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## Concentration of proteins and protein fractions in blood plasma of chickens hatched from eggs irradiated with low dose gamma radiation

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### ABSTRACT

This study was performed to investigate the effect of egg irradiation by low dose gamma rays on the concentration of total proteins and protein fractions in the blood plasma of chickens hatched from irradiated eggs. The eggs of heavy breed chickens were irradiated with a dose of 0.15 Gy gamma-rays (<sup>60</sup>Co) before incubation. Blood samples were taken from the right jugular vein on the 1<sup>st</sup> and 3<sup>rd</sup> days, or from the wing vein on day 7 after hatching. The total protein concentrations in the blood plasma was determined by the biuret method using Boehringer Mannheim GmbH optimized kits. The protein fractions (albumin,  $\alpha$ 1-globulin,  $\alpha$ 2-globulin,  $\beta$ - and  $\gamma$ - globulins) were estimated electrophoretically on cellulose acetate strips. The total protein concentration was significantly decreased in the blood plasma of chickens hatched from irradiated eggs on days 3 ( $P<0.05$ ) and 7 ( $P<0.001$ ). The albumin concentration in the blood plasma of chickens hatched from irradiated eggs was significantly decreased on the 7<sup>th</sup> day ( $P<0.001$ ), and the concentration of  $\alpha$ 2-globulin was decreased on days 1 ( $P<0.05$ ) and 7 ( $P<0.001$ ).  $\beta$ - and  $\gamma$ -globulins concentration was significantly decreased on the 7<sup>th</sup> day of life ( $P<0.05$ ). The obtained results indicate that a low dose of gamma radiation has an inhibitory effect upon the concentration of total proteins and protein fractions in the blood plasma of chickens hatched from irradiated eggs before incubation, during the first week of their life.

**Key words:** gamma radiation, low dose, chickens, blood plasma, total proteins, protein fractions

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## Introduction

Radiation is injurious to living beings, and any dose of ionizing radiation is 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 in living organisms. This includes, for example, stimulation of immunological functions (LIU et al., 1987; KOJIMA et al., 2000; SHIN et al., 2010) growth rate (LUCKEY, 1982; SUZUKI et al., 2001; LIANG et al., 2011), adaptive response (MATSUMOTO et al., 2007) and prolongation of life span (CARATERO et al., 1998; INA and SAKAI, 2004; LACOSTE-COLLIN et al., 2007).

In our earlier paper (KRALJEVIĆ et al., 2009) we showed that irradiation of commercial broiler parent stock hatching eggs immediately before incubation with a dose of 0.15 Gy gamma radiation led to an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the blood plasma of the chickens hatched from those eggs, during the first 10 days of life. Since the aminotransferase activities in the blood plasma of the chickens hatched from eggs irradiated with low level gamma rays before incubation was increased, one may presume that the concentration of serum total proteins and protein fractions of those chickens may be also increased. Namely, it is well known that aminotransferases play an important role in protein metabolism because they catalyse the transfer of an amino group from amino acid to keto acid (GUYTON and HALL, 2006). On the other hand, it is also well known that protein concentration increases in chickens after hatching (PATTERSON, 1962; HUANG et al., 2006).

Therefore, in this paper we wanted to investigate the concentration of total proteins and protein fractions in the blood plasma of chickens hatched from eggs irradiated before incubation, with a dose of 0.15 Gy of gamma radiation, during the first week of life.

## Materials and methods

*Animals.* The experiment was performed on hybrid chickens of the 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 hatched from non-irradiated eggs. The chickens were kept in wire-cages with latticed floors, covered with cardboard during the first three days after hatching. 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 chickens were fed with a commercial mash produced by Poljoprerada d.d. Zagreb, Croatia, which, as well as water, was given *ad libitum*.

*Irradiation and dosimetry.* The eggs (n = 600) were irradiated before incubation with a dose of 0.15 Gy gamma radiation from a panoramic <sup>60</sup>Co source (activity about 3 PBq) at the Ruđer Bošković Institute (Zagreb, Croatia). The dose rate was about 23.84 mGy/s,

and the 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). The dose is specified as the 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 a commercial incubator, Vistoria (Pavia, Italy), capacity 22100 eggs for 19 days. The incubators had automatic controls of temperature (37.6 °C), and humidity (48% relative humidity), and an incubation rack, turning each hour. On the 19<sup>th</sup> day of incubation the eggs were transferred to hatching trays located in the same incubator.

*Samples.* Blood samples were drawn from the right jugular vein on the 1<sup>st</sup> and 3<sup>rd</sup> day, or from the wing vein on the 7<sup>th</sup> day of life. During each drawing of blood, each blood sample included 10 birds, picked out at random. The blood was heparinized and the cells were separated from plasma by centrifugation at 2,000 x g.

