

## THE EFFECT OF RADIOPROTECTORS PROTECTION ABILITY IN PHEASANTS AFTER ALIMENTARY RADIO-CONTAMINATION WITH $^{137}\text{Cs}$

by

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Considering that previous studies were mainly focused on a protection of domestic animals from alimentary Cs-contamination, the objective of the present study was to investigate the possibilities of ammonium – iron (III) hexacyanoferrate (II) – AFCF and clinoptilolite as radioprotectors. Pheasants of species *Phasianus colchicus* were used as trial animals. The total number of 2 -month old pheasants was 100. The pheasants were divided into five groups; each group was contaminated with 750 Bq of  $^{137}\text{Cs}$  per bird in a single experiment. The experiment consisted of 5 phases during which the animals were given AFCF (0.2 g) and clinoptilolite (2 g) either in water solution or mixed with food, according to the phase. The level of contamination was determined by gamma – spectrometry in breast meat, leg meat, liver and gizzard. The results show that the best protection effect was obtained when AFCF is used in the water solution *i. e.* 92.7%; while AFCF mixing with food demonstrated 74.9% of protection; clinoptilolite proved less protective with 69.3% – in the water solution, and 50.5% when mixed with food.

*Key words:* radiocontamination,  $^{137}\text{Cs}$ , radioprotection, AFCF, zeolite, pheasant, meat, internal organ

### INTRODUCTION

During the evolution, humans and animals were exposed to environmental radiation, with no influence on the dynamic equilibrium of ecological system. On the other hand biosphere is nowadays contaminated with man-made radionuclides (mostly due to energy production). It is changing ecological characteristics, of one or several cycles of ecosystem. It is therefore necessary to control levels of natural and artificial radionuclides in the environment. This is the only way to preserve healthy ecosystem.

In accidental circumstances, population protection from the alimentary contamination, is a prime goal of radiation hygiene.  $^{90}\text{Sr}$ ,  $^{131}\text{I}$ , and  $^{137}\text{Cs}$ , which have metabolical and structural analogs in biosystems, pose the most significant risk.

After the Chernobyl accident in 1986, the atmosphere was contaminated with radionuclides such as  $^{134}\text{Cs}$  and  $^{137}\text{Cs}$  [1]. These organotrope isotopes, long living radionuclides, are potential ecological factor of risk.

Due to constant presence in the environment, radionuclides enter in the “food-chain” and make the animal food products the most frequent source of human contamination by radionuclides [2].

The increase of concentration of  $^{137}\text{Cs}$  is the most significant in animals which live and feed in natural conditions and are directly exposed to radioactive contamination. These animals represent the bioindicators of the environmental contamination. Game is an important part of the environment, that binds human population and natural environment. Alimentary contamination is present in the animals which take contaminated food and water in industrial and individual farms. The highest level of  $^{137}\text{Cs}$  contamination is found in flesh of fish, large and small wild animals, as well as domestic graze animals (sheep, cow and goat).

Numerous efforts are made all over the world in order to reduce the negative effects of ionizing radiation. A part of today's research is aimed at reducing alimentary contamination of animals, thus diminishing the health risk of consumers. Chemical substances that bind and immobilize radioisotopes in intestinal tract of animals, and eliminate them from the body in the case

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of alimentary contamination, are of essential importance in meat and dairy industry. In the last decades, hexacyanoferrates are known as substances that selectively bind cesium ions. Based on the latter data, such substances are used as an antidote for cesium absorption reduction in the animal tissues [3-5].

After the Chernobyl accident, the radioprotector that was most often used for domestic animals, was ammonium iron (III) hexacyanoferrate (II) AFCF [6-18] and clay minerals – clinoptilolite [9, 14, 16, 18, 19].

Considering that researches in the past focused on animal protection from alimentary contamination by  $^{137}\text{Cs}$ , the objective of the present study was to investigate application of AFCF in wild animals – pheasants. At the same time the intention was to determine and compare the protection effect of clinoptilolite in pheasants after alimentary contamination with  $^{137}\text{Cs}$ .

## MATERIAL AND METHODS

The pheasants used for this experiment, were from the species *Phasianus colchicus*. The experimental work included two months old female birds from the “Široka greda” pheasant farm located near Padinska Skela, Serbia. The pheasants were raised in artificial farm conditions based on mass production of eggs laid by artificially raised hens, incubating the young, and keeping them until they were transported to the hunting ground.

