Acta Veterinaria (Beograd), Vol. 59, No. 5-6, 503-511, 2009.

DOI: 10.2298/AVB0906503K UDK 619:612.014.482.4

BODY WEIGHT AND ENZYMES ACTIVITIES IN BLOOD PLASMA OF CHICKENS HATCHED FROM EGGS IRRADIATED WITH LOW LEVEL GAMMA RAYS BEFORE INCUBATION

KRALJEVIĆ P*, VILIĆ M*, MILJANIĆ S** and ŠIMPRAGA M*

*Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Croatia; **Division of Materials Chemistry, Ruđer Bošković Institute, Zagreb, Croatia

(Received 12th February 2009)

An attempt was made to determine the effect of eggs irradiation by low dose gamma radiation upon body weight (BW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) of chickens hatched from irradiated eggs. Our aim was also to investigate the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the blood plasma of those chickens.

The eggs of heavy breed chickens were irradiated with a dose of 0.15 Gy gamma radiation (^{60}Co) before incubation. Along with the chickens which were hatched from irradiated eggs, there was a control group of chickens hatched from nonirradiated eggs. All other conditions were the same for both groups of chickens. BW of chickens was measured by a single weighting of chickens on the 1st and 42nd day of the fattening period. An average BWG was calculated from the obtained results during the whole fattening period (i.e. from the 1st until the 42nd day). FC was measured each day during the fattening time and total feed consumption was calculated. On the basis of FC and BW, FCR was calculated (FC/BWG). Blood samples were taken from the right jugular vein on the 1st and 3rd day, or from the wing vein on days 5, 7, 10, 20, 30 and 42. The activity of all enzymes was determined spectrophotometrically by using reagents according to recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine.

BW of chickens hatched from irradiated eggs was statistically significant higher than in the controls during the fattening period; on day 42 of fattening, BW of the experimental chickens was 90 g (i.e. 4.8 %) higher than in the controls ($P<0.05$). FC during the fattening period did not essentially differ in the experimental and the control group. The AST activity was significantly increased in blood plasma of chickens hatched from irradiated eggs on days 3 and 10 ($P<0.05$), ALT activity was increased in the same chickens only on the 10th day ($P<0.05$). The activity of ACP in the blood plasma of the same chickens was increased on day 42 ($P<0.001$) and the activity of ALP in the blood

plasma of chickens hatched from irradiated eggs was decreased on day 42 ($P<0.001$).

The obtained results indicate that low doses of gamma radiation have a stimulative effect upon metabolic processes in chickens hatched from eggs irradiated before incubation, which is proved by increase of BWG and BW, as well as by increase of AST, ALT and ACP activities in blood plasma.

Key words: gamma radiation, low dose, chickens, body weight, blood plasma, enzymes

INTRODUCTION

Radiation is injurious to living beings, and any dose of ionizing radiation has been believed to be detrimental, even in extremely low doses. However, in literature, there are many results which have shown that low dose radiation can stimulate many physiological processes of living organisms. This includes, for example, stimulation of growth rate (Luckey, 1982), activation of immune function (Kojima *et al.,* 2000), suppression of reactive oxygen species-related diseases (Kojima *et al*., 2000a), resistance to high dose irradiation (Yonezawa *et al*., 1990), and prolongation of life span (Caratero *et al.,* 1998). The effects of irradiation of hatching eggs with x-ray or gamma ray on hatchability and performance of the chickens, have intrigued scientists since the early days of poultry industry. So, Essenberg (1935) irradiated hatching eggs, incubated for 19-243 hours, with x-ray doses varying from 0.3 to 6 Gy. Small doses (<0.8 Gy) accelerated the embryonic development process and doses above 0.8 Gy retarded the developmental process. Bless and Romanoff (1943) found that a moderate dose has a stimulating effect on the early embryonic development (earlier hatch). The uniformity in the development of the exposed embryos however was less than that in the unexposed embryos. Simon (1984) had a commercialized x-ray system to irradiate hatching-eggs (dose about 0.20 Gy) with the following effects: shortened incubation time (1-2 days), about 8% more chicks, a 4% lower mortality during rearing, and increased body weight 30-60 g after six week rearing. Zakaria (1991) investigated the effect of doses of 0.05 to 2.1 Gy gamma irradiation before incubation on hatchability and body weight of hatched broiler chickens under large-scale commercial condition, and found that gamma irradiation by doses of 0.05 to 1.6 Gy before incubation did not affect embryo development, whereas a dose of 2.1 Gy was harmful. Gerrits and Dijk (1992) irradiated broiler hatching eggs with low x-ray doses (0.04-0.17 Gy) in accordance with conditions determined by Simon (1984), and found no significant improvement on hatchability or performance of the chickens. Since literature results on effects of low doses of ionizing radiation upon growth and body weight of chickens are contradictory, in this paper we wanted to investigate the effect of a dose of 0.15 Gy of gamma radiation upon body weight (BW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR which was calculated as FC/BW) in chickens hatched from eggs irradiated before incubation. Besides, we

