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Reproductive Performance of Male Rainbow Trout *Oncorhynchus mykiss* (Walbaum, 1792) Feeding with Food from Two Different Manufacturers

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Abstract

This study was carried out to compare the growth and development of testicles in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) at the Royal fish and Magazin Maprim fish farms using pellet fish feed of various manufacturers. Two groups with 30 individuals of the same age were examined. The males from the Royal fish farm were fed with Skretting feed while the Magazin Maprim fish farm used Coppens feed. Fish were fed twice a day at 5% body weight basis. Physicochemical parameters of water were within acceptable values for this species. The values of total length and total weight of the males from the Royal fish farm fed with Skretting feed showed a significant statistical difference compared to the males from the Magazin Maprim fish farm fed with Coppens feed. Also, mean weight of the testicles and gonadosomatic index of the males fed with Skretting feed did not show a statistically significant difference compared to the weight of the testicles and gonadosomatic indexof the males fed with Coppens feed. Histological assay of the testicles of rainbow trout subjected to experimental feeds showed a normal cell growth and spermatogenesis in both groups. The results indicate that there are differences in

the growth of rainbow trout between the observed groups in favor of Skretting feed manufacturer, which should be taken into account when selecting the feed for a more productive farming.

Keywords: *rainbow trout, growth, gonadosomatic index, testicles histology, pellet fish food.*

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1. Introduction

Aquaculture in Bosnia and Herzegovina is in constant development, representing the fastest growing sector of animal agriculture. The growing trend of production has continued from year to year according to the available data of B&H Agency for Statistics. The most important fish species raised at fish farms in the Federation of Bosnia and Herzegovina is rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). It was introduced in BiH for the first time in 1894, stocked some of our salmonid rivers (Korjenić, 2010). It is raised in concrete and cage ponds, which are two different biological environments in terms of the physicochemical properties of water. Under conditions of intensive farming, rainbow trout grows at a faster pace; it is extremely adaptable to the living environment and is significantly more resistant to parasitic and bacterial diseases relative to other species that reproduce and grow under artificial conditions. Also, its nutritional quality is excellent (Davies & Bromage, 2002; Davidson et al., 2011; Vranić et al., 2011). A right choice of parental material under intensive breeding conditions ensures high production parameters of the rainbow trout offspring (Vehviläinen et al., 2012). Intensive production of this fish species requires several factors that should allow the most favorable conditions for life and a fast growth of fish population, i.e. to contribute to a faster achievement of the planned production and placement of the final product on the market. The previous studies have shown that photoperiod, temperature and amount of dissolved oxygen i.e. their values during spawning significantly affect the production in aquaculture (Randall, 2001; Pornsoping et al., 2007; Jažić et al., 2011). Technology of breeding rainbow trout must provide elementary conditions: clean water that fulfills the oxygen requirement (9-11mg/l), an appropriate water temperature (8-12°C), pH of water (6,5-8,5) and a sufficient flow of water and a systematic diet with different types of industrial and natural feeds (Ćuk et al., 2006; Marković et al., 2006; McMillan et al., 2012). In artificial breeding of rainbow trout, diet is without any doubt the most difficult and complex undertaking. Complexity of the problem with diet comes not so much from the type and amount of feed, but more from the fact that it is

conditioned by a range of different factors. A high productivity is based on a balanced and proper diet with all components that support intensive growth and prevent diseases (Klontz, 1991; Willoughby, 1999; Akbary et al., 2010).

Interesting biology and extreme economic significance of rainbow trout are the reasons behind intensive studies of many authors from our region (Mikavica et al., 2005; Muhamedagić et al., 2010; Savić et al., 2013b). These authors mainly examined the growth (length and weight) of rainbow trout relative to diet or different conditions of habitat (Pelo et al., 2012). In addition, the studies of the health of salmonids, the quality and nutritional values of meat, a significance in human diet and monitoring of morphometric and hematological indexes were carried out (Jeremić et al., 2003; Baltić et al., 2009; Jažić et al., 2011; Vranić et al., 2011).

Starting with an assumption that production and economic performance of salmonid species are affected significantly by artificial breeding, the goal of this study was to compare the growth, gonadosomatic index (GSI) and histological properties of the testicles in rainbow trout fed with pellet feed manufactured by two manufacturers, Coppens and Skretting.

2. Material and Methods

The studies monitored two groups of 30 rainbow trout males. The age of the specimen was determined on the basis of fish scales. Sampling of the rainbow trout males was carried out at two fish farms: Royal fish and Magazin Maprim. The Royal fish farm is located at Grabovica lake (lat: 43.626; lon: 17.751), hydroaccumulation of Neretva River, with intensive breeding in the floating cages. The Magazin Maprim fish farm with a classic intensive fish farming in concrete ponds is located at Ramički creek (lat: 43.89; 18.131), which flows into Bosna River (Figure 1.).