*Total proteins and protein fraction analyses.* Total proteins and the protein fractions, i.e. albumin,  $\alpha$ 1-globulin,  $\alpha$ 2-globulin,  $\beta$ - and  $\gamma$ -globulins, were estimated in the blood plasma. Total protein concentrations were analyzed spectrophotometrically on a Helios Delta Vis spectrophotometer (Thermo Spectronic, Cambridge, UK) by the biuret method, using a commercial kit (Boehringer Mannheim GmbH, Mannheim, Germany). The concentration of protein fractions was estimated electrophoretically on cellulose acetate strips (Celloge<sup>l</sup>®, MALTA Chemetron, Milan, Italy) and determined by Global-scan densitometer (MALTA Chemetron, Milan, Italy).

*Statistical analysis.* Results of concentration of total proteins and protein fractions in the blood plasma were expressed as mean  $\pm$  standard error of mean (SE) and the significance of differences between the control and irradiated groups was analyzed using STATISTICA (STATSOFT, 2007). After testing for normal distribution (Kolmogorov-Smirnov test of normality), the results were tested by Student's *t*-test where a P value <0.05 was selected to indicate significance.

## Results

The results of the concentration of total proteins and albumins in the blood plasma of chickens, hatched from eggs irradiated with 0.15 Gy gamma rays before incubation, are presented in Fig. 1.

The concentration of total proteins was significantly decreased in the blood plasma of chickens hatched from irradiated eggs on days 3 ( $P < 0.05$ ) and 7 ( $P < 0.001$ ) compared to the control group. The level of albumin in the blood plasma of chickens hatched from irradiated eggs was significantly decreased on day 7 ( $P < 0.001$ ) compared to the control group.

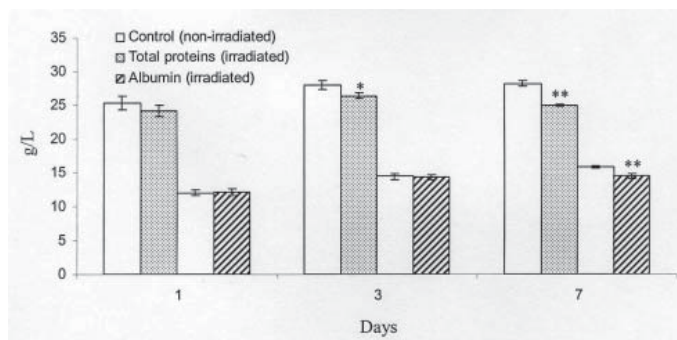


Fig. 1. Total proteins and albumin concentration (g/L) in the blood plasma of chickens hatched from non-irradiated eggs (open bars) and chickens hatched from irradiated eggs with 0.15 Gy gamma rays before incubation (hatched bars). Results are expressed as mean  $\pm$  SE of 10 blood samples. Each blood samples included 10 animals picked at random. \* $P < 0.05$ ; \*\* $P < 0.01$ .

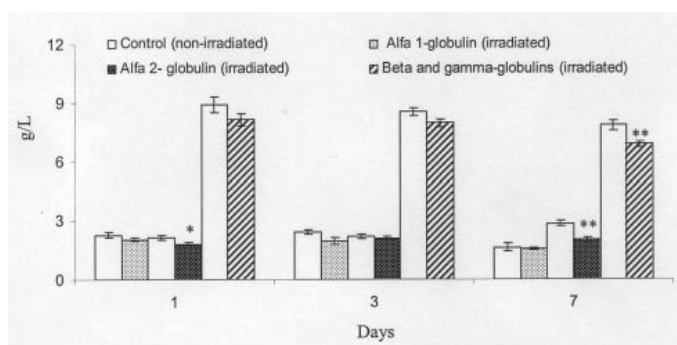


Fig. 2.  $\alpha$ 1-globulin,  $\alpha$ 2-globulin,  $\beta$ - and  $\gamma$ -globulins concentrations (g/L) in the blood plasma of chickens hatched from non-irradiated eggs (open bars) and chickens hatched from irradiated eggs with 0.15 Gy gamma rays before incubation (hatched bars). Results are expressed as mean  $\pm$  SE of 10 blood samples. Each blood sample included 10 animals picked at random. \* $P < 0.05$ ; \*\* $P < 0.01$ .

The results of the concentration of  $\alpha$ 1-globulins,  $\alpha$ 2-globulins and  $\beta$ - and  $\gamma$ -globulins in the blood plasma of chickens hatched from eggs irradiated with 0.15 Gy gamma rays before incubation are presented in Fig. 2.