also investigated the effects of low dose gamma radiation upon aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), alkaline phosphatase (ALP: EC 3.1.3.1) and acid phosphatase (ACP; EC 3.1.3.2) in blood plasma of those chickens. Namely, it is a well known fact that aminotransferases and phosphatases are very important for the increase in body weight of all mammals and birds. Aminotransferases play an important role in protein metabolism because they catalyse the transfer of an amino group from amino acid to a keto acid (Coles, 1980; Guyton and Hall, 2006). Phosphatases, however, might be involved in the calcification of bones, because they act either as hydrolases in which case inorganic phosphate is liberated, or as phosphotransferases which transfer the liberated phosphate radical directly to an acceptor molecule such as sugar (Wilkinson, 1976).

MATERIALS AND METHODS

Animals

The experiments were performed on hybrid chickens of heavy Gent breed (line Cobb 500) of both sexes. The chickens were hatched from eggs irradiated before incubation (experimental group). Along with experimental chickens, there was a control group of chickens which were hatched from non-irradiated eggs. The chickens were kept in wire-cages and feed with a commercial mash produced by Poljoprerada d.d., Zagreb, Croatia, which, as well as water, was given *ad libitum.* Throughout the experimental period the temperature and relative humidity were recorded in the hen house and their values were adjusted to optimal limits for chickens of this age. The microclimate was appropriate, since the concentrations of CO_2 and NH_3 did not exceed 0.20% and 0.003%, respectively.

Irradiation and dosimetry

The eggs (n=600) were irradiated before incubation by a dose of 0.15 Gy gamma radiation from panoramic ^{60}Co source (activity about 3 PBq) at the Ruđer Bošković Institute (Zagreb, Croatia). The dose rate was about 23.84 mGy/s, and a source axis-to-egg axis distance was 3.06 m. Dosimetric measurements were performed with an ionization chamber type 2581 and a Farmer Dosimeter type 2570 (NE Technology Limited). Dose is specified as absorbed dose to water (measured in free air) (Miljanić *et al.,* 1994; Miljanić and Ranogajec-Komor, 1996).

Incubation

Irradiated and non-irradiated eggs were set in the commercial incubator Victoria (Pavia, Italy), capacity 22100 eggs for 19 days. Incubators had automatic controls of temperature (37.6 ^oC), humidity (48 % relative humidity), and an incubation rack turning each hour. Incubators were regularly checked for temperature, humidity, and incubation rack turning. On the 19th day of incubation the eggs were transferred to hatching trays located in the same incubator.

Body weight and growth

BW of chickens (n=60) was measured by a single weighing of chickens on the 1st and 42nd day of fattening. Twelve hours before weighing, the chickens were refused food and measuring was performed at the same time. An average increase of body weight (BWG) was calculated from the obtained results during the whole fattening period (from the 1st until the 42nd day). FC was measured each day during the fattening period and total feed consumption was calculated. On the basis of FC and BWG, FCR was calculated (FC/BWG). During the experiment, three chickens died, so that the final feed consumption per chicken was corrected for the amount of feed consumed by those chickens.

