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Feed Variation and Biometrical Factors in Tilapias (*Oreochromis niloticus*) Exposed to ^{60}Co Gamma Radiation

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Abstract. Ionizing radiation has been widely used for cancer treatment and diagnostic exams in medicine, besides being used in the fields of energy generation and industry. This work aimed to verify the occurrence of biological effects of high doses of gamma radiation using biometric tests in Nile tilapia. 32 fishes were divided into four groups: a control group and three groups irradiated with different values of absorbed dose (20 Gy, 40 Gy and 80 Gy). The fish showed high resistance to the deleterious effects of gamma radiation. Future studies may prove the use of fish for monitoring radiation contaminated environments.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a fish that can be introduced outside its natural range, adults can reach 60 cm in length and up to 4.3 kg, it can live for up to 9 years. It generally feeds in shallow waters, tolerates temperatures from 8 to 42°C and can tolerate high and low salinity^[1].

The discovery of radioactivity changed the medical routine and found applications in biology, industry, agriculture, archaeology, energy generation and other areas of human activity. In this study Nile tilapia fish were irradiated with different doses of ionizing radiation.

The use of ionizing radiation demands technical and scientific knowledge, otherwise the result can be catastrophic – as Chernobyl and Fukushima nuclear accidents^[2]. Telecobalt machines are prominently used for cancer treatment; cobalt-60 is a synthetic radioactive isotope of cobalt with a half-life of 5.27 years. The use of cobalt-60 in irradiation demands the calculus of absorbed dose constant; allowing the calculation of the equivalent dose, which depends on distance and source activity^[3].

The biological reactions to ionizing radiation appear as a natural response of the body to a physical agent causing a chemical effect resulting in a biological effect. In this study fish were irradiated with different doses of ionizing radiation and it was performed biometric tests. The biometric tests can use physiology, chemistry or behavior aspects; the selection of a particular biometric test depends on extraction of relevant feature sets^[4]. In this work the biometric included weight, length and feed intake tests.

MATERIALS AND METHODS

The stock population of Nile tilapia (*Oreochromis niloticus*) was juvenile fish held in an indoor 1200 liter tank for about 2 months. The tank was supplied with constant aeration with biological filter (recirculation system) and a dechlorinated water flow. During this time, the averaged temperature was 24±1°C and the water was maintained with low levels of ammonia (<0.25 ppm) and nitrite (<0.50 ppm). The photoperiod was set from 6:00 hours to 18:00 hours. Thirty two males were chosen by body size, standard average weight of 46.8 g and length of 11.8 cm.

During the first 10 days of adaptation the fish were conditioned in a single fish aquarium with 19 liters capacity (22 x 22 x 40 cm), each aquarium was visually isolated from the others with an opaque sheeting and fish were fed two times a day with commercial feed (35% crude protein) at a food intake rate of 3% body weight. The water quality was monitored weekly and maintained in optimum condition; during the experiment the water pH ranged from 6.6 to 6.9, water-dissolved oxygen ranged from 6 mg/l to 7 mg/l, and nitrite and ammonia were kept lower than 0.5 ppm and 0.25 ppm, respectively.

The food remained available for 1 hour, then waste food and excreta were removed and the aquarium cleaned. The amount of food not ingested by the fish was calculated by drying the remaining pellets and weight in a balance. The fish were separated in four groups: a control group, no irradiated, and three other groups irradiated with 20, 40 and 80 Gy, respectively.

A telecobalt machine used for cancer treatment was used to irradiate the fish; it was calculated the required exposure time to achieve the stipulated doses and the dose was given all at one session, as a single dose. Besides the time calculation, the doses were doubled check by measuring the doses using a calibrated ionization chamber for ^{60}Co radiation energy, it was followed the protocol TRS-398 of absorbed dose determination in external beam radiotherapy published by the International Atomic Energy Agency (IAEA).

Fish were irradiated by gamma radiation in individual plastic containers with a 1.5 L capacity of water. In order to avoid different stress factor all the fish were positioned to the ^{60}Co equipment, even the ones that were not irradiated. The radiation field was set to cover the whole extension of the container and all of the radiometric parameters were set in order to ensure uniformity of the radiation field.

After been irradiated fish were weighed and measured to determine biometric parameters and then they were placed in their respective aquariums. These were observed for 30 days after being irradiated and the amount of food intake was calculated every day, furthermore every 10 days – totalizing three periods – a new biometric test (weight and length) was performed.

RESULTS AND DISCUSSION

The feed intake can be an indication of deleterious effects of radiation as tissues that have a higher proliferative capacity (such as mucous membranes of the digestive tract) can be more easily injured after being irradiated. As the amount of daily feed (3% of fish weight) on offer should be consumed, the fact of feed still remain in the aquarium is a sign that the digestive system is injured. The feed intake average for each group during the 30 days is compared in figure 1.