The birds brought from the farm were kept in cages, five female birds in each cage. Feeding and watering the animals was as they take. The birds were fed with 20% proteins complete fodder output mix.

In order to examine the protection ability of complex salt – AFCF and natural mineral clinoptilolite in the case of alimentary contamination with  $^{137}\text{Cs}$  the pheasants were divided into groups for single experiment.

The single experiment consisted of five phases. Total number of animals was 100. Twenty animals were divided into 4 groups in the each phase with 5 birds in every group. The first group of pheasants was sacrificed 2 h after giving  $^{137}\text{Cs}$ , while the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups after 4 h, 8 h, and 24 h, respectively.

In the first phase of a single experiment (control group) a natural course of distribution and elimination of radioactive cesium  $^{137}\text{Cs}$  in meat and internal organs were followed.

In other phases the level of  $^{137}\text{Cs}$  activity concentration in a single application of  $^{137}\text{Cs}$  water solution was constant (750 Bq/ml) while the method of application of radioprotectors AFCF (0.2 g) and clinoptilolite (2 g) was changed. The protectors were administered orally via gastric tube or mixed food in the form of a pellet.

The level of  $^{137}\text{Cs}$  activity concentration was determined by gamma-spectrometry in breast meat, leg meat and internal organs, gizzard, and liver. All the samples were minced and homogenized. The mass of breast meat and leg meat samples were 100-200 g, while the mass of internal organs gizzard and liver were 10-30 g.

Statistical analysis was performed using MS Excel, applying the following statistical methods: AUC (Area Under Curve calculated using Trapezoidal Rule), Student's t-test (paired, one-tailed), Chi2-test.

## RESULTS AND DISCUSSION

The food was also measured before it was given to the pheasants, hence the obtained results are expected to be accurate. The results of pellet mixture investigation gave the  $^{137}\text{Cs}$  activity concentration from 0.5 Bq/kg-0.6 Bq/kg which was within the range of low level background radioactivity (less than 1,0 Bq/kg). On the basis of these results, as well as data, from the literature it can be concluded that  $^{137}\text{Cs}$  activity concentration in food has no effect on the activity concentration of this radionuclide in meat or internal organs of the pheasants.

The zero group was neither administered  $^{137}\text{Cs}$  in water solution nor radioprotectors. The birds were fed with standard pheasant mixtures at the farm. In the examined samples a low level  $^{137}\text{Cs}$  of activity concentration was found both in meat and internal organs (0.3 Bq/kg-0.5 Bq/kg). Subsequently, it can be stated that feeding the pheasants with low-contaminated food and keeping them in strictly controlled ambient conditions results in birds whose eatable parts (meat and internal organs) meet the radiation-hygiene regulations for our country.

The  $^{137}\text{Cs}$  activity concentration in pheasant's leg meat, breast meat, liver, and gizzard fed with and without protectors are given in figs. 1, 3, 5, and 7, respectively.

According to the results obtained in the single experiment the best effect for leg meat was achieved by simultaneously giving  $^{137}\text{Cs}$  and radioprotector AFCF by tube. The lowest measured activity concentration was 18.3 Bq/kg (fig. 1).

Average percentage of protection achieved by simultaneously giving  $^{137}\text{Cs}$  and AFCF radioprotector by tube was 90%, which in time – 24 h gradually increased to 91.9% (fig. 2). Stated data are in compliance with those stated by others authors [19-21].

The highest activity value was obtain during the application of clinoptilolite mixed with food (247 Bq/kg). It has gradually increased and reached its highest level for the birds sacrificed after 24 hours.

The protection level which has been made using AFCF-tube is the highest and resulted in a small percentage of the presence of  $^{137}\text{Cs}$  in the leg meat, while

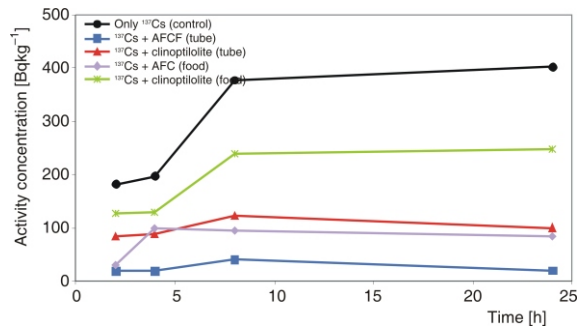


Figure 1. <sup>137</sup>Cs activity concentration in pheasant's leg meat after single alimentary contamination and protection with AFCF and clinoptilolite in Bq/kg

