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Effects of Low Dose Gamma Radiation on Plasma Proteins in Chickens Hatched from Eggs Irradiated before Incubation

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ABSTRACT

Introduction: Biological effects after a single prenatal exposure to ionizing radiation, reflecting significant heterogeneities in the responses in different species with respect to radiation type, dose, dose rate and time of exposure. Moreover, current knowledge and obtained results for poultry exposed to low dose ionizing radiation are inconsistent and almost lacking at present. The aim of this study was to determine the effect of low dose gamma radiation on protein profile in blood of chickens irradiated *in ovo*.

Material and Methods: Fertilized chicken eggs in the experimental group were exposed to 0.3 Gy gamma radiation one hour before incubation, and control group was sham irradiated. Blood samples were taken on 1st, 3rd, 5th, 7th and 10th days of life. The plasma proteins fractions were separated by electrophoresis, while total protein and albumin levels were determined using the spectrophotometric method.

Results: The obtained differences between control and experimental groups for total protein and albumin concentrations were statistically nonsignificant during our research. Total globulins were increased 10th day of chick's life due to an increase in alpha globulins ($P < 0.05$). In contrast, gamma globulins were decreased in one-day-old chickens exposed to ionizing radiation ($P < 0.05$).

Conclusion: Our study indicates a significant effect of low-dose ionizing radiation on protein synthesis after *in ovo* exposure, although more research is needed to determine underlying molecular mechanisms triggered by low-dose gamma radiation.

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Introduction

It is well known that ionizing radiation, at any dose, can cause biological damage in both, human and non-human species. As the use of ionizing radiation in medicine and industry increases, so does the concerns for human and animal health. The contradictory findings from low dose / low dose rate radiation studies fuel scientific debate [1, 2], while cross-species data extrapolation remains a challenge due to complex biological responses and various radiosensitivity in different species. The main research challenge is that low dose radiation effects are subtle and not easy to differentiate from those triggered by other cellular stressors [3]. Moreover, the effects of exposure to various radiation sources (background radiation, diagnostic exposure, nuclear fallout) strongly depends on different factors, such as type of radiation, total dose, dose rate, tissue exposed, interval over which dose is received, or on efficiency of repairing mechanisms [4]. It is also known that radiation-induced biological effects differ among species and strains [5]. Mammals are considered the most radiosensitive, followed by birds, fish, amphibians, reptiles, crustaceans, insects, and more primitive organisms [6]. Early developmental stages are identified as highly radiosensitive in general, but the effect strongly depends on the stage of embryo/foetus development [7]. Over the last few decades researchers are focused at providing answers what radiation effects are associated with specific low dose, and whether is there a coherence of effects across different species [8]. However, commonly used small laboratory animals do not represent the genetic diversity of non-laboratory animals and human populations, thus it remains to be seen what health outcomes will be observed in other species and strains in response to low doses. The radioecological studies conducted after major nuclear accidents revealed that animals may be exposed to a higher degree than humans depending on their activities in contaminated area [8, 9]. All above underscores the need to study radiation responses in different taxonomic and developmental models, which may have a profound effect on many aspects of radiation protection, including global environment and biodiversity protection.

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International organisations such as the International Commission on Radiological Protection and the United Nations Scientific Committee on the Effects of Atomic Radiation considers low doses to be those of 0.1 Gy and below when considering cancer risks in humans, and 0.5 Gy and below when considering non-cancer diseases, while dose rates are defined as dose rates below 0.1 mGy/min [10]. Bearing in mind that radiosensitivity varies greatly between species, the meaning of "low dose" radiation should be considered as species-specific [11]. Although some agencies and researchers use 0.1 Gy as the upper limit for low dose radiation, many cellular, animal, and human studies reported biopositive (hormetic) effect for up to five times higher doses under the same title [12, 13]. Doses below 0.5 Gy can, for example, stimulate homeostatic control systems [14], reduce inflammation [15], boost immunity [16], prolong lifespan [17] or promote oxidative stress damage removal [18]. Nonetheless, there are very few published results of the low dose radiation effects in poultry. Despite all the inconsistency and lack of data for poultry, previous studies have shown that ionizing radiation at low doses can increase chicken (*Gallus gallus domesticus*) growth and hatchability [19], change enzymes activity in blood [20], or trigger various changes in metabolism of calcium, phosphorus, glucose, and cholesterol [21]. Moreover, in chickens hatched from irradiated eggs, the 0.3 Gy dose can stimulate the immune response [22], change the number/ratio of white blood cells [23], and alter antioxidative defence [24, 25].

