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Clinical picture, haematological parameters and pathomorphological findings in fattening chickens after application of a lethal quantity of ^{32}P

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ABSTRACT

Due to its quality and relatively low price as a result of a simple and short production cycle, poultry meat occupies a very high position in the consumption of foodstuffs of animal origin. It is just because of such simple and rapid production that, under the circumstances of larger-scale radioactive contamination, poultry meat might become the main source of protein of animal origin. This fact, as well as the fact that no investigation into the effects of radioactive phosphorus (^{32}P) on fattening chickens has been conducted so far, induced us to investigate the impact of a lethal quantity of this isotope on chickens at slaughter age. The investigation was performed on chickens, hybrids of the Jata heavy breeds of both sexes, aged 50 days and with a mass ranging from 1500 to 2000 g. The experiment was based on the application of radioactive phosphorus ^{32}P at 333 MBq/kg of body mass. Test animals were clinically examined, blood samples were taken for haematological analysis and, immediately after death, dissection and pathohistological examination were performed. Based on the obtained results it was concluded that radioactive phosphorus ^{32}P, when applied to 50-day-old chickens of the Jata heavy breed at 333 MBq/kg of body mass, causes: 1) anaemia, manifested by a decrease in erythrocyte count on the 5th day and decreased and haematocrit values 7 days post-contamination; 2) leukopoenia, in a decrease in lymphocyte count on the 1st day; decreased eosinophil count on the 3rd day, and decreased heterophil count 5 days post-contamination; 3) thrombocytopoenia, on the 5th day post-contamination; 4) onset of clinical signs of radiation sickness on the 6th day, and death of all contaminated animals on the 9th and 10th days post-contamination contamination. Pathoanatomic examination of dead animals revealed dotted bleeding sites on heart and mucous membrane of digestive tract, as well as changes in parenchymal organs. Pathohistological examination of tissues and organs confirmed the findings of pathoanatomic examinations, which indicated the changes caused by radioactive radiation.

Key words: chickens, radioactive phosphorus ^{32}P, haematology, clinical picture, pathomorphological changes

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Introduction

Due to its good quality and relatively low price as a result of a simple and short production cycle, poultry meat takes a very high position in the consumption of foodstuffs of animal origin. It is just because of such simple and rapid production that, under the circumstances of larger-scale radioactive contamination, poultry meat might become the main source of protein of animal origin. We have already experienced such radioactive contamination, both in war and in peace, and in the light of recent global geopolitical trends this possibility is once more becoming more topical. In particular, people are becoming increasingly aware that terrorist organisations in their New Terrorism doctrine, in addition to chemical and biological terrorism, include radiological and nuclear terrorism as well. There are three ways of possible terrorist nuclear action. First, they could use fissile material, i.e. plutonium (^{239}Pu) or highly enriched uranium (HEU) and attempt to fabricate so-called "improvised nuclear devices" (IUD). Second, they could use radioactive material and disperse it in the environment in order to cause radioactive contamination. Third, they could attack a nuclear plant - for example, a nuclear power plant - either to cause radioactive contamination or to steal radioactive material for their nuclear terrorist actions (ČIŽMEK, 2005; AUGUSTINE et al., 2005).

This fact, as well as the fact that no investigation of the effects of fissionable products, in particular radioactive phosphorus (^{32}P), on fattening chickens has been conducted so far, induced us to investigate the impact of a lethal quantity of this isotope on chickens at slaughter age.

Materials and methods

Animals. The investigation was conducted on healthy chickens, hybrids of the Jata heavy breed of both sexes, at age of 50 days and mass ranging from 1500 to 2000 g. The birds were divided into two groups of five chickens each. They were kept in two separate cages and an identification mark was applied to each animal. Microclimate conditions in the test room were optimum for their particular age. Chickens were fed commercially available mixture BRO-finisher for broilers (Feed Plant Agroemona-Domžale, Slovenia), which had been fed to the chickens since their 13^{th} day of age. Subsequently, commercial fed was given.

Radioactive isotope. In our investigation we used radioactive phosphorus isotope ^{32}P (Amersham International plc., England), which, due to its physical properties, is frequently used in radiobiological research (ROBERTS and SMITH, 1997; ENSINGER et al., 2005). A single injection of the radionucleid was applied to breast muscles of the test chickens, at a rate of 333 MBq/kg of body mass. The same quantity of physiological solution (0.9 ml/kg of body mass) was applied in a similar way and at the same site to the control group of chickens.

Clinical examination. The animals were subjected to clinical examination on a daily basis, in the morning and in the afternoon, eight days before and ten days after the application of the radionucleid to the test group of animals. The examination included general appearance and behaviour of the animals, respiration, response to extraneous stimuli (hand-clapping), eating and drinking, as well as colour and consistency of faeces.

Haematological examination. Blood samples for haematological tests were taken by venipuncture from wing vein (v. cutanea ulnaris) into Wintrobe tubes containing dry anticoagulant before the application, and thereafter on 1st, 3rd, 5th, 7th and 10th days after the application of ^{32}P. Blood examination included leukocyte, erythrocyte and thrombocyte counts, and haematocrit values.

