

Research regarding the influence of royal jelly on sex reversal in zebra fishes (*Danio rerio*)

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Abstract. In this study we observed the influence of royal jelly on sex reversal in zebra fishes (*Danio rerio*). Experiments were made in the laboratories of aquaculture from Faculty of Animal Science and Biotechnologies, Timișoara in the period 01.03.2010 – 15.05.2010 on 225 individuals. The dynamics of standard body weight, the standard length and the maximum body high at all individuals from three experimental variants were evaluated. It was identified the number of males and females by direct examination of the body and microscopic observation of histological samples. In the first experimental variant the food was mixed with 0.02 g powdered royal jelly and recorded at the end of the experiment 70.76% females and 29.24% males. In the second experimental variant, a double dose of powdered royal jelly was mixed in the feed, this leading to the increasing of females in the group to 81,19%. In the control variant the percentage of females was 50.7% and the percentage of males was 49.3%.

Key Words: royal jelly, zebra fish, sexual inversion, sex reversal.

Zusammenfassung. In dieser Studie wurde der Einfluss von Weissmilch auf die sexuelle Inversion von Zebrafischen (*Danio rerio*) untersucht. Das Experiment wurde im Fischzuchtlabor der Tierzucht und Biotechnologie Fakultät Timisoara durchgeführt, und zwar in der Zeit von 01.03.2010 –bis 15.05.2010 an 225 Fischen. Das Körpergewicht, Standardkörperlänge und maximale Höhe wurde bei jedem Individuum der drei Versuchsvarianten gemessen. Die Zahl der Weibchen und Männchen wurde durch Außenkontrolle und histologische Präparation festgestellt. In der Versuchsanordnung 1, wo 0.02 g Weissmilch ins Futter zugegeben wurde, erreichte die Weibchenzahl 70.76 % und die Männchenzahl 29.24 %. Die Versuchsanordnung 2, wo die Weissmilchzugabe verdoppelt wurde, erreichten die Weibchen 81.81 % und die Männchen nur 18.19 %. Im Vergleich dazu wurden in der Kontrollversuchsanordnung ohne Weissmilchzugabe 50.7 % Weibchen und 49.3 % Männchen gefunden.

Stichworte: Weissmilch, Zebrafisch, sexuelle Inversion.

Rezumat. În această lucrare am urmărit influența lăptișorului de matcă asupra inversiunii sexuale la peștele zebură (*Danio rerio*). Experimentele s-au efectuat în cadrul laboratorului de acvacultură al Facultății de Zootehnie și Biotehnologii din Timișoara, în perioada 01.03.2010 - 15.05.2010, pe un efectiv de 225 de exemplare. Pe parcursul experimentului s-a urmărit evoluția greutatei corporale, lungimea standard și înălțimea maximă a corpului la toți indivizii din cele trei variante experimentale. S-a identificat numărul de femele și masculi prin examinarea exteriorului și prin efectuarea și examinarea la microscop a preparatelor histologice. Lotul experimental 1, în hrana căruia s-a încorporat 0,02 g lăptișor de matcă liofilizat, a înregistrat 70,76% femele și 29,24% masculi iar lotul experimental 2, la care s-a adăugat doză dublă, a înregistrat 81,81% femele și 18,19% masculi, în comparație cu lotul martor la care procentul de femele a fost de 50,7%, iar cel de masculi de 49,3%.

Cuvinte cheie: lăptișor de matcă, pește zebură, inversiune sexuală.

Introduction. The sex of many fish species has regularly genetic determination (Dunham 2004; Păsărin 2010), at developing embryo. There are some fish species, in which the sex is influenced by the environment in which they live, for example: temperature, pH, hardness, density of the population etc (Păcală et al 2006; Amiri-Moghaddam et al 2010).

The zebra fishes are sexually mature after three months, but the sex can be determined at 21-23 days after fertilization. Before the sex differentiation, all individuals have gonads similar to ovaries, which develop in 10-20 days after fertilization.

Between day 21 and 30 after fertilization, starts the development of differentiated gonads in the same time with the apoptosis of primordial ovaries (Uchida et al 2002). Having these in view and taking in consideration the fact that at bees, in the absence of queen, feeding on royal jelly the worker bees genitals became functional, the aim of this research was to observe if the addition of royal jelly in fish feeding influence the zebra fish sex differentiation.

Material and Method. The experiment was carried out in the laboratory of aquaculture of the Faculty Animal Science and Biotechnologies, from Banat's University of Agricultural Science and Veterinary Medicine, in the period 1.03.2010 – 15.05.2010 on a group of 225 individuals (Figure 1).



Figure 1. The organization plan of the experiment (original photo).

Because of the fact that water's physical-chemical factors play a major role in body growth and development, we controled the main physical-chemical parameters: luminosity, water temperature, total water hardness, pH, dissolved oxygen, saturation in oxygen, nitrites (NO_2^-), nitrates (NO_3^-). Table 1 presents the mean values of water's physical-chemical parameters.