The concentration of  $\alpha$ 2-globulins in the blood plasma of chickens hatched from irradiated eggs was decreased on day 1 ( $P < 0.05$ ) and 7 ( $P < 0.001$ ) compared to the control group, and the concentration of  $\beta$ - and  $\gamma$ -globulins was significantly decreased in the blood plasma of the same chickens on day 7 ( $P < 0.05$ ).

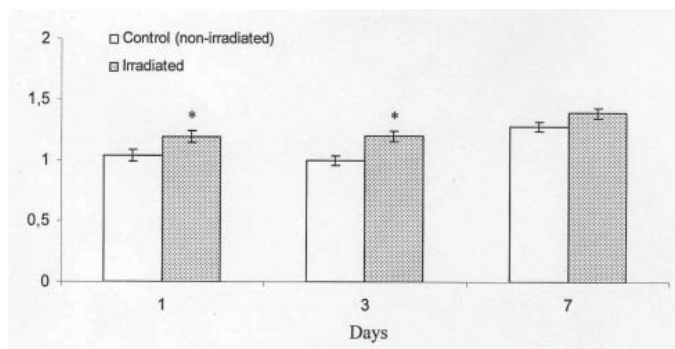


Fig. 3. Albumin/globulin ratio in the blood plasma of chickens hatched from non-irradiated eggs (open bars) and chickens hatched from irradiated eggs with 0.15 Gy gamma rays before incubation (hatched bars). Results are expressed as mean  $\pm$  SE of 10 blood samples. Each blood samples included 10 animals picked at random. \* $P < 0.05$ .

The results of the albumin/globulin ratio in the blood plasma of chickens hatched from eggs irradiated with 0.15 Gy gamma rays before incubation are presented in Fig. 3.

The albumin/globulin ratio in the blood plasma of chickens hatched from irradiated eggs was increased on day 1 ( $P < 0.05$ ) and 3 ( $P < 0.05$ ) compared to the control group.

### Discussion

The obtained results indicate that the concentration of total proteins and protein fractions in the blood plasma of chickens hatched from eggs irradiated with a dose of 0.15 Gy gamma-rays before incubation was decreased during the first week of life, compared to the chickens hatched from non-irradiated eggs.

These results differ from the results referring to AST and ALT activity in the blood plasma of the same chickens during the first seven days of life (KRALJEVIĆ et al., 2009). Namely, ALT activity in the blood plasma of chickens hatched from eggs irradiated with a dose of 0.15 Gy gamma radiation before incubation did not change during the first week, while the AST was significantly increased only on the 3<sup>rd</sup> day of life.

On the other hand, when comparing the concentration of total proteins with the concentration of protein fractions in the blood plasma of the same chickens, it could be seen that the concentrations of albumin and globulins were also decreased during the first 7 days of life. This decrease in the concentrations of albumin and globulins in the blood plasma may probably cause the decrease in the concentration of total proteins in the blood plasma of chickens. Namely, these two protein fractions (albumin and globulins)

constitute most of the total serum protein concentration in the plasma of normal animals and chickens (PATTERSON et al., 1962). However, the statistically significant decrease in the concentrations of albumin and globulins during the first week of life might be due to the reduction in the synthesis of protein fractions, especially albumin, in the liver, caused by the inhibitory effect of low dose gamma rays upon the liver. This hypothesis is based on the same data obtained by HEIM and SCHECHTMAN (1954) and STURKIE (1986), who showed that the function of the liver, without regard to how much it is morphologically developed, is not completely established until the 10<sup>th</sup> day of its embryonic development. Therefore, the possibility cannot be excluded that the irradiation of eggs by low dose gamma rays at the beginning of embryonic development affected liver function in the first week of life, in the sense of decreasing its anabolic activity. Namely, it is well known that the increase in body mass is rapid during the post-hatching and juvenile stage (STURKIE, 1986) and that chick livers increase the synthesis of serum proteins at these stages (PATTERSON et al., 1962, GRABOWSKI, 1966; FILIPOVIĆ et al., 2007). For example, GRABOWSKI (1966) showed that the protein content of blood serum rises very slowly during chick embryo development and more rapidly after hatching; at day 4 after hatching the concentration of proteins in chick serum is approximately one-fourth higher compared to the level on hatching day. On the other hand, PATTERSON et al. (1962) showed that a chick embryo two days before hatching has fewer albumins than globulins, while a two-day-old chick shows an increasing amount of serum albumin. Furthermore, to evaluate metabolic status and health, apart from the concentration of protein fractions, it is important to know the albumin/globulin ratio (A/G) (KANNEKO, 2008). Namely, often the total protein concentration is within physiological range, whereas the A/G ratio is changed. Therefore the A/G ratio also has great clinical significance. According to our results, where the total protein concentration was decreased while the A/G ratio was increased, it may be concluded that the level of gamma fractions, which includes immunoglobulins and those synthesized in the cells of the reticuloendothelial system, was increased. This is consistent with the results of antibody titer against the Newcastle disease virus in chickens, after exposure to low dose ionizing radiation. Namely, VILIĆ et al. (2009) showed that egg exposure to low dose radiation before incubation and on the 19<sup>th</sup> day of incubation could enhance maternal antibody titer against the Newcastle disease virus (NDV) in newly hatched chicks, as well as antibody titer synthesis in chickens after vaccination. In our study, all the chicks were also vaccinated against NDV on the first day after hatching with a commercial Newcastle disease vaccine (Pestikal<sup>®</sup> La Sota SPF, Veterina d.o.o., Zagreb, Croatia) by nebulisation. Therefore it is not excluded that the increased antibody transport from the yolk sac into embryo circulation during embryonic development, or the direct effect of chicken B-cell development after vaccination on the first day may enhance the A/G ratio in chickens hatched from eggs irradiated before incubation. Finally, we suppose