Priorities for improving radiation biology studies include developing biomarkers for radiation-induced health effects [3]. Recent study revealed that multiple plasma proteins are altered in response to doses up to 0.5 Gy, indicating plasma proteins as useful biomarker for radiation effects following low dose exposure in mammals [26]. Obviously, the plasma protein concentrations are important parameter for health assessment in veterinary medicine considering protein roles in inflammatory and immune reaction, as well in the process of healing and tissue repair. Chicken plasma proteins have many similarities with those of mammals, but also several differences, like the widespread presence of prealbumin, the lowest concentration of gamma globulin, or more marked response to inflammatory stimuli in the beta globulin field [27]. To our knowledge, no prior studies have investigated gamma radiation effect on chicken plasma proteins for doses above 0.15 Gy, thus the aim of this study was to evaluate the acute effect of 0.3 Gy dose gamma radiation on plasma protein levels in chickens irradiated before incubation.

Materials and Methods

Ethics committee approval

All procedures with animals were conducted according to relevant law and institutional policies. Approval for conducting the experimental study was issued by the Ethics Committee of the Veterinary Faculty, University of Zagreb, under reference number: 251-61-01/139-15-19.

Eggs and irradiation

Fertilized chicken eggs (N=700) from the meat-producing Ross 308 line were purchased from the Valipile d.o.o. hatchery located in Sesvetski Kraljevac, Croatia. One hour prior to incubation, the experimental group of 360 fertile chicken eggs was exposed to 0.3 Gy gamma radiation from the panoramic cobalt-60 source (Ruđer Bošković Institute, Zagreb, Croatia). The control group of 340 eggs was subjected to a simulated irradiation (sham irradiation). A detailed description of the source and dose distribution in the irradiation chamber is published elsewhere [28]. The dose rate during irradiation was approximately 23.84 mGy/s. The source-to-egg distance was maintained at 291 cm. Farmer-type 2581 ionization chamber and a Farmer dosimeter (model 2570) from NE Technology Limited were used for dosimetry calibration and transit dose measurements. The dose is expressed as absorbed dose to water. Following irradiation, the eggs were transported to the hatchery (Valipile d.o.o., Croatia) and set to an automatic incubator (Victoria s.r.l., Pavia, Italy). Throughout the study, temperature, lighting, and humidity were monitored and maintained within precise parameters in compliance with established standards and regulations. On day 19, the eggs were moved from the incubator to hatching trays. After hatching, chicks of both sexes were transferred to the Veterinary Faculty at the University of Zagreb, Croatia, where they were housed in a controlled environment and under continuous veterinary supervision until the end of the study. Water and commercial feed were available *ad libitum*, in accordance with the chicks' growth and development stage.

Sampling and blood analysis

For the plasma proteins analysis blood samples (N=10) were taken from the right jugular vein on 1st, 3rd, 5th, 7th and 10th day after hatching. Spectrophotometric estimation of total protein and albumin levels was carried using Olympus AU 600 biochemical analyzer (Olympus Diagnostica GMBH), and Herbos dijagnostika d.o.o. (Sisak, Croatia) optimized kits. The protein fractions (albumin, alpha-, beta- and gamma-globulins) were estimated electrophoretically on cellulose acetate strips (Cellogel®, MALTA Chemetron, Milan, Italy) and determined by Global-scan densitometer (MALTA Chemetron, Milan, Italy).

Statistical analysis

Statistical analysis was conducted using the SAS 9.4 statistical software (Statistical Analysis Software 2002-2012, SAS Institute Inc., Cary, USA). Shapiro-Wilk test was used to check normality of variables. Independent testing of samples between the groups was performed using the Student's t-test for each day separately. Results are presented as mean values and 95% confidence intervals. The level of statistical significance was set at P<0.05.