Pathomorphological examination. Immediately after death, test animals were dissected and subjected to pathohistological examination, which included the parts of the liver, lungs, cloacal bursa, duodenum, pancreas, heart, spleen, kidney and adrenal gland. For the pathohistological examination, the organ parts were fixated in 10% formalin solution, embedded in paraffin, cut to a thickness of 4 μm and stained using the haematoxylin-eosine method. Animals from the control group were subjected to the same examinations and sacrificed by cervical dislocation at the end of day 10 of trial.

Statistical analysis. Results of haematological examinations were statistically analysed and presented in graph form as mean values ± mean deviation from the mean; significance of differences was determined by Student's *t*-test.

Results

Clinical examination. Clinical examination of chickens before application of the radionucleid did not reveal any deviations in the examined parameters, in either group of animals. On the day of application of radioactive phosphorus ^{32}P, as well as during the subsequent 10 days, no changes in the examined parameters were observed in animals of the control group. In the test group of chickens, on the day of application of ^{32}P and during the subsequent five days, the findings of clinical examination were similar to those established in the control group. On the sixth day after application of ^{32}P, feed and water intake in contaminated chickens lowered, and on the seventh day ceased completely. Also, their faeces became watery and markedly white in colour. On the eighth day all chickens in this group stayed pressed together, with ruffled feathers. Two had slack wings, gasped with their beaks open and did not respond to hand-clapping. On the ninth day these two birds died. The same morning a third bird from this group died, after having shown the same symptoms as the first two. The remaining two animals kept close together, with their feathers ruffled and did not respond to their environment. On the morning of the 10th day, another animal was found dead, and the last one, with respiratory signs and clotted blood coming from cloaca, died in the early afternoon.

Haematological examination. Erythrocyte count. Changes in the erythrocyte count in the blood of investigated chickens are presented in Table 1. No significant changes in erythrocyte count in peripheral circulation were observed on the first and third day after radionucleid application. However, on the fifth day a significant fall was observed ($P<0.005$). The same trend was noted on the seventh day after the application of ^{32}P, and the statistical significance of changes was also at the level of 0.005. On the last trial day, the erythrocyte count in the last surviving bird from the test group reached a lowest value of as little as 0.91×10^{12}/L, while average erythrocyte count in the control group was 2.62×10^{12}/L of blood.

Table 1. Erythrocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	2.63	2.48	2.05	2.67	2.26	2.57	2.57	2.39	2.02	1.91	1.76	0.91
2	2.63	2.58	2.07	1.76	1.93	2.53	2.39	2.57	2.09	1.79	1.45	-
3	2.3	2.63	2.48	2.36	2.37	2.73	2.36	2.46	2.36	1.80	1.76	-
4	2.74	2.42	2.45	2.48	2.12	2.68	2.64	2.59	2.37	2.0	1.85	-
5	2.61	2.83	2.17	2.11	2.48	2.52	2.36	2.21	2.01	1.52	1.2	-
M	2.58	2.59	2.24	2.28*	2.23*	2.62	2.46	2.44	2.17	1.8*	1.6*	-
S.D.	0.17	0.14	0.19	0.32	0.19	0.1	0.13	0.14	0.16	0.16	0.24	-
S.E.	0.07	0.06	0.08	0.14	0.08	0.04	0.06	0.6	0.07	0.07	0.11	-

* Statistical significance of difference between mean values (M) of test and control group of chickens ($P<0.05$).

Haematocrit value. Changes in haematocrit value in the blood of investigated chickens are presented in Table 2. In the test group, no significant changes in haematocrit value were observed on the first, third and fifth days after radionucleid application. A statistically significant fall of haematocrit values was observed on the seventh day, with the difference between the two groups being at a level of 0.005. On the last day of the trial period, haematocrit value in the only surviving animal was as low as 11%, while average haematocrit value in the peripheral circulation of the birds in the control group was 33.4%.

Thrombocyte count. Changes of total thrombocyte count in the blood of investigated chickens are presented in Table 3. In the test group, no significant changes in thrombocyte count were observed on the first and third days after radionucleid application. It was only on the fifth day after radionucleid application that a significant fall in thrombocyte count

was noted ($P<0.001$). The same trend was observed on the seventh day after application of ^{32}P with the difference between the two groups also being at a level of 0.001. On the tenth day of the test, total thrombocyte count in the only surviving animal was 0.77×10^9/L, while average thrombocyte count in the control group was 50.71×10^9/L of blood.