Samples

Blood samples were drawn from the right jugular vein on the 1st and 3rd day, or from the wing vein on 5, 7, 10, 20, 30 and 42 days of fattening. During each drawing of blood, each blood sample included 7 animals which were picked out at random. The blood was heparinized and the cells were separated from plasma by contrifugation at 2,000 x g.

Enzymatic assays

The dynamics of activity changes of AST, ALT, ALP and ACP in blood plasma was investigated using reagents according to recommendation of International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The activities were measured on the Olympus AU600 spectrophotometer (Tokyo, Japan). The temperature of the reaction was kept at 25 ^{o}C using a water bath.

Statistical analysis

Results of enzyme activities in the blood plasma were expressed as mean and standard error of mean (SE), and were statistically analysed in STATISTICA (STATSOFT, 2005) using Student's t-test whereas P value <0.05 was selected to indicate significance.

RESULTS

The results of BW, BWG, FC and FCR of chickens which were hatched from eggs irradiated with 0.15 Gy gamma rays before incubation are presented in Table 1.

An average BW of chickens after hatching did not significantly differ in the experimental and control group of chickens. At the end of fattening period, i.e. after 42 days, BW of chickens in experimental group was 90 g (i.e. 4.8%) higher than in the control group. This difference was statistically significant ($P<0.05$). In the period from 1 up to 42 days of the experiment, BWG in experimental group of chickens occurred to be for 4.97% higher than in the control group; in the experimetal group it was 1939.12±267.63 g whereas in the control group it turned to be 1847.33±169.48 g. The difference is also statistically significant at the level of reliability of 0.05. FCR in the experimental period was not statistically different between the investigated groups; in the experimetal group of chickens it was 2.07 kg, whereas in the control group it was 1.96 kg.

Table 1. Body weight (BW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) of chickens hatched from eggs irradiated with 0.15 Gy gamma rays before incubation

Days of fattening	n	BW (g) $\bar{X} \pm SD$	BWG (g) $\bar{X} \pm SD$	FC (g)	FCR (kg/kg)
1	C 60	41.62±2.64	–	–	–
	E 60	39.89±3.16	–	–	–
42	C 60	1888.95±169.40	–	–	–
	E 57	1979.01±269.63*	–	–	–
1-42	C 60		1847.33±169.48	3615.38	1.96
	E 57		1939.12±267.63*	4008.33	2.07