Results

Concentration of total proteins, albumin, total globulins, and the albumin to globulin (A/G) ratio in the blood plasma of chickens hatched from eggs irradiated before incubation at a dose of 0.3 Gy gamma radiation, and in the blood plasma of chickens hatched from non-irradiated eggs are presented in Table 1, while concentration of globulin fractions are presented in Table 2. Plasma total protein and albumin levels in blood of chickens hatched from irradiated eggs revealed a slow increase in the first week of chick's life, but the differences between control and experimental groups were statistically insignificant during the whole study period. In response to gamma radiation, a significant increase in plasma globulins ($P < 0.05$) was found on the 10th day of chick life. On the same day, the experimental group had the highest mean value of total globulins 14.42 g/L with a 95% confidence interval of the mean (95% CI) 13.33-15.51. In the control group the highest total globulins value was found in one-day-old chicks, with mean value 14.96 g/L (95% CI:13.87-16.05). Concentrations of alpha globulins in both groups of chickens revealed a tendency of the gradual increase until the last day of the experiment, while the concentration of alpha globulins was significantly increased ($P < 0.05$) only on the 10th day of chick life. The mean values of beta-globulins in both groups showed a tendency of the gradual increase during the experiment, ranged from 2.62 g/L (95% CI:2.33-2.95) to 3.53 (95% CI:3.23-3.85) in the control group, and from 2.81 g/L (95% CI: 2.52-3.14) to 3.10 g/L (95% CI:2.80-3.42) in the experimental group.

Table 1. Concentration of total proteins, albumin, total globulins, and the albumin to globulin ratio (A/G) in the blood plasma of chickens hatched from eggs irradiated before incubation at a dose of 0.3 Gy gamma radiation (experimental group) and in the blood plasma of chickens hatched from non-irradiated eggs (control group)

		MEAN VALUE (95% confidence interval)				
		Age (days)				
Parameter	Group	1	3	5	7	10
Total protein (g/L)	C	28.90 (27.00-30.80)	29.00 (27.10-30.90)	27.70 (25.80-29.60)	28.60 (26.70-30.50)	27.90 (26.00-29.80)
	E	26,70 (24.80-28.60)	27.90 (26.00-29.80)	27.30 25.40-29.20)	29.60 (27.70-31.50)	29.40 (27.50-31.30)
Albumin (g/L)	C	13.94 (12.94-15.02)	14.29 (13.28-15.36)	14.54 (13.54-15.62)	15.44 (14.43-16.51)	15.49 (14.49-16.57)
	E	12.80 (11.80-13.88)	13.62 (12.62-14.70)	14.21 (13.20-15.28)	15.80 (14.79-16.87)	14.98 (13.98-16.06)
Total globulin (g/L)	C	14.96 (13.87-16.05)	14.71 (13.63-15.80)	13.16 (12.07-14.25)	13.16 (12.07-14.25)	12.41 (11.32-13.50)
	E	13.90 (12.81-14.99)	14.28 (13.19-15.36)	13.09 (12.01-14.18)	13.80 (12.72-14.89)	14.42* (13.33-15.51)
A/G	C	0.93 (0.87-1.00)	0.97 (0.90-1.04)	1.11 (1.05-1.18)	1.18 (1.11-1.24)	1.25 (1.18-1.32)
	E	0.92 (0.86-0.99)	0.96 (0.89-1.03)	1.09 (1.02-1.16)	1.16 (1.09-1.22)	1.04* (0.98-1.11)

Values marked with * are significantly different (P<0.05) between groups
C – Control group; E – experimental group; A/G – albumin to globulin ratio

Table 2. Concentration of alpha globulin, beta globulin, and gamma globulin (g/L) in the blood plasma of chickens hatched from eggs irradiated before incubation at a dose of 0.3 Gy gamma radiation (experimental group) and in the blood plasma of chickens hatched from non-irradiated eggs (control group)

		MEAN VALUE (95% confidence interval)				
		Age (days)				
Parameter	Group	1	3	5	7	10
Alpha globulin (g/L)	C	1.33 (1.08-1.59)	1.72 (1.47-1.98)	1.75 (1.50-2.01)	1.73 (1.49-2.00)	1.92 (1.67-2.18)
	E	1.36 (1.12-1.62)	1.80 (1.55-2.06)	1.73 (1.49-1.99)	1.91 (1.67-2.17)	2.30* (2.06-2.56)
Beta globulin (g/L)	C	2.81 (2.52-3.14)	2.82 (2.53-3.15)	2.74 (2.45-3.07)	3.20 (2.92-3.52)	3.10 (2.80-3.42)
	E	2.62 (2.33-2.95)	2.77 (2.47-3.09)	2.82 (2.53-3.15)	3.27 (2.98-3.59)	3.53 (3.23-3.85)
Gamma globulin (g/L)	C	10.82 (10.43-11.24)	2.74 (2.38-3.20)	2.13 (1.78-2.61)	1.72 (1.38-2.21)	1.71 (1.37-2.20)
	E	9.92* (9.53-10.34)	2.68 (2.31-3.14)	1.94 (1.59-2.42)	1.81 (1.47-2.30)	1.63 (1.29-2.12)