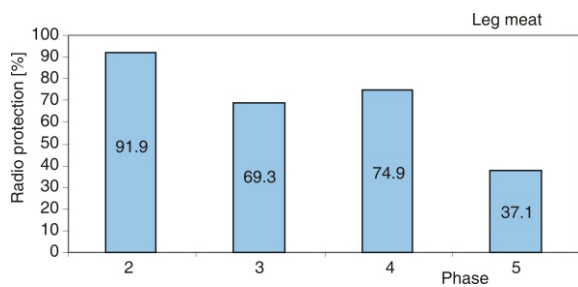


Figure 2. Efficiency of AFCF and clinoptilolite in <sup>137</sup>Cs deposition reduction in pheasant's leg meat after single alimentary contamination and protection (% of 100% activity of <sup>137</sup>Cs compared to the control group without protector)

the smallest percentage of efficiency was 37.1% when clinoptilolite mixed in food was given to the pheasants.

The level of <sup>137</sup>Cs activity concentration in breast meat, as well as in leg meat, kept constantly raising with time from the moment of contamination and protection (fig. 3). The lowest concentration (6.9 Bq/kg) was measured in the second phase 4 hours after the AFCF administration with tube. The highest level of activity concentration (101.9 Bq/kg) has been measured in the 5<sup>th</sup> phase when clinoptilolite mixed in food was given and measured in birds slaughtered after 24 hours.

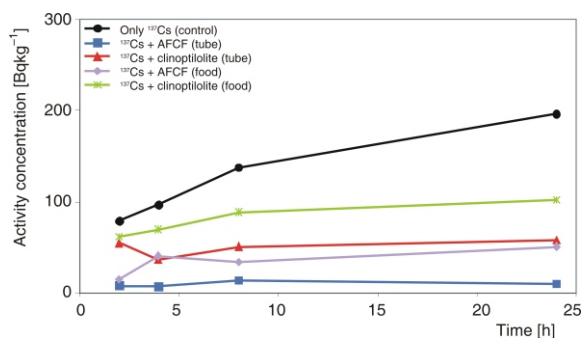


Figure 3. <sup>137</sup>Cs activity concentration in pheasant's breast meat after single alimentary contamination and protection with AFCF and clinoptilolite in Bq/kg

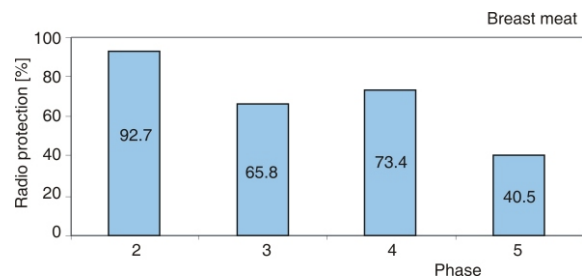


Figure 4. Efficiency of AFCF and clinoptilolite in <sup>137</sup>Cs deposition reduction in pheasant's breast meat after single alimentary contamination and protection (% of 100% activity of <sup>137</sup>Cs compared to the control group without protector)

The highest protection in breast meat after 24 hours was 92.7% (fig. 4). It was achieved applying AFCF by the tube, which is in accordance with the results obtained by others authors [9, 18, 20]. The minimum percentage of protector efficiency 40.5% was measured for birds in the fifth phase, where the clinoptilolite was mixed in the food.

The experiment results in the liver showed that <sup>137</sup>Cs activity concentration is gradually diminishing in all cases, with the lowest activities measured 24 hours after the contamination and protection (fig. 5). The lowest activity was in the second phase (19.7 Bq/kg) when AFCF radioprotector was simultaneously given with tube administered <sup>137</sup>Cs. The highest activity was measured in the fifth phase (1040 Bq/kg) in birds slaughtered after 2 hours.

The highest protection in liver was 92.2% in the second phase, 24 hours after contamination (fig. 6). The minimum percentage of radioprotector efficiency was after 24 hours in the fifth phase and stopped at 50.5%.