Table 1

Water quality parameters in breeding basins (mean values)

<i>Water's physical-chemical parameters</i>							
Luminosity	Temperature	Hardness	pH	Dissolved oxygen	Saturation in oxygen	Nitrites (NO_2^-)	Nitrates (NO_3^-)
237±18.21 lx	22.71±0.14°C	7 dH°	8.33±0.1	10.8±0.1 mg/L	94.9 %	0.02±0.07 mg/L	2±0.05 mg/L

The biological material was exposed to a photoperiod of 12/24 hours (light/day), and the mean light intensity at water surface was 237±18.21 lx. Luminosity was determined with EXTECH 401025 luxmeter, with measuring limits of 0-50.000 lx, with an accuracy of 5%. Data were recorded three times a day (at 8⁰⁰, 14⁰⁰ and 20⁰⁰).

The mean water temperature values during the experimental period was 22.71±0.14°C, and the mean oxygen values were 10.8±0.1 mg/L dissolved oxygen and 94.9 % saturation in oxygen. The determination of the above mentioned parameters was performed one a day with the help of a portable oxigenometer.

Basin hygienisation was done manually, twice a day, in order to avoid water qualitative depreciation. The water volume replaced/day was 10% of the total water volume/basin.

The biological material was divided in three experimental batches:

- The control batch (C.B.) was fed with STECO CRUMBLE HE (58% crude protein) for 14 days. After this period live food (*Artemia salina*) was administered;
- The first experimental batch (E.B.1.) was fed with the same feed, adding 0.02 g powdered royal jelly for 14 days. After this period live food (*Artemia salina*) was administered;
- The second experimental batch (E.B.2.) was fed with the same feed, adding this time 0.04 g powdered royal jelly for 14 days. After this period live food (*Artemia salina*) was administered, as in cases of the other batches (see Table 2).

At the control batch, administration of feed was made after the feed was minced through trituration. In the experimental variants 1 and 2, the lyophilized royal jelly was incorporated in 0.3 g combined forage STECO CRUMBLE HE, previously minced through trituration. The statistical processing of results was performed with the help of the software MINITAB 14.

Table 2

The experimental design

Period	Experimental batches		
	C.B.	E.B.1.	E.B.2.
0-14 days	Feed	Feed + 0.02 g powdered royal jelly	Feed + 0.04 g powdered royal jelly
15-18 days	Switchover at live food (<i>Artemia salina</i>)	Switchover at live food (<i>Artemia salina</i>)	Switchover at live food (<i>Artemia salina</i>)
18-75 days	<i>Artemia salina</i>	<i>Artemia salina</i>	<i>Artemia salina</i>

Results and Discussion. During this experiment, we supervised the evolution of body weight, standard body length (from the peak of the mouth to the base of the caudal fin) and maximal body height (in the tallest body region) in all individuals from the three experimental variants.

Then we compared statistically the experimental variants which were fed combined forage supplemented with lyophilized royal jelly with the control variant, fed only combined forage, regarding all the characters studied.

We identified the number of females and males by examining their exterior and by examining at the microscope the correspondent histological preparations.

In Table 3 are presented data obtained after administration of powdered royal jelly to zebra fish. It is easy to observe that in the control batch the number of females was almost equal with the number of males, resulting a sex-ratio of almost 1:1 (36 females and 35 males). In the case of experimental batch 1, from 65 individuals, 46 was females and 19 was male (2.42:1). The highest percentage of females was recorded in experimental batch 2, 81.81% (see Table 4).

Table 3

Results obtained after powdered royal jelly administration at zebra fish

Specification	C. B.	E.B.1.	E. B. 2.
Number of individuals at the beginning of experiment	75	75	75
Number of individuals at the end of experiment	71	65	66
Females	36	46	54
Males	35	19	12
Malformations	-	2	4
Mortality	4	8	5

From all the three experimental batches, in the experimental batch 1 was observed the highest mortality (8 fish, compared to 4 fish in control batch and 5 fish in experimental

batch 2). Data from Table 5 reflect the fact that at all studied characteristics, at all three experimental batches, females and males recorded rather similar values. Males body weight varied between 0.4055 g (E.B.2.) and 0.4164 g (C.B.) and that of females between 0.5054 g (C.B.) and 0.5149 (E.B.1.).

Table 4

Percentage of males and females after feeding with royal jelly enriched feed

<i>Experimental batches</i>	<i>Males (%)</i>	<i>Females (%)</i>
Control batch	49.3	50.7
Experimental batch 1	29.24	70.76
Experimental batch 2	18.19	81.81

Table 5

The results regarding the standard length, maximum body depth and body weight at studied experimental batches

<i>Experimental batches</i>	<i>Standard body length (cm)</i>		<i>Maximum body depth (cm)</i>		<i>Body weight (g)</i>	
	males	females	males	females	males	females
Control batch	2.602	2.766	0.613	0.800	0.416	0.505
Experimental batch 1	2.588	2.840	0.660	0.845	0.409	0.514
Experimental batch 2	2.650	2.837	0.663	0.886	0.405	0.508

From histological preparation, at males from experimental batches were not noticed histological changes which can be attributed to powdered royal jelly. At females belonging to experimental batches 1 and 2 registered changes at the level of ovaries which can be attributed to feeding with powdered royal jelly (Figures 2-4).