Values marked with * are significantly different (P<0.05) between groups
C – control group; E – experimental group

The obtained differences between control and experimental groups were statistically non-significant. The mean values of gamma-globulins in one-day old chickens were 10.82 g/L (95% CI:10.43-11.24) in the control group and 9.92 g/L (95% CI:9,53-10,34) in the experimental group. The difference between groups showed significantly decreased ($P < 0.05$) gamma-globulins in the irradiated group of chickens. Following steep decrease after day one, the gamma-globulins reached minimum values on the 10th day of chick's life. Recorded values in the control and the experimental group were 1.71 g/L (95% CI:1.37-2.20) and 1.63 g/L (95% CI:1.29-2.12), respectively.

Discussion

Our study indicates significant inhibitory effect of 0.3 Gy gamma rays on gamma-globulins in blood of one-day-old chicks irradiated before incubation, while the same dose stimulated alpha-globulin synthesis 10th day after hatching. The results for total protein and albumin concentrations in plasma of irradiated chickens were statistically non-significant compared to controls ($P < 0.05$) during the first 10 days of chick's life. These findings are not consistent with the results obtained by Vilić *et al*. [29] who reported the inhibitory effect of low-dose on all protein fractions in the first week of chick's life after *in ovo* irradiation with 0.15 Gy. Greater effect at low dose compared to higher low dose implied that radiation-induced protective pathways in exposed chicken embryo may be more efficient in the specific low dose range. These findings are in line with the conclusion reached by Calabrese [30] who study the dose–effect relationships and found that the most of them are not linear but rather have a threshold or may even be hormetic. Possible explanation may be different radiation effects on defence mechanisms, such as DNA repair, free radical scavenging, or damaged cells removal (reviewed by Tubiana *et al*., [31]), who may be overwhelmed by one dose, and stimulated by other. It is known that inflammatory reaction plays an important role in general response to low doses [32], while the mechanisms are probably mediated by reactive oxygen species [33]. Even multiple response patterns are previously described in exposed tissues and cells, our results are difficult to explain within the context of eggs exposed before incubation (when organs are not developed), because it is unknown whether a low-dose gamma radiation affected gene expression, DNA – protein interaction, or initiated a series of biochemical and molecular signalling events that may damage essential macromolecules (DNA, lipids, and proteins), repair the damage, or cause permanent physiological changes [34]. Proteomic analysis confirmed that many processes take place at molecular level in a fertilized egg, including synthesis of proteins important for the organism defence [35]. The experimental study where chick embryos were irradiated with 0.3 Gy gamma-radiation on the 19th day of incubation confirmed alteration of antioxidant status in liver [24]. Moreover, it is suggested that both, oxidative stress and inflammatory response modify mitochondrial redox balance, cellular response, and repair mechanisms [36, 37]. A similar conclusion was reached by Lumniczky *et al*. [38] who reviewed interactions between ionizing radiation and the immune system, and found both, the evidence of permanently modified immune response associated with pathophysiological consequences, and beneficial, anti-inflammatory effects. It may be reasonably expected that the alpha protein fractions in our research were involved in inflammation process induced by low-dose radiation exposure. However, having in mind that globulins are involved in other metabolic pathways, for example lipid and iron metabolism [39], additional research is needed to identify specific metabolic processes associated with radiation exposure.

Conclusion

This study confirmed that low dose ionizing radiation change plasma protein concentrations in chickens hatched from eggs exposed before incubation. Our findings signal the need for additional studies to identify control genes, and to better understand reactive oxygen metabolism and molecular pathways in response to different doses applied *in ovo*.