Figure 1. Geographic distribution of investigated fish farms in B&H

Pellet fish feed manufactured by Coppens (Holland) was used at the Magazin Maprim fish farm, while Skretting (Italy) feed was used at the Royal fish farm. Fish were fed twice daily on a 5 % body weight basis.
The measurements carried out at the fish farms included the basic physicochemical parameters of the quality of water (temperature, concentration of dissolved oxygen and pH values) and were done using a measurement device Multi 340i/SET (Germany).

2.1. Morphometric parameters and gonadosomatic index (GSI)

Sampled fish males were measured for the following morphometric parameters: total body length, total body weight and testicular weight. Total body length is a total length of a body, from head to the end of the tail. This parameter was measured using ichtiometer. Body weight of the males and the testicular weight were determined using a digital analytical scale (Tehtnica ET 1111) with a precision of two decimals, at the Histology Laboratory of the Faculty of Science Sarajevo. The data collected was used to calculate the gonadosomatic index (GSI) using the formula (Bolger & Connolly, 1989):

$$GSI = \text{gonad weight (g)}/\text{total fish weight (g)}*100$$

2.2. Histological analysis of the testicles

For the histological examination, after the separation from the surrounding tissues, the testicles were fixated in 10% formaldehyde. Microscopic preparations were made and analyzed in the Histology and Embryology Laboratory of the Veterinary Faculty in Sarajevo. Sample processing, from fixation to molding with paraffinn, was carried out on a rotational tissue processor (MICROM model STP 120). After molding, the samples were cut using digital microtome (LEICA RM 2145), several serial cuts from 0,5 to 1,5 micrometer thick and stained with standard method of hematoxylin-eosin (Bancroft & Cook, 1994). Examination of the histological preparations was done using a light microscope equipped with camera MOTIC TYPE 102M, under magnification of 100, 200 and 400x. The analyses of histological structures were carried out using a special program Motic Images Plus 2.0 ML.

2.3. Statistical analysis

Obtained results of measurement were statistically analysed using software Statistica 8.0 (©Copyright StatSoft, Inc. 1984-2007). The results of the morphometric measurements were presented as arithmetic means of measurements with a

standard deviation accompanied by minimum, maximum and coefficient of variation (%), (Microsoft Excel, 2007). *One way* ANOVA was applied to compare the observed parameters, after which a post-hoc Newman Keuls test followed. Linear regression analysis was used for modelling relationship between gonadosomatic index (GSI), total length, body weight and testicular weight. The analyses applied the level of a statistical significance of $P < 0.05$.

3. Results

3.1. Composition of pelleted fish feed and the parameters of the quality of water

Values of nutrients in pelleted fish feed was used in the diet of rainbow trout at the examined fish farms, are presented in (Table 1.). The table shows that fish foods are approximately similar in composition.

Table 1. Comparative illustration of pelleted fish feed composition

Manufactured	Pelleted fish feed	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Phosphorus (%)	Vitamin A IU/kg	Vitamin D3 IU/kg
Skreting (Italy)	Skreting optiline HE 2P	42	26	3	4,0	0,6	5000	1 000
Cooppens (Holland)	Ultra troco	43	28	1	8,3	0,9	15 000	3 000

The measured values of physicochemical parameters of water at the examined fish farms were within acceptable values for the rainbow trout breeding (Table 2.).

Table 2. Values of physicochemical parameters of water at the examined fish farms

Fish farm	Magazin Maprim	Royal fish
Temperture	11 °C	8 °C
Concentration of dissolved oxygen	10 mg/l	9 mg/l
Water pH	8.3	8.15

3.2. Morphometric parameters and gonadosomatic index (GSI)

Descriptive statistics of the morphometric parameters, weight testicular and gonadosomatic index is presented in (Table 3.). Our results show that the average values of a total length (34.80±1.86 cm) and a total body weight (563±53 g) of the males from the Royal fish farm fed with Skretting feed differ significantly from the values of a total length (26.70±2.05 cm) and a total body weight (237±62.46 g) of the males from the Magazine Maprim fish farm that used Coppens feed. The average testicular weight (13.56±4.18 g) of the males fed with Skretting feed did not show any significant difference as compared to the testicular weight (8.38±3.96 g) of the

males fed with Coppens feed. The gonadosomatic index (2,39±0,58) of the males from the Royal fish farm did not differ statistically from the gonadosomatic index (3,47±1,06) values of the males from the Magazin Maprim fish farm.