Considering the day 0 as being the irradiation process day it is possible to compare the control group with the other groups. The fish that received 20 Gy decreased the consumption of feed in 11.8% in the first 10 days, in 9.2% in 10-20 days and 14.3% in 20-30 days, when compared to control group. The group that received 40 Gy showed a decrease of 21.4% in 0-10 days, 46.7% in 10-20 days and 62.5% in 20-30 days when compared to control group. The group that received 80 Gy showed a 58.9% feed consumption reduction in 0-10 days, 99.9% in 10-20 days and there were no more living fish exposed to 80 Gy at day 30. On the fifth day after irradiation, the fish that received 80 Gy could not eat pellets of feed, swallowing and throwing then out; one explanation for this change of feeding behavior may be the lesions in the oral cavity after radiological exposure, commonly called mucositis, fish that received 40 Gy had also this symptom.

The body weight of groups that received 40 and 80 Gy had a significant difference within 10 days after being irradiated when compared the control group. The lethality of radiation exposure presented by the absorbed dose of the fish is presented in figure 2, 14 fish died during the experiment – 1 from 20 Gy group, 5 from 40 Gy group and 8 from 80 Gy group. In the group of fish irradiated with 40 Gy 62.5% died between the twelfth and eighteenth day, in the group of fish irradiated with 80 Gy all of the fish died between the eighth and twelfth day; there so it is suggested that the time to death is corresponding to the dose.

The biometrical tests provided data from Table 1 and 2. Table 1 compares values of mean measured weight for each group. Table 2 compares the mean body lengths for each group. The gaining of weight and length did not take the same growth rate in the irradiated groups when compared to control group.

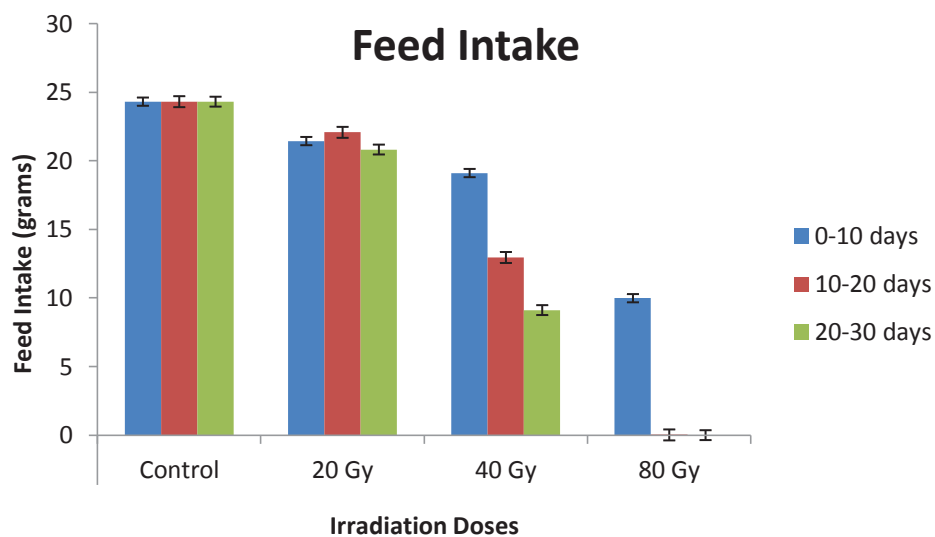


FIGURE 1. Average of feed intake for each group during the 30 days after the irradiation.

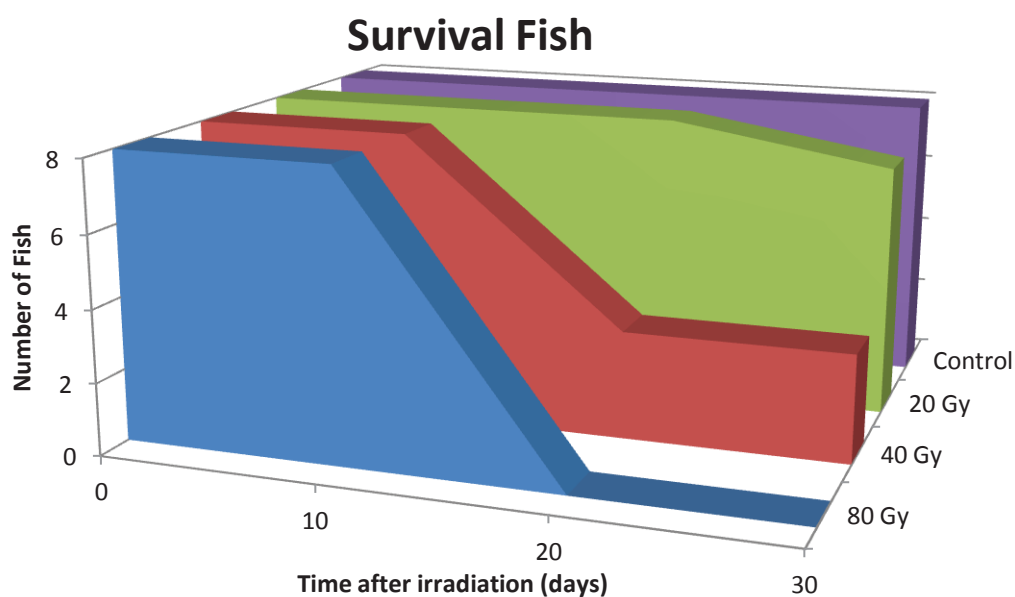


FIGURE 2. Comparison of the number of deaths for the time after irradiation, displayed by groups.