C = control group; E = experimental group; *P<0.05

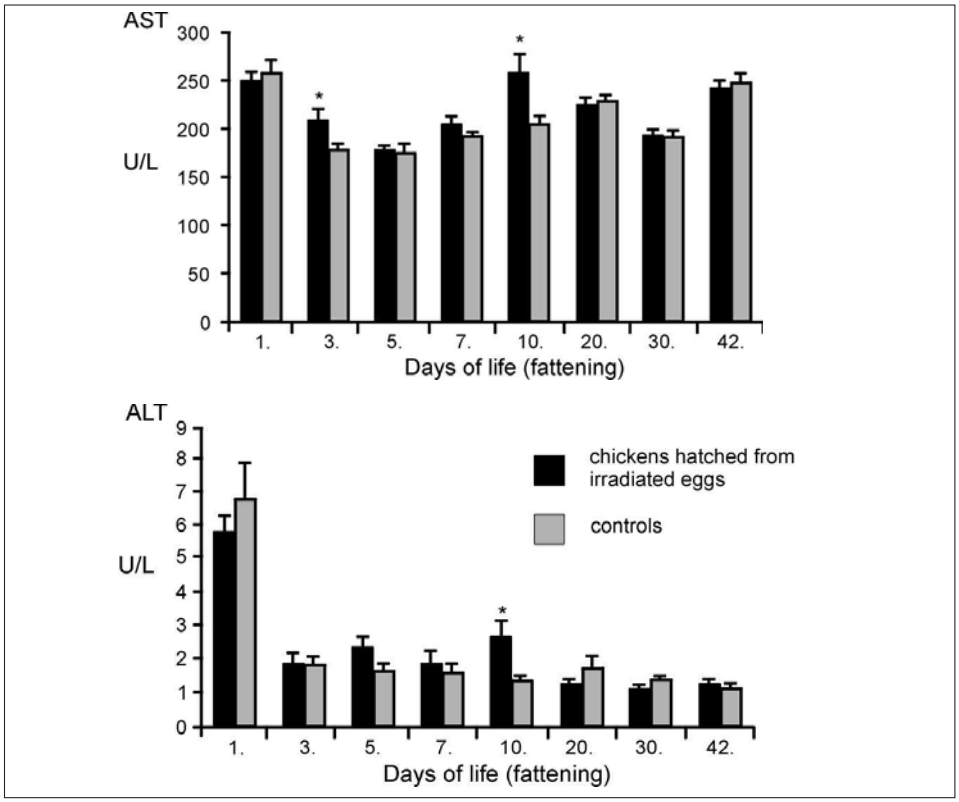

Figure 1. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in the blood plasma of chickens hatched from eggs irradiated with 0.15 Gy gamma radiation before incubation. Results are expressed as mean ± SE of 10 blood samples. Each blood sample included 7 animals picked out at random. *P<0.05.

The results of AST and ALT activity in the blood plasma of chickens which were hatched from eggs irradiated with 0.15 Gy gamma rays before incubation are presented in Figure 1.

AST activity was significantly increased in the blood plasma of chickens hatched from irradiated eggs on days 3 and 10 ($P<0.05$). ALT activity in the blood plasma of the same chickens was increased on day 10 ($P<0.05$).

The results of ACP and ALP activity in the blood plasma of chickens which were hatched from eggs irradiated with 0.15 mGy gamma rays before incubation are presented in Figure 2.

ALP activity was significantly decreased in the blood plasma of chickens hatched from irradiated eggs on day 42 ($P<0.001$) and ACP activity in the blood plasma of the same chickens was increased on day 42 ($P<0.001$).

