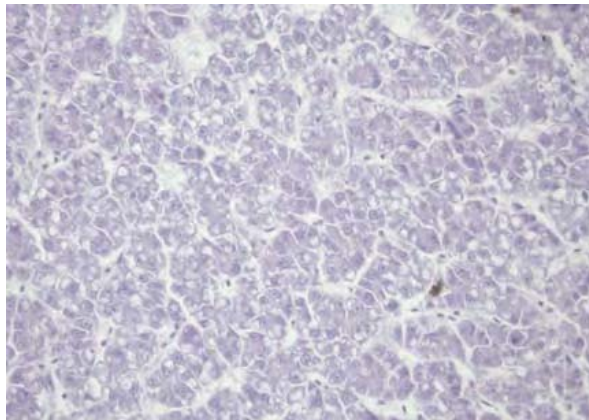


Figure 4. Hepatocytes vacuolization

In conclusion, in both A/W and S/S part of the season signs of fatty liver degeneration appeared in trout fed commercial feed with the highest lipid (26 %) and protein level (48 % and 44 % for feed 2 and 5, respectively). High lipid content in the compound feed could cause fatty degeneration of the liver (C a b a l l e r o et al. 2003; D u et al. 2008). Nuclear picnosis is an effect of fat accumulation in liver cells and their degeneration (T a k a s h i m a and H i b i y a, 1995). Livers of trout fed feed type 5 in both periods (A/W and S/S) had the best growth rate although with liver fatty degeneration. Best food conversion ratio and best Fulton's condition coefficient were observed, again, in cages with trout fed feed 5 and 2 that had a highest lipid level (S a v i c et al. 2008). A z e v e d o et al. (2004) found that food consumption increases with decrease of protein content and increase of lipids.

Hepatocytes nuclear area measurement

During the first period (A/W) as shown on Tab.3 in all cages hepatocyte nuclear area had an increasing trend from the beginning of the experiment. Statistically significant ($\alpha = 0.05$ and $\alpha = 0.01$) increase of the average hepatocytes nuclear area was determined

using t-test, with maximum increase in the cage 6, where the increase of average hepatocytes nuclear area of $12.25 \mu\text{m}^2$ was found at the end of the experiment compared to its beginning. Least increase of average hepatocytes nuclear area of $6.11 \mu\text{m}^2$ (end of experiment compared to the beginning) was found in cage 5.

Table 3. Average hepatocytes nuclear area values (μm^2), average differences (μm^2), coefficient of variation and t - test (Two-Sample Assuming Equal Variances) between the start and end of the autumn – winter period (A/W)

Cage	\bar{x}_{start}	\bar{x}_{end}	$\bar{x}_{\text{start}} - \bar{x}_{\text{end}}$	V_{start}	V_{end}	$t_{\text{calc.}}$	t_{tab}	
							0.05	0.01
1	20.308	32.283	-11.98**	13.434	17.838	-27.42**	1.96	2.59
2	22.689	31.449	-8.76**	11.737	16.906	-21.56**		
3	22.057	30.357	-8.30**	12.536	23.986	-15.39**		
4	21.217	28.856	-7.64**	11.995	16.664	-20.62**		
5	22.955	29.067	-6.11**	12.451	13.414	-19.02**		
6	18.627	30.876	-12.25**	14.445	14.229	-35.26**		

Average hepatocyte nuclear area at the end of the second experiment, as shown on Tab.4, decreased in all cages compared with values at experiment start. The slightest difference of average hepatocytes nuclear area of $0.75 \mu\text{m}^2$ was found in cage number 5, while the biggest difference was $6.67 \mu\text{m}^2$ in cage number 1. In the second experiment a statistically significant decreasing trend in hepatocytes nuclear area, compared to the control was detected ($\alpha = 0.05$ and $\alpha = 0.01$).