Table 3. Total length, body weight, weight testicular and gonadosomatic index of rainbow trout in fish farms

Fish farm	Statistical parameter	Total length (cm)	Body weight	Testicular weight	Gonadosomatic index
Floating cages ponds	Mean	34.80*	562.80*	13.56	2.39
	Standard deviation	1.86	53.31	4.18	0.58
	Minimum	31.50	476.00	9.63	2.02
	Maximum	36.00	609.00	20.64	3.42
	Coefficient of variation - %	5.34	9.47	30.83	24.32
Concrete ponds	Mean	26.70*	237.00*	8.38	3.47
	Standard deviation	2.05	62.46	3.96	1.06
	Minimum	24.50	159.00	4.09	2.23
	Maximum	28.50	304.00	14.37	4.73
	Coefficient of variation - %	7.68	26.36	47.23	30.43

Asterisk in superscript denotes significantly different mean values between groups (* P ≤0.05); P - values obtained by Newman Keuls test

3.3. Relationship between the gonadosomatic index (GSI) and body parameters of *Oncorhynchus mykiss*

The gonadosomatic index (GSI) of male *Oncorhynchus mykiss* fed Skretting feed showed a linear relationship with the total length (cm), total weight (g) and testicular weight (g). Power regression equation for the gonadosomatic index (GSI) and total length (cm), total weight (g) and testicular weight (g) of male *Oncorhynchus mykiss* fed Skretting feed is as follows:

$$GSI=0.0987\times-1.0482 \ (r^2=0.0998, P>0.05)$$
$$GSI=0.0057\times-0.8156 \ (r^2=0.2731, P>0.05)$$
$$GSI=0.1355\times-0.5494 \ (r^2=0.952, P>0.05)$$

The gonadosomatic index (GSI) of male *Oncorhynchus mykiss* fed Coppens feed showed a linear relationship with the total length (cm), total weight (g) and testicular weight (g). Power regression equation for the gonadosomatic index (GSI) and total length (cm), total weight (g) and testicular weight (g) of male *Oncorhynchus mykiss* fed Coppens feed is as follows:

$$GSI=0.1986\times-1.8316 \ (r^2=0.1485, P>0.05)$$
$$GSI=0.005\times-2.2856 \ (r^2=0.0875, P>0.05)$$
$$GSI=0.2155\times-1.6649 \ (r^2=0.6525, P>0.05)$$

3.4. Histological analysis of testicles of rainbow trout

Histological changes of the testicles during the maturation were similar in both examined groups. Interstitial tissue was well developed while the lobules were clearly separated. Tunica was very thin (Figure 2.). All stages of spermatogenesis classified as spermatocytogenesis, meiosis and spermiogenesis as well as the cells that belong to the respective stages were clearly visible in the semen tubes (Figure 3.). There were also slight differences in a degree of the testicular maturation between the observed groups, and they are related to the spermiogenesis process that progressed in the semen tubes of the testicles of rainbow trout fished at the Royal fish farm.

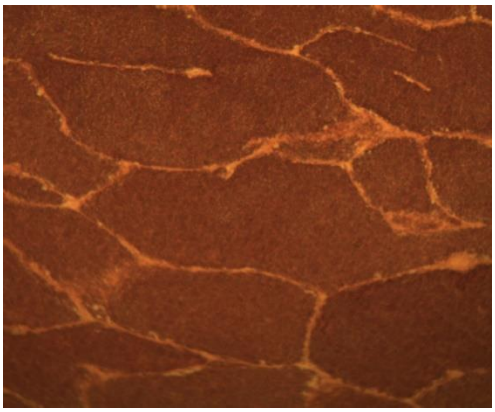

Figure 2. Lobularity: expressed testicular parenchyma (HE×200)

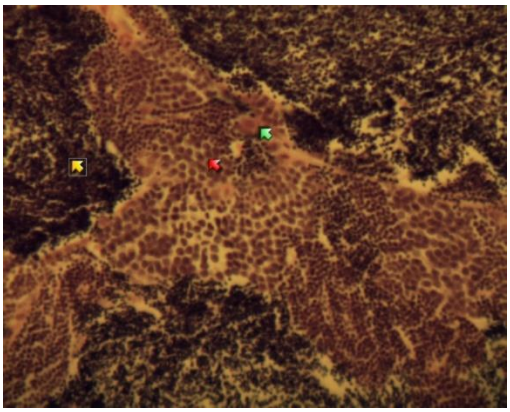

Figure 3. Spermatogenesis: green arrow - spermatogonia A; red arrow - spermatogonia B; yellow arrow - sperms (HE×400)

4. Discussion

Over the course of the previous period, the market in FBiH offered pellet fish feed of various manufactures with significant oscillations in terms of the percentage of raw materials in their composition. To assess the quality of the fish feed, besides the data on its chemical composition, the main criteria is a growth pace. Rainbow trout has one significant peculiarity that achieves a high biomass growth in a short span of time. Nutritional values of meat and reaching high values of biomass are sufficient reasons why rainbow trout has become one of dominant species at the world fish meat market. A growth of rainbow trout i.e. a pace of the length and weight growth in differing abiotic factors of the breeding environment and application of different feeds was the subject of many studies (Uysal, 2002; Guzel & Arvas, 2011; Kizak et al., 2011; Pelo et al., 2012; Sevgili et al., 2012; Savić et al., 2013a).