The highest <sup>137</sup>Cs activity concentration were obtained in the experiment with the gizzard. Similar to experiments with liver <sup>137</sup>Cs activity concentration gradually decreases over time, so the lowest activities were measured for the birds slaughtered 24 hours after contamination and protection (fig. 7). The lowest mea-

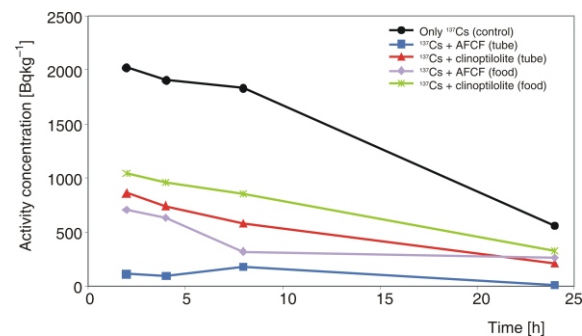
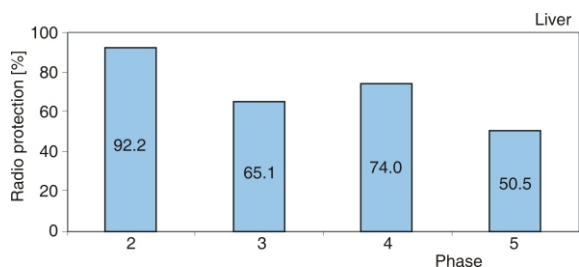
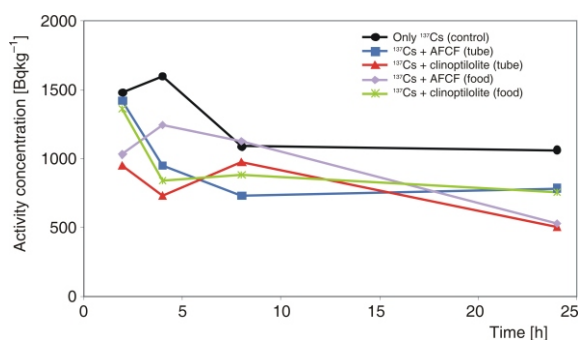


Figure 5. <sup>137</sup>Cs activity concentration in pheasant's liver after single alimentary contamination and protection with AFCF and clinoptilolite in Bq/kg



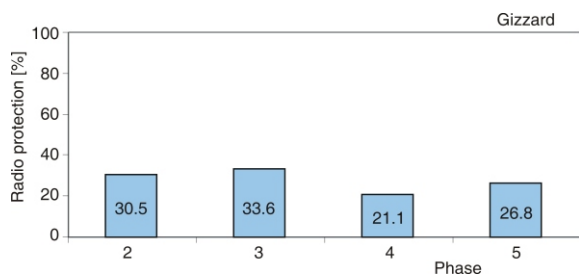
**Figure 6. Efficiency of AFCF and clinoptilolite in  $^{137}\text{Cs}$  deposition reduction in pheasant's liver after single alimentary contamination and protection (% of 100% activity of  $^{137}\text{Cs}$  compared to the control group without protector)**



**Figure 7.  $^{137}\text{Cs}$  activity concentration in pheasant's gizzard after single alimentary contamination and protection with AFCF and clinoptilolite in Bq/kg**

sured  $^{137}\text{Cs}$  activity concentration was in the third phase (511 Bq/kg) when the clinoptilolite was given through the tube. The highest activity was measured in the second phase and was 1420 Bq/kg.

In the second phase,  $^{137}\text{Cs}$  + AFCF (tube), which has so far shown the lowest  $^{137}\text{Cs}$  activity concentration, we registered for the first time a small protective effect on gizzard compared with meat and liver. The percentage of removed activity 30.5% in the second phase was lower than the percentage of activity removed in the third phase (fig. 8), which proved to be the best protection and the effect was 33.6%.



**Figure 8. Efficiency of AFCF and clinoptilolite in  $^{137}\text{Cs}$  deposition reduction in pheasant's gizzard after single alimentary contamination and protection (% of 100% activity of  $^{137}\text{Cs}$  compared to the control group without protector)**

## CONCLUSIONS

The maximum effect of protection was achieved by a single contamination of pheasants with water solution of  $^{137}\text{Cs}$  and simultaneous administration of the water solution of AFCF by tube. The achieved effect of protection in leg meat, breast meat, and liver was 91.9%-92.7%, while in gizzard it was lower, i. e. 30.5% in the 2<sup>nd</sup> phase. The latter results are in compliance with those stated by others authors [10, 18, 21, 22]. Reduced protection effect was obtained by a simultaneous administration of the  $^{137}\text{Cs}$  water solution and AFCF mixed in pheasant's food in the form of a pellet (73.4%-74.9%) for leg meat, breast meat and liver, while for gizzard it was 21.1%.