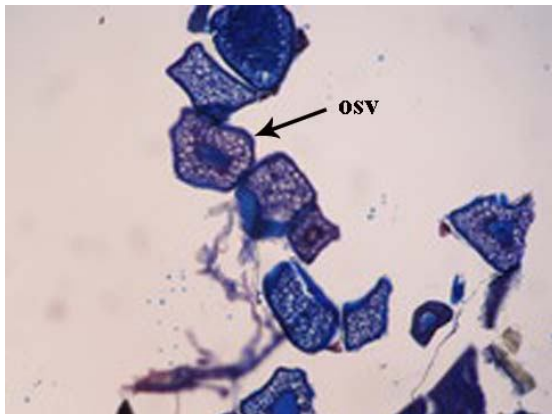


Fig. 2. Female belonging to control variant; prevail secundar ovocites vitellus (osv) (original).

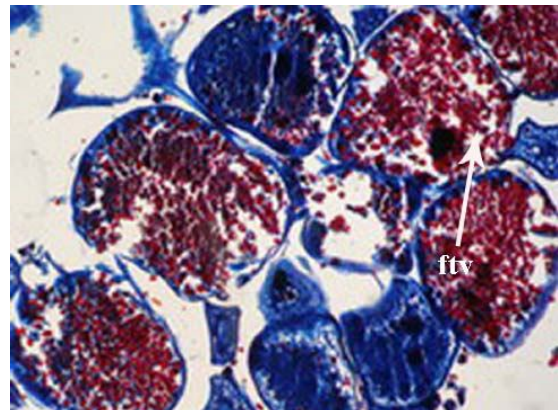


Fig.3. Female belonging the first experimental variant; prevail big follicles with tertian vitellus (ftv)(original).

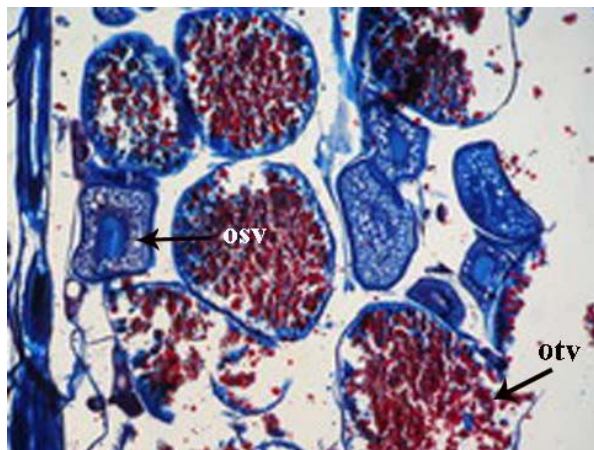


Fig.4. Females from the second experimental variant; prevail big ovocites with tertian and secondary vitellus (osv,otv) (original).

Considering that the histological examination was performed at the end of the experiment (75 days), we could conclude that the administration of royal jelly accelerated the apparition of sexual maturity in the females from the experimental groups.

Conclusions

1. Successive to the administration of lyophilized royal jelly in zebra fish feed, the number of females increased remarkably. The experimental group 1, whose feed was supplemented with 0.02 g royal jelly, recorded 70.76% females and 29.24% males, and the experimental group 2, whose feed was supplemented with double amounts, 81.81% females and 18.19% males. In the control group, the female percentage was 50.7%, and the male one was 49.3%.
2. The royal jelly administration in zebra fish feed did not influence body weight, standard length and maximal body height in any of the experimental variants, compared with the control variant; between these, there are statistically insignificant differences.
3. From a histological viewpoint, the males from the experimental variants did not present any histological changes that could be attributed to the lyophilized royal jelly intake.
4. In the case of females, in the experimental variants, the lyophilized royal jelly caused important changes at histological level, compared with the control variant. In the experimental variant 1, we could predominantly observe big follicles with tertiary vitelus, in the experimental group 2 big ovocytes with secondary and tertiary vitelus and in the control variant, we could observe ovocytes with secondary vitelus. We may conclude that the administration of royal jelly accelerated the apparition of sexual maturity in the experimental variants, compared with the control variant.
5. The lyophilized royal jelly may be successfully used in the induction of sexual inversion in zebra fish and also for the reduction of sexual maturity age in the females belonging to this species.

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Received: 10 August 2010. Accepted: 18 October 2010. Published online: 21 October 2010.

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How to cite this article:

Pătruică S., Grozea A., Dumitrescu G., Bănăţean-Dunea I., Ciochină L., Nicula M., Dăianu A., Szucs S., Boca L., 2010 Researches regarding the influence of royal jelly on sex reversal in zebra fishes (*Danio rerio*). AACL Bioflux **3**(3):255-260.