TABLE 1. Each group average weight value (g), during the experimental period. The value of standard deviation is within the brackets and “X” means that all of the fish died.

Dose	Days			
	0	10	20	30
0 Gy	49.39 (2.87)	65.19 (3.97)	70.84 (6.25)	81.35 (3.48)
20 Gy	48.86 (4.00)	59.98 (4.85)	64.61 (9.02)	73.7 (9.04)
40 Gy	47.22 (3.16)	54.89 (6.84)	60.47 (11.51)	69.07 (11.65)
80 Gy	47.49 (5.24)	50.65 (6.21)	X	X

TABLE 2. Each group average body length value (cm), during the experimental period. The value of standard deviation is within the brackets and “X” means that all of the fish died.

Dose	Days			
	0	10	20	30
0 Gy	12.01 (0.40)	12.96 (0.35)	13.74 (0.53)	14.17 (0.62)
20 Gy	11.75 (0.56)	12.73 (0.51)	13.13 (0.61)	13.61 (0.58)
40 Gy	11.63 (0.55)	12.13 (0.59)	12.73 (0.92)	13.20 (0.96)
80 Gy	11.83 (0.51)	11.90 (0.58)	X	X

During the measures from the biometrical tests it was evident the difference in the weight and size between animals from control group to irradiated ones. Tissue lesions were observed in the head, body and eyes in exposed fish (40 and 80 Gy) between the eighth and thirteenth day. Some of these lesions were cellular material growth, also the kind of color pattern characterized by vertical stripes along the body changed. In Figure 3 there are 3 pictures of mucositis, earlier tumor and lesion in fishes irradiated with 80 Gy and 40 Gy.

In Nile tilapia it was observed the occurrence of mucositis in the groups that received doses of 40 and 80 Gy. The latency period of solid tumor observation took 8 to 18 days in these fish. Two fish irradiated with 40 Gy survived and had not shown any mucositis in oral cavity. Three fish irradiated with 40 Gy died without presenting any damages at it is mucous membrane, this fact suggests that probably the deaths occurred due to an opportunistic infection as the radiation destroyed the hematopoietic stem cells of the fish.

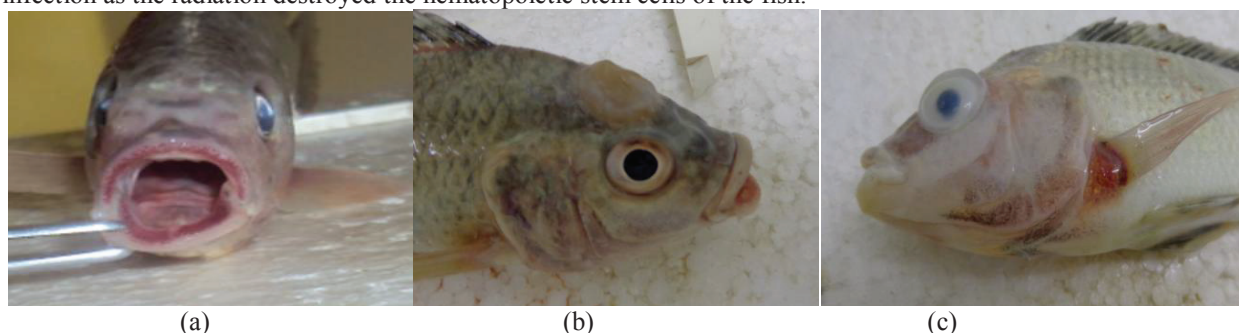


FIGURE 3. (a) Lesions in the mouth of the fish irradiated with 80 Gy in the eighth Day after irradiation, indicating mucositis, (b) Fish presenting early tumor on the eighth day after irradiation with 80 Gy and (c) Early lesion and bulging eyes apparent in a fish irradiated with 40 Gy on the thirteenth day after irradiation.

CONCLUSION

A significant difference was found between the death rate in fish irradiated with 40 and 80 Gy and the not irradiated group (control). It is known that the radiation damages tissues that have a higher proliferative capacity, as mucous membrane and hematopoietic stem cells, but it was remarkable the amount of radiation that the exposed fish were able to survive, especially when compared to the doses human can be exposed and survive – considering that mortality rate for human exposed to levels exceeding 8 Gy is 100% [5].

Probably the self regulating mechanisms in Nile tilapia can explain this surviving rate. Future studies involving effects of ionizing radiation in fishes can provide a biological model to monitoring nuclear areas, providing important information about possible radiobiological changes [6,7]. Finally, studies showing the effects of ionizing radiation exposure as stressor in fish are required as the use of nuclear energy increases.

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