The use of clinoptilolite as radioprotector showed reduced protection effect. At the same time, single application of  $^{137}\text{Cs}$  and clinoptilolite by tube gave good protection effect for leg meat, breast meat, and liver from 65.1%-69.3%, while for gizzard it was lower than 33.6%. When clinoptilolite was mixed with the food the protection effect was lower and for leg meat, breast meat, and liver it was 37.1%-50.5% and 26.8% for gizzard. The above stated data are in compliance with those stated by others authors [14, 23-25].

The highest protection efficiency was achieved using AFCF radioprotector per application while using mineral clinoptilolite as radioprotector gave a reduced amount of protective effect.

## AUTHOR CONTRIBUTIONS

Selection of pheasants was performed by D. Z. Živanov. Animal feeding and application of radioactive  $^{137}\text{Cs}$ , as well as samples preparing, were done by B. M. Mitrović and M. Č. Vićentijević. The samples measurement was carried out by D. Ž. Vuković. Gamma spectrometer calibration was done by G. K. Pantelić. The manuscript was written by M. Č. Vićentijević, D. Ž. Vuković, and V. J. Vuković. The figures were prepared by V. J. Vuković and all authors analysed and discussed the results.

## REFERENCES

- [1] Hecht, H., Radioactive Pollution of Wild and Farm Animals after the Accident at Chernobyl (in Germany), *Fleischwirtschaft*, 68 (1988), 4, pp. 508-513
- [2] Pantelić, G., et al.,  $^{137}\text{Cs}$  Activity in Cattle Feed and Milk, after the Chernobyl Accident, *Acta Veterinaria*, 49 (1999), 5-6, pp. 371-378
- [3] Vitorović, G. S., et al., Radioactive Contamination of Food Chain around Coal Mine and Coal-Fired Power Stations, *Nucl Technol Radiat*, 27 (2012), 4, pp. 388-391
- [4] Nigrović, V., Enhancement of the Excretion of Radiocesium in Rats by Ferric Cyanoferrate (II), *Int. J. ad. Biol.*, 7 (1963), pp. 307-309
- [5] Havlicek, F., The Effect of Cyanoferrate on the Excretion of Radiocesium in Rats and Goats (in Germany), *Strahtentherapie*, 134 (1967), pp. 123-129