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References

1. Vaiserman A, Koliada A, Zabuga O, Socol Y. Health Impacts of Low-Dose Ionizing Radiation: Current Scientific Debates and Regulatory Issues. Dose-response. 2018;16(3):1559325818796331.
2. Preston RJ, Boice JD Jr, Brill AB, Chakraborty R, Conolly R, Hoffman FO, et al. Uncertainties in estimating health risks associated with exposure to ionising radiation. J. Radiol. Prot. 2013;33:573–88.
3. National Academies of Sciences, Engineering, and Medicine. Leveraging Advances in Modern Science to Revitalize Low-Dose Radiation Research in the United States. Washington, DC: The National Academies Press; 2022.
4. Hall EJ, Giaccia AJ. Radiobiology for the radiologist. 8th ed. Philadelphia: Wolters Kluwer; 2019.
5. Møller AP, Mousseau TA. (2015): Strong effects of ionizing radiation from Chernobyl on mutation rates. Sci. Rep. 2015;5:8363.
6. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Sources and effects of ionizing radiation. Report to the General Assembly, with Scientific Annex. New York: United Nations; 1996:27-43.
7. Honjo Y, Ichinohe T. Stage-Specific Effects of Ionizing Radiation during Early Development. Int. J. Mol. Sci. 2020;21(11):3975.
8. International Atomic Energy Agency (IAEA). Environmental consequences of the Chernobyl accident and their remediation: twenty years of

experience / report of the Chernobyl. Vienna: IAEA; 2006:4-137.