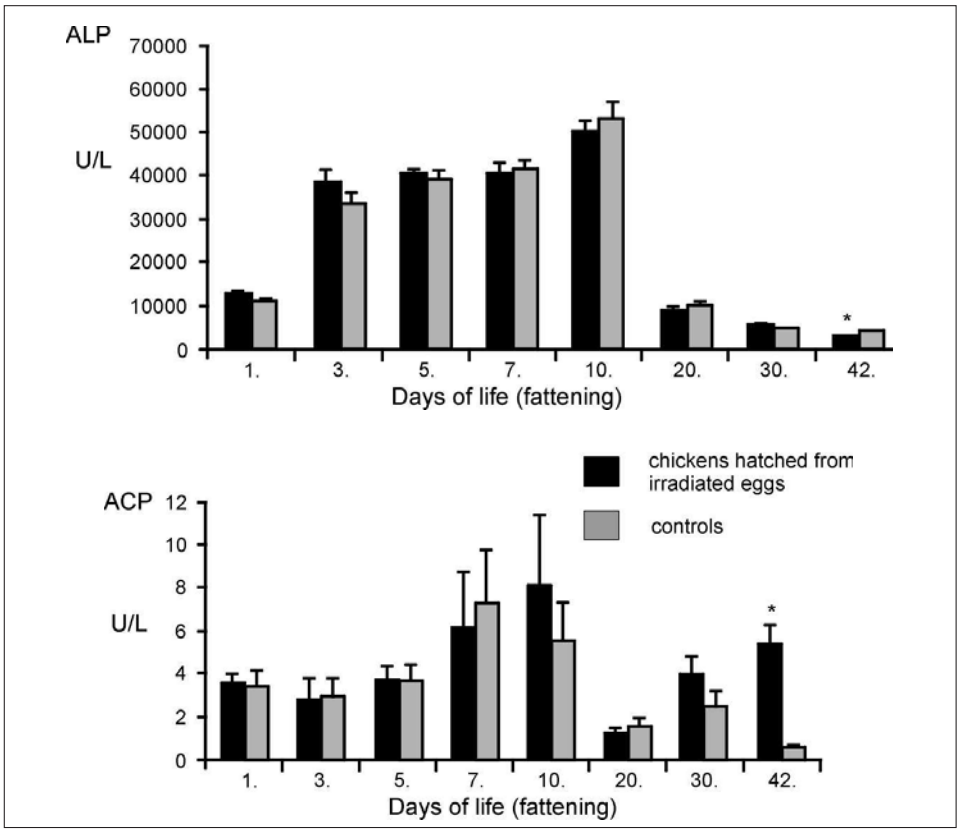

Figure 2. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activity in the blood plasma of chickens hatched from eggs irradiated with 0.15 Gy gamma radiation before incubation. Results are expressed as the mean ± standard error (SE) of 10 blood samples. Each blood sample included 7 animals which were picked out at random. *statistically significant with $P<0.05$

DISCUSSION

Obtained results indicate that BWG in chickens hatched from eggs irradiated by a dose of 0.15 Gy gamma radiation before incubation was higher during the fattening period than it was in the chickens hatched fron non-irradiated eggs. As a result of a higher BWG, BW turned to be also higher in the experimental group at the end of fattening period. At the same time, the FCR did not significantly differ between the experimental and control group. The results also indicate that irradiation of eggs of commercial broiler chickens with a dose of 0.15 Gy gamma radiation before incubation causes the increase of AST, ALT and ACP activity, as well as a decrease of ALP activity in blood plasma of chickens hatched from irradiated eggs. It is obvious that gamma irradiation of eggs before incubation with a dose of 0.15 Gy has an effect upon the metabolism of chickens hatched from irradiated eggs, which is proved by an increase of activity of most investigated enzymes and by a higher increase of BW of chickens at the end of the fattening period.

The increase of AST, ALT and ACP activity in the blood plasma of chickens hatched from irradiated eggs could be explained by the stimulating effect of low dose gamma radiation upon enzyme synthesis or upon higher cell proliferation. This hypothesis is based on the well known fact that increased enzyme activity in blood plasma, especially if the cells of different tissues are not damaged, depends on: (a) the extent of enzyme synthesis, (b) speed of cell proliferation, and (c) declaration of clearance from blood plasma (Zilva *et al*., 1992). The results of Watanabe *et al.* (2002) are in favour of the hypothesis that, in this case, cell proliferation could possibly occur. Namely, investigating the activity of extracellulary regulated kinases ½ (ERK1/2) and activity p53 protein in normal human diploid cells after irradiation of low dose 2-5 cGy x-radiation, the above mentioned authors showed that such doses stimulate proliferation of normal human diploid cells.

It is more difficult to explain the statistically significant decreases of ALP activity on the 42nd day of life, i.e. at the end of the fattening period. The fact is that ALP is widely distributed in the body, and is found in high concentrations in the bone tissue (in the osteoblasts), intestinal mucosa, renal tubule cells and liver. Each of these tissues has a distinctly different isoenzyme of ALP (Coles, 1980). In our investigations, we studied the activity of total ALP in blood plasma of chickens and we did not determine the activity of any of the isoenzymes. On the other side it is known that intensive growth of broiler chickens occurs between 35 and 42 days. In this study we found that growth of chickens hatched from irradiated eggs is enhanced and we suppose that it is due to their more intensive metabolism. This is supported by enhanced activity of AST and ALT in blood plasma of those chickens, because they participate in protein metabolism. That is why both, the growth of chickens hatched from irradiated eggs and consequently the bone growth of those chickens, ended earlier than the growth of chickens hatched from nonirradiated eggs. Therefore, there is a lower activity of ALP in blood plasma of chickens hatched from irradiated eggs on the 42nd day of life.