- [6] Margenthal, A., Feeding Trial to Test Ammonium-Ferric-Cyano-Ferrate (II) for Decorporation of Radiocesium in Sheep (in Germany), *Inaugural Dissertation-Hannover*, Germany, 1988
- [7] Bailer, B., Accelerating Excretion of Radiocesium in Sheep by Feeding with Ammonium Iron Hexacyanoferrate (in Germany), *Inaugural Dissertation-Hannover*, Germany, 1988
- [8] Dresow, B., et al., *In vivo* Binding of Radiocesium by Two Forms of Prussian Blue and by Ammonium Iron Hexacyanoferrates (II), *J. Toxicol Clin Toxicol*, 31 (1993), 4, pp. 563-569
- [9] Giese, W., Countermeasures for Reducing of Radiocesium to Animal Derived Food, *The Sci. of Total Envir.*, 85 (1989), pp. 317-326
- [10] Vitorović, G., The Application of Ammonium-Ferric (III) – Hexacyano-Ferrate (II) in the Protection of Chicken Meat from Contamination with Radiocesium., Ph. D. thesis, Faculty of Veterinary Medicine, University of Belgrade, 1992
- [11] Hove, K., Hansen, H. S., Reduction of Radiocesium Transfer to Animal Products Using Sustained Release Boli with Ammoniumiron (III) Hexacyanoferrate (II), *Acta Vet. Scand.*, 34 (1993), 3, pp. 287-297
- [12] Hansen, H. S., et al., The Effect of Sustained Release Boli with Ammoniumiron (III) Hexacyanoferrate (II) on Radiocesium Accumulation in Sheep Grazing Contaminated Pasture, *Health Phys.*, 71 (1996), 5, pp. 705-712
- [13] Ratnikov, A., et al., The Use of Hexacyanoferrates in Different Forms to Reduce Radiocesium Contamination of Animal Products in Russia, *The Science of the Total Environment*, 223 (1998), 2-3, pp. 167-176
- [14] Poschl, M., Balas, J., Reduction of Radiocesium Transfer to Broiler Chicken Meat by a Clinoptilolite Modified with Hexacyanoferrate, *Radiat. Environ. Biophys.*, 38 (1999), 2, pp. 117-124
- [15] Paasikallio, A., et al., The Effect of Ammonium Ferric Hexacyanoferrate on Reducing Radiocesium Transfer from Grass Silage to Sheep, *Agricultural and Food Science in Finland*, (2000), pp. 135-147
- [16] Vićentijević, M., et al., Comparative Ratio of AFCF and Clinoptilolite Efficiency Obtained through Multiple <sup>137</sup>Cs Contamination of Pheasants, *Proceedings*, Symposium on Clinical Pathology and Treatment of Animals, Budva, Montenegro, 2003, pp. 238-240
- [17] Vićentijević, M., et al., AFCF Efficiency in Pheasants after Multiple <sup>137</sup>Cs Contamination, *Proceedings*, XXIV Symposium of Radiation Protection Society of Serbia and Montenegro, Zlatibor, Serbia, 2007, pp. 129-133
- [18] Vitorović, G., The Application of Ammonium Ferric (III) Hexacyanoferrate (II) in Protection of Chicken Meat from Contamination with Radiocesium, *Acta Vet.*, 43 (1993), 5-6, pp. 351-358
- [19] Vitorović, G., et al., The Effect of Clinoptilolite on <sup>137</sup>Cs Binding in Broiler Chickens, *Agricultural and Food Science in Finland*, 11 (2002), 2, pp. 137-141
- [20] Mitrović, B. M., et al., The Effect of Hexacyanoferrate Compounds and Clinoptilolite after a Single Alimentary Contamination of Chicken with <sup>137</sup>Cs, *Proceedings*, XVIII European Symposium on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs Products Prague, 2007, pp. 283-284
- [21] Giese, W., The Behavior of Radiocesium in the Laboratory and Domestic Animals as well as Possibilities for the Decrease of Radioactivity and Radiation Exposure, Habilitation, Veterinary University, Hannover, Germany, 1971
- [22] Vićentijević, M., et al., Efficiency Clinoptilolite In Case a Multiple Alimentary Contamination of the Pheasants with <sup>137</sup>Cs, *Biotechnology in Animal Husbandry*, 22 (2006), 3-4, pp. 105-114
- [23] Balas, J., Transfer of Radiocesium to the Meat of Broiler Chicken and the Possibility of Affecting it, Abstract, Ph. D. thesis, University of Agriculture and Forestry in Brno, Czech Republic, 2000
- [24] Slavata, B., Testing Radioprotection Ability of Ammonium Iron (III) Hexacyanoferrate (II) and Clinoptilolite in Broilers Exposed Alimentary Contamination with <sup>137</sup>Cs, M. Sc. thesis, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, 2003
- [25] Such, P., et al., The Effect of a Clinoptilolite-Based Feed Supplement on the Performance of Broiler Chickens, *Czech J Anim Sci.*, 51 (2006), 4, pp. 168-173

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**ЕФЕКАТ ПРОТЕКЦИОНЕ СПОСОБНОСТИ РАДИОПРОТЕКТОРА  
КОД ФАЗАНА ПОСЛЕ АЛИМЕНТАРНЕ РАДИОКОНТАМИНАЦИЈЕ <sup>137</sup>Cs**

Имајући у виду да су досадашња истраживања била усмерена ка заштити домаћих животиња од алиментарне контаминације цезијумом, наш циљ је био да се истраже радиопротекторске могућности амонијум – гвожђе (III) хексацијаноферата (II) – AFCF и клиноптиолита. Као експерименталне животиње користили смо фазане из реда *Phasianus colchicus*. У експерименту је коришћено 100 фазана старости два месеца. Фазанке су биле подељене у пет група, свака група је контаминирана са 750 Bq <sup>137</sup>Cs по животињи у једнократном експерименту. Експеримент се састојао од 5 фаза у току којих су фазанке добијале AFCF (0.2 g) и клиноптиолит (2 g), у воденом раствору или умешано у храни у зависности од фазе. Ниво контаминације одређен је гама спектрометријски у светлом месу, тамном месу, јетри и мишићном желуцу. Резултати показују да је најбољи ефекат заштите постигнут помоћу AFCF-а (92.7%) дат у виду воденог раствора; мешање AFCF-а са храном дало је 74.9% заштите. Клиноптиолит у виду воденог раствора дао је ефекат 69.3%, и 50.5%, када је умешан у смешу за исхрану фазана.

*Кључне речи:* радиоконтаминација, <sup>137</sup>Cs, радиопротекција, AFCF, зеолити, фазан, месо, унутрашњи орган