Based on the data on the growth pace of rainbow trout fed with pellet feed of various manufacturers, it was concluded that the examined types of the feed contained all

optimal values of nutritive materials necessary for the growth, and the growth pace depended on the variations of physicochemical parameters of water.

Pelo et al. (2012) has showed that the composition of the feed and a number of feedings during the day play a key role in a growth of biomass of rainbow trout raised in a cage pond at water temperature between 7°C and 9°C and that growth of rainbow trout depends on small variations of physicochemical properties of water. The most intensive growth rainbow trout achieved at constant water temperature while significant temperature variations did not have a favorable impact on the biomass production. Similar results were achieved in the study by Ekanem et al. (2012). This group of authors came to a conclusion that various kinds of feed tested under the same physicochemical parameters of water lead to different mass growth, as a consequence of a different share of feed ingredients. Monitoring the impact of Unical Aqua feed and commercial feed manufactured by Coppens on body mass growth, gonadosomatic index (GSI) and morphology of gonads of African catfish, C. gariepinus, was carried out by Eyo et al. (2014). Based on their findings, the examined kinds of feed were quite alike in terms of their performance; only in terms of availability, Unical Aqua feed was more cost effective, which is why it is recommended to fish breeders. However, there was a significantly positive correlation among GSI index, gonads weight, total body length and total body weight in fish fed with both feeds, within physiological values of physicochemical parameters of water. The results of these authors are quite similar to the results of our study. The study that aimed to analyze the impact of the meal size on the growth of rainbow trout progeny (*Oncorhynchus mykiss*) was carried out by Savić et al. (2013). An increase in a meal size by 10% above the recommended values does not lead to any significant differences, but in in case this increase is above 10% statistically significant differences will appear in the body mass. Increasing the daily diet norms for rainbow trout should be in line with their genetic growth potential and conditions of the living environment.

The studies showed that environmental factors such as water temperature, photoperiod, amount of dissolved oxygen and pH values of water significantly affect the intensity of gametogenesis (Campbell et al., 1994; Contreras-Sinchez et al., 1998). Their value at times of spawning is extremely important for productive aquaculture (Bromage & Roberts, 1995). Pavlov et al. (2013) cite that the differences in the structure of reproductive system of rainbow trout can be attributed to hormonal effects and abiotic environmental conditions. The intensity of spermatogenesis in rainbow and brown trout, sampled at different locations, was the subject of the examination carried out by Mlaćo et al. (2014). Some significant microstructural differences were identified in the structure of the testicles as well as

in the intensity of spermatogenesis, which indicates that microclimate environment conditions and diet are important factors impacting the reproduction of salmonids. Based on the results of our study, we can agree with the claims of the previous studies. Hiro & Yamamoto (1987) noticed a significant increase of the number of mature sperm cells in Oncorhynchus keta during migration, which indicates that a gametes will mature faster in a colder water. This fact can explain a advanced process of spermiogenesis at the Royal fish farm (water temperature 8°C) as opposed to the Magazin Maprim fish farm (water temperature 11°C). In addition to water temperature and the type of feed, the fish farms used different types of breeding. Higher weight growth was recorded at the Royal fish farm with cage breeding compared to Magazin Maprim with concrete pond breeding. Our results are consistent with the literature data (Mikavica et al., 2005; Ćuk et al., 2006; Pelo et al., 2012; Pilić et al., 2018).

5. Conclusions

This study has shown that there are statistically significant differences in the growth of rainbow trout (*Oncorhynchus mykiss*) in favor of Skretting manufacturer. The values of gonadosomatic index (GSI) and the weight of the testicles along with histological properties of the testicles did not show any significant differences between the observed groups. In line with the results, we believe that choosing the right feed decreases the breeding period, increases productivity and has a positive impact on the fish health and the quality of meat.

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8. Authors Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Selma Pilić, Anesa Jerković-Mujkić, Amela Katica, Nadžida Mlaćo; data collection: Selma Pilić, Enad Korjenić, Aldijana Mušović; analysis and interpretation of results: Selma Pilić, Adi Vesnić, Mahir Gajević: draft manuscript preparation: Selma Pilić, Anesa Jerković-Mujkić, Amela Katica, Nadžida Mlaćo, Enad Korjenić, Adi Vesnić, Aldijana Mušović, Mahir Gajević. All authors reviewed the results and approved the final version of the manuscript.

9. Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.