9. Møller AP, Nishiumi I, Suzuki H, Ueda K, Mousseau TA. Differences in effects of radiation on abundance of animals in Fukushima and Chernobyl. Ecol. Indic. 2013;24:75-81.
10. Multidisciplinary European Low Dose Initiative (MELODI). Strategic Research Agenda − 2019. Available online at http://www.melodi-online.eu/. Accessed 17 Sep 2022.
11. Vaiserman A., Cuttler JM., Socol Y. Low-dose ionizing radiation as a hormetin: experimental observations and therapeutic perspective for age-related disorders. Biogerontology. 2021;22(2):145–64.
12. Siegel JA, Greenspan BS, Maurer AH, Taylor AT, Phillips WT, Van Nostrand D, et al. The BEIR VII Estimates of Low-Dose Radiation Health Risks Are Based on Faulty Assumptions and Data Analyses: A Call for Reassessment. J Nucl Med. 2018;59(7):1017-9.
13. Feinendegen LE. Evidence for beneficial low level radiation effects and radiation hormesis. Br. J. Radiol. 2005;78: 3–7.
14. Pollycove M. Radiobiological basis of low-dose irradiation in prevention and therapy of cancer. Dose-response. 2006;5(1):26-38.
15. Arenas M, Gil F, Gironella M, Hernandez V, Jorcano S, Biete A, et al. Anti-inflammatory effects of low-dose radiotherapy in an experimental model of systemic inflammation in mice. Int. J. Radiat. Oncol. Biol. Phys. 2006;66:560–7.
16. Ina Y, Sakai K. Activation of immunological network by chronic low-dose-rate irradiation in wild-type mouse strains: analysis of immune cell populations and surface molecules. Int. J. Radiat. Biol. 2005;81(10):721-9.
17. Cornelius C, Graziano A, Calabrese Ej, Calabrese V. Hormesis and vitagenes in aging and longevity: mitochondrial control and hormonal regulation. Horm. Mol. Biol. Clin. Investig. 2013;16(2):73-89.
18. Ermakov AV, Konkova MS, Kostyuk SV, Egolina NA, Efremova LV, Veiko NN. Oxidative stress as a significant factor for development of an adaptive response in irradiated and nonirradiated human lymphocytes after inducing the bystander effect by low-dose X-radiation. Mutat. Res. − Fund. Mol. M. 2009;669(1–2):155-61.
19. Zakaria H. Effect of low doses of gamma irradiation prior to egg incubation on hatchability and body weight of broiler chickens. Brit. Poultry Sci. 1991;32(1):103-7.
20. Kraljević P, Vilić M, Miljanić S, Šimpraga M. Body weight and enzymes activities in blood plasma of chickens hatched from eggs irradiated with low-level gamma rays before incubation. Acta Vet. 2009;59(5-6):503-11.
21. Kraljević P, Šimpraga M, Vilić M, Miljanić S. Effect of Low Dose Gamma Radiation on Some Biochemical Indicators in the Blood Plasma of Chickens. In: Obelić B, Ranogajec-Komor M, Miljanić S, Krajcar Bronić I, editors. Proceedings of IRPA Regional Congress on Radiation Protection in Central Europe. Radiation Protection and Health; 20-25 May 2001; Zagreb: Croatian Radiation Protection Association. 2002;3-12.
22. Vilić M, Gottstein Ž, Ciglar Grozdanić I, Matanović K, Miljanić S, Mazija H, et al. Effect of low dose gamma-radiation upon Newcastle disease virus antibody level in chicken. Int. J. Radiat. Res. 2009;7(1):27-31.
23. Pejaković Hlede J, Vince S, Žura Žaja I, Majer M, Vilić M. Effect of low dose gamma irradiation on number and ratio of some peripheral blood cells in hatched chicks. Veterinaria. 2021;70(1):27-36.
24. Vilić M, Aladrović J, Beer Ljubić B, Miljanić S, Kraljević P. Effect of low dose gamma-radiation upon antioxidant enzymes in chick embryo liver. Arch. Geflügelk. 2010;74 (4):274- 8.
25. Vilić M, Aladrović J, Beer Ljubić B, Žura Žaja I, Gottstein Ž, Pejaković Hlede J, et al. The temporal dynamics of antioxidants and lipid peroxidation in chick embryo livers after low- dose gamma irradiation. Vet. Arhiv. 2020;90(2):169-84.
26. Ge C, Liang Y, Zhang Y, Su F, Chen L, Ma F, et al. Plasma Proteins As Biodosimetric Markers of Low-DoseRadiation in Mice. Dose-response. 2021;19(2):15593258211016257.
27. Melillo A. Applications of serum protein electrophoresis in exotic pet medicine. Vet. Clin. Exot. Anim. 2013;16(1):211-25.
28. Majer M, Roguljić M, Knežević Ž, Starodumov A, Ferenček D, Brigljević V, Mihaljević B. Dose mapping of the panoramic 60Co gamma irradiation facility at the Ruđer Bošković Institute − Geant4 simulation and measurements. Appl. Radiat. Isotopes. 2019;154:108824.
29. Vilić M, Kraljević P, Žura Žaja I, Pejaković Hlede J, Miljanić S, Šimpraga M. Concentration of proteins and protein fractions in blood plasma of chickens hatched from eggs irradiated with low dose gamma radiation. Vet. Arhiv. 2014;84(4):401-9.
30. Calabrese EJ. The Emergence of the Dose-Response Concept in Biology and Medicine. Int. J. Mol. Sci. 2016;17(12):2034.
31. Tubiana M, Feinendegen LE, Yang C, Kaminski JM. The Linear No-Threshold Relationship Is Inconsistent with Radiation Biologic and Experimental Data. Radiology 2009;251(1)13-22.
32. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Biological mechanisms of radiation actions at low doses. A white paper to guide the Scientific Committee′s future programme of work. New York: United Nations; 2012:9-32.
33. Riley PA. Free Radicals in Biology: Oxidative Stress and the Effects of Ionizing Radiation. Int. J. Rad. Biol.1994;65(1):27-33.
34. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. Cancer Metast. Rev. 2004;23(3-4):311–22.
35. Padliya ND, Qian M, Roy SM, Chu P, Zheng H, Tess A, et al. The impact of fertilization on the chicken egg yolk plasma and granule proteome 24 hours post-lay at room temperature: capitalizing on high-pH/low-pH reverse phase chromatography in

conjunction with tandem mass tag (TMT) technology. Food Function. 2015;6(7):2303−14.

36. Dąbrowska N, Wiczkowski A. Analytics of oxidative stress markers in the early diagnosis of oxygen DNA damage. Adv. Clin. Exp. Med. 2017;26:155–66.
37. Czarny P, Wigner P, Galecki P, Sliwinski, T. The interplay between inflammation, oxidative stress, DNA damage, DNA repair and mitochondrial dysfunction in depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 2018;80:309–21.
38. Lumniczky K, Impens N, Armengol G, Candéias S, Georgakilas Ag, Hornhardt S, et al. Low dose ionizing radiation effects on the immune system. Environ. Int. 2021;149:106212.
39. Kaneko JJ, Harvey JW, Bruss ML. Clinical Biochemistry of Domestic Animals. 6th ed. San Diego: Academic Press; 2008.