Our results mostly agree with the results obtained by Todorov and Drijanovski (1984). Investigating the effects of low doses of gamma radiation on the activity of AST, ALT, ALP, ACP, lactate dehydrogenase (LDH) and activity of isoenzyme LDH in blood plasma of turkeys and pheasants hatched from eggs irradiated by a dose of 0.15 Gy gamma radiation before incubation, those authors found out that all investigated enzymes were increased. The only difference between their and our results is that in their investigation they found that the ALP activity in the blood plasma of turkeys and pheasants hatched from irradiated eggs was increased, whereas in our experiments it was decreased. We suppose that this is due to different birds we use in our experiments.

In conclusion, irradiation of commercial broiler eggs before incubation with a dose of 0.15 Gy gamma radiation has a stimulative effect upon the metabolism of chickens hatched from those eggs, which is proved by increase of BWG and BW, as well as by an increase of AST, ALT and ACP activities in blood plasma.

ACKNOWLEDGEMENTS:
This investigation was financed by the Ministry of Science, High Educations and Sport of the Republic of Croatia. The authors wish to thank the Ministry for their financial support.

Address for correspondence:
Prof. dr. sc. Petar Kraljević
Zavod za fiziologiju i radiobiologiju
Veterinarski fakultet Sveučilišta u Zagrebu
Heinzelova 55
HR-10000 Zagreb
Croatia
E-mail: kraljev@vef.hr

REFERENCES

1. *Bless AA, Romanoff AL,* 1943, Stimulation of the development of the avian embryo by x-rays, *J Cell Compar Physl, 21, 117-21.*
2. *Caratero A, Courtade M, Bonnet L, Planel H, Caratero C,* 1998, Effect of a continuous gamma irradiation at a very low dose on the life span of mice. *Gerontology,* 44, 272-6.
3. *Coles HE,* 1980, Veterinary clinical Pathology, 3th ed. Saunders Company, Philadelphia-London-Toronto, 205-9.
4. *Essenberg JM*, 1935, Effect of x-rays on the incubation period, sexual development, and egg-laying in white and brown leghorn chickens, *Poult Sci,* 14, 284-93 and 317.
5. *Gerrits AR, Dijk DJ,* 1992, Effect of X-ray irradiation of hatching eggs on hatching time, hatchability and broiler weight, *Arch Geflügelk*, 56, 179-81.
6. *Guyton CA, Hall TE,* 2006, Textbook of medical Physiology, 11th ed. Elsevier Saunders, Philadelphia, 852-8.
7. *Kojima S, Matsumori S, Ishida H, Yamaoka K,* 2000, Possible role of elevation of glutathione in the acquisition of enhanced proliferation of mouse splenocytes exposed to small-dose gamma-rays, *Int J Radiat Biol,* 76, 1641-7.
8. *Kojima S, Shimomura H, Matsumori S*, 2000a, Effect of pre-irradiation with low-dose gamma-rays on chemically induced hepatotoxicity and glutathione depletion, *Anticancer Res*, 20, 1583-8.
9. *Luckey TD*, 1982, Physiological benefit from low levels of ionizing radiation, *Health Phys,* 43, 771-89.
10. *Miljanić S, Ražem D, Ranogajec-Komor M,* 1994, Dosimetric calibration of an annular ^{60}Co gamma ray source, *J Radioan Nucl Ch Ar*, 185, 101-8.
11. *Miljanić S, Ranogajec-Komor M,* 1996, Estimation of photon energy of ^{60}Co in water using CaF_2: Mn and Fricke dosimeters, *Radiat Prot Dosim*, 66, 283-8.