that egg irradiation before incubation could be responsible for the increased A/G ratio, as well as decreased levels of total protein and albumin at the same time.

In conclusion, irradiation of commercial broiler eggs before incubation with a dose of 0.15 Gy gamma-rays caused a decrease in the concentration of total proteins and protein fraction in the blood plasma of chickens hatched from those eggs, during the first week of life. This decrease in the concentration of total proteins and protein fractions, especially alpha fraction, may be a result of the inhibitory effect of the dose of gamma rays used before incubation upon the anabolic activity of the liver, but it seems to have a stimulative effect on humoral immunity in chickens.

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**VILIĆ, M., P. KRALJEVIĆ, I. ŽURA ŽAJA, J. PEJAKOVIĆ HLEDE, S. MILJANIĆ, M. ŠIMPRAGA: Koncentracija bjelančevina i bjelančevinskih frakcija u krvnoj plazmi pilića izleženih iz jaja ozračenih malom dozom gama-zračenja. Vet. arhiv 84, 401-409, 2014.**

**SAŽETAK**

U našem prethodnome radu pokazano je da se u krvnoj plazmi pilića, izleženih iz jaja ozračenih dozom od 0,15 Gy gama-zračenja prije inkubacije, povećava aktivnost aspartat-aminotransferaze i alanin-aminotransferaze tj. dvaju enzima koji imaju važnu ulogu u metabolizmu bjelančevina. Stoga je u ovom radu istražen učinak ozračivanja jaja dozom od 0,15 Gy gama-zračenja prije inkubacije na koncentraciju ukupnih bjelančevina i bjelančevinskih frakcija u krvnoj plazmi pilića izleženih iz ozračenih jaja. Jaja teške pasmine pilića ozračena su dozom od 0,15 Gy gama-zračenja iz radioaktivnog izvora kobalt-60 (<sup>60</sup>Co) prije inkubacije. Krv za analizu vađena je iz desne jugularne vene u dobi od prvog i trećeg dana, te iz krilne vene 7. dana. Koncentracija ukupnih bjelančevina određivana je u skupnom uzorku krvne plazme od 10 pilića uzetim slučajnim odabirom, biuret-metodom na spektrofotometru rabeći gotove kompletne reagencije proizvođača Boehringer Mannheim GmbH. Bjelančevinske frakcije (albumini,  $\alpha_1$  globulini,  $\alpha_2$  globulini te  $\beta$ - i  $\gamma$ -globulini) određivane su elektroforezom na trakicama geliranog celuloznog acetata. Koncentracija ukupnih bjelančevina u krvnoj plazmi pilića izleženih iz ozračenih jaja bila je statistički značajno smanjena trećeg ( $P < 0,05$ ) i 7. dana života ( $P < 0,001$ ). Koncentracija albumina u krvnoj plazmi pilića izleženih iz ozračenih jaja bila je značajno smanjena 7. dana ( $P < 0,001$ ), a koncentracija  $\alpha_2$  globulina bila je smanjena prvog ( $P < 0,05$ ) i 7. dana starosti ( $P < 0,001$ ). Koncentracija  $\beta$ - i  $\gamma$ -globulina u krvnoj plazmi pilića izleženih iz ozračenih jaja bila je značajno smanjena 7. dana života ( $P < 0,05$ ). Dobiveni rezultati pokazuju da male doze gama-zračenja imaju inhibicijski učinak na koncentraciju ukupnih bjelančevina i bjelančevinskih frakcija u krvnoj plazmi pilića, izleženih iz ozračenih jaja prije inkubacije, tijekom prvih sedam dana života.

**Ključne riječi:** ionizacijsko zračenje, mala doza, tovni pilići, ukupne bjelančevine, bjelančevinske frakcije

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