Table 4. Average hepatocytes nuclear area values (μm^2), average differences (μm^2), coefficient of variation and t - test (Two-Sample Assuming Equal Variances) between the start and end of the spring – summer period (S/S)

Cage	\bar{x}_{start}	\bar{x}_{end}	$\bar{x}_{\text{start}} - \bar{x}_{\text{end}}$	V_{start}	V_{end}	$t_{\text{calc.}}$	t_{tab}	
							0.05	0.01
1	26.172	19.499	6.67**	13.39	9.89	27.35**	1.96	2.59
2	25.433	19.636	5.80**	18.10	11.39	18.75**		
3	23.975	18.294	5.68**	16.19	12.80	20.39**		
4	24.067	19.154	4.91**	14.18	11.94	19.28**		
5	20.298	19.552	0.75*	20.25	12.80	2.52*		
6	24.637	20.189	4.45**	15.47	14.59	14.68**		

Using F-test (two factorial experiment 2×6 ; two experiments $\times 6$ feed types; hepatocytes nuclear area) a statistically significant ($\alpha = 0.05$ and $\alpha = 0.01$) difference in hepatocytes nuclear area was determined (Tab.5). The results have shown that average hepatocytes nuclear area is highly affected by the season i.e. water temperature and daily feed quantity. With increase of temperature and feed quantity hepatocytes nuclear area decreased and it increases with the decrease of temperature and feed quantity.

Table 5. Effect of the season and feeding on hepatocytes nuclear area at the end of first and second experiment and factor interaction - two factorial model (ANOVA).

Type of variation	<i>F</i> calc.	<i>F</i> tab.	
		0.05	0.01
Factor A (experiment)	6.438.86**	3.84	6.64
Factor B (hepatocytes nuclear area)	22.79**	2.22	3.02
Interaction	15.54**		

Similar results were obtained in the experiment that lasted 60 days from 17/05/2005 to 17/07/2005 (S/S). This study was carried out in the same lake of the hydro accumulation Bočac using the same commercial feeds (P o l e k s i c et al. 2006). It was a 60 days experiment, but average values of hepatocytes nuclear area decreased as in the S/S period of the 90 days experiment year later.

In rainbow trout fatty acid composition and temperature are important for the metabolism of lipids (T o c h e r et al. 2004). The results obtained in the present and the previous study (P o l e k s i c et al. 2006) are pointing out changes in hepatocytes metabolism along the season. The reason for the nuclear area increase could be attributed to increased protein synthesis, and therefore enlarged hepatocytes nuclei in the period of exhaustion of carbohydrates and lipids as energy sources in A/W period. In the S/S period when temperature raise and feed quantity increase, protein synthesis and therefore nuclear area diminish. It is known that the nuclear area of hepatocytes is directly related to DNA content (N u n e z et al. 2000). Moreover, the energy source during decreased feeding in fish varies among species: some using mainly glycogen while others use lipids or proteins. Energy mobilization during food restriction may be affected by other factors, including ambient temperature and may provoke alterations in tissue structure (S o u z a et al. 2001).

The ability to adapt to the changes in resource availability by mobilization of energy-providing substrates occurs in order to support the body's requirements, although the use of lipids, protein and glycogen leads to cellular modifications in fish tissues (S o u z a et al. 2001, R i o s et al. 2007).

CONCLUSION

The results of the study of liver histology have shown that in trout fed six different commercial diets mainly normal histological structure prevailed with some alterations that could be attributed to the season and feed composition.

In order to adapt to environmental conditions a season-dependent change in average hepatocytes nuclear area occurs in the rainbow trout.

Effect of temperature and feed quantity was negatively correlated to hepatocytes nuclear area: average values of the nuclear area of hepatocytes decreased with the increase of temperature and feed quantity, and it increased when temperature and feed quantity decreased, regardless of feed composition.

Acknowledgements

The study was supported by projects: Reinforcement of Sustainable Aquaculture ROSA (FP7 REGPOT, No. 205135).

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