12. *Statsoft*, 2005, Statistica (data analysis software system). Version 7.1.
13. *Simon J*, 1984, cit. according *Gerrits AR, Dijk DJ,* 1992, Effect of X-ray irradiation of hatching eggs on hatching time, hatchability and broiler weight, *Arch Geflügelk* 56, 179-81.
14. *Todorov B, Drijanovski P*, 1984, Enzyme activity in blood plasma of turkey and pheasant received from irradiated with small doses gamma-rays eggs. Final programme and book of abstracts of XVth annual meeting of European Society of Nuclear Methods in Agriculture. Piacenza, European Society of Nuclear Methods in Agriculture.
15. *Watanabe M, Suzuki K, Kodama S,* 2002, Specific gene expression by extremely low-dose ionizing radiation which related to enhance proliferation of normal human diploid cells, *International Congress Series,* 1236, 237-9.
16. *Wilkinson JH,* 1976, The principles and practice of diagnostic enzymology, Edward Arnold, London, 129-44.
17. *Yonezawa M, Takeda A, Misonoh J,* 1990, Acquired radioresistance after low dose X-irradiation in mice, *J Radiat Res*, 3, 256-62.
18. *Zakaria AH,* 1991, Effect of low doses of gamma irradiation prior to egg incubation on hatchability and body weight of broiler chickens, *Brit Poultry Sci*, 32, 103-7.
19. *Zilva JF, Pannall PR, Mayne PD,* 1992, Clinical chemistry in diagnostic and therapy [in Croatian]. 3rd ed. Školska knjiga, Zagreb, 307.

TJELESNA MASA I AKTIVNOSTI ENZIMA U KRVNOJ PLAZMI PILIĆA IZLEŽENIH IZ JAJA OZRAČENIH MALOM DOZOM GAMA-ZRAČENJA PRIJE INKUBACIJE

KRALJEVIĆ P, VILIĆ M, MILJANIĆ SAVETA i ŠIMPRAGA M

SADRŽAJ

U ovome se radu želelo istražiti učinak ozračivanja jaja malom dozom gama-zračenja na tjelesnu težinu (BW), prirast (BWG), potrošak hrane (FC) i konverziju (FCR) u pilića izleženih iz ozračenih jaja. Cilj istraživanja također je bio istražiti aktivnost aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), kisele fosfataze (ACP) i alkalne fosfataze (ALP) u krvnoj plazmi tih pilića.

Jaja teške pasmine pilića ozračena su dozom od 0,15 Gy gama-zračenja prije inkubacije. Tjelesna masa pilića mjerena je pojedinačnim vaganjem pilića 1. i 42. dana tova. Prosječni prirast je izračunat iz rezultata dobivenim tijekom čitavoga razdoblja tova. Konzumacija hrane je mjerena svaki dan tijekom tova i izračunat je ukupni potrošak hrane. Konverzija hrane je izračunata na temelju potroška hrane i tjelesne mase (FC/BWG). Uzorci krvi za analizu enzima vađeni su 1. i 3. dana tova iz jugularne vene, a 5, 7, 10, 20, 30. i 42. dana tova iz krilne vene. Aktivnost svih enzima određivana je spektrofotometrijski koristeći reagense u skladu s preporukama Međunarodne federacije za kliničku kemiju i laboratorijsku medicinu (IFCC).

Tjelesna masa pilića izleženih iz ozračenih jaja bila je tijekom tova statistički značajno veća nego u kontrola ($P<0,05$). Aktivnost AST značajno je porasla u krvnoj plazmi pilića izleženih iz ozračenih jaja 3. i 10. dana tova ($P<0,05$). Aktivnost ALT bila je povećana 10. dana tova ($P<0,05$), a aktivnost ACP porasla je 42. dana tova ($P<0,001$).

Dobiveni rezultati pokazuju da male doze gama-zračenja imaju stimulacijski učinak na metaboličke procese u pilića izleženih iz jaja ozračenih prije inkubacije.