Total leukocyte count. Changes of total leukocyte count in the blood of investigated chickens are presented in Table 4. In the test group, however, a statistically significant fall

Table 2. Haematocrit value in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	37	35	33	33	34	33	37	31	29	25	20	11
2	34	25	30	25	29	34	33	28	28	25	20	-
3	34	30	32	32	33	33	35	31	30	26	21	-
4	41	36	30	32	33	35	38	33	32	32	25	-
5	42	36	32	29	29	32	-	30	27	21	16	-
M	37.60	32.40	31.40	30.20	31.60*	33.40	35.75	30.60	29.20	25.80	20.40*	-
S.D.	3.78	4.32	1.20	2.93	2.15	1.02	2.22	1.20	1.72	3.54	2.87	-
S.E.	1.69	1.93	0.54	1.31	0.96	0.46	0.99	0.54	0.77	1.58	1.28	-

*Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.05$).

Table 3. Thrombocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	42.89	53.11	21.65	57.11	53.56	64.22	33.56	44.67	11.33	16.89	5.78	0.77
2	32.22	43.10	20.22	61.78	85.56	48.89	50.60	54.89	23.33	31.78	12.89	-
3	25.10	32.89	44.00	48.22	46.89	33.33	42.40	44.00	58.00	11.56	6.44	-
4	26.66	48.22	44.44	46.22	43.89	60.00	30.89	32.22	34.67	23.11	10.00	-
5	27.55	32.67	26.00	41.33	59.30	47.11	42.00	34.00	22.89	23.56	2.00	-
M	30.88	42.00	31.24	50.93	57.82	50.71	39.89	42.00	30.04	21.38*	7.42*	-
S.D.	7.22	5.77	10.77	7.45	14.88	10.84	7.85	8.21	15.81	6.55	3.73	-
S.E.	3.23	1.83	4.82	3.33	6.65	4.85	3.51	3.67	7.07	2.93	1.67	-

*Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.001$).

was noted as early as the first day after radionucleid application ($P<0.025$). The decreasing trend continued in the following days of the test period. On the third, fifth and seventh days the difference was at a level of 0.001. On the last, i.e. tenth, day of the test period, total leukocyte count in the only surviving animal in the test group reached its lowest value of 0.22×10^9/L, while average leukocyte count in the control group was 32.28×10^9/L of blood.

Table 4. Total leukocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	46.70	28.70	25.80	24	20.90	42.90	24.20	20.90	6.20	5.10	0.89	0.22
2	22.90	28.90	33.80	27.10	20.40	26.90	29.20	24.20	15.30	3.30	0.60	-
3	43.30	44.90	38.90	25.80	44.40	28.20	34.70	18.70	18.90	1.30	0.89	-
4	30.70	34.00	38.70	48.70	43.30	40.70	41.30	20.90	10.40	2.40	0.67	-
5	29.60	54.10	30.70	37.80	34.20	22	49.80	33.10	6.20	2	0.44	-
M	34.64	36.32	33.58	32.68	32.64	32.28	35.84	23.56*	11.40**	2.82**	0.7**	-
S.D.	9.99	7.34	4.97	9.35	10.41	8.18	10.07	5.08	5.03	1.31	0.17	-
S.E.	4.47	3.28	2.22	4.179	4.66	3.66	4.50	2.27	2.25	0.6	0.08	-

*Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.05$). ** Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.001$).

Table 5. Lymphocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	20.55	12.63	12.38	12.00	14.84	30.46	16.46	9.61	1.12	4.39	0.89	0
2	4.81	13.58	12.84	17.34	4.90	17.76	8.76	9.92	0.92	2.74	0.56	-
3	4.33	11.67	14.39	10.31	23.53	4.59	24.63	5.61	0.76	1.25	0.87	-
4	12.28	16.66	21.67	29.22	24.25	20.34	29.74	5.85	0.42	2.11	0.63	-
5	7.67	20.74	14.35	21.17	14.36	9.24	26.89	5.29	0.50	1.64	-	-
M	9.93	15.06	15.33	18.01	16.38	17.48	21.30	7.26*	0.74**	2.43*	0.59**	-
S.D.	6.73	3.30	3.35	6.80	7.09	8.20	8.58	2.06	0.26	1.10	0.32	-
S.E.	3.01	1.48	1.50	3.04	3.17	3.67	3.84	0.92	0.12	0.49	0.14	-

* Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.05$). ** Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.001$).

Lymphocytes. Changes in lymphocyte count in the blood of the investigated chickens are presented in Table 5. The reported figures indicate that a fall in lymphocyte count was noted as early as the first day after radionucleid application and which continued to decrease throughout the test period. On the very first day of the trial, statistically significant differences between the control and test group were established ($P<0.005$). On the third day, fifth and seventh days the significance was at levels of 0.001, 0.005 and 0.001, respectively. On the tenth day after contamination no lymphocytes were found in the blood of the only surviving bird.

Table 6. Heterophil leukocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	23.36	12.05	11.87	10.56	5.02	6.86	6.53	9.2	5.02	0.4	0	0
2	17.40	12.72	20.28	7.59	15.50	7.53	8.76	14.04	13.78	0.05	0.24	-
3	37.24	30.53	24.12	13.93	17.76	17.48	9.37	12.14	18.13	0.19	0.02	-
4	17.19	14.62	14.32	15.58	16.46	19.54	9.50	15.05	9.57	0.18	0.04	-
5	17.94	22.10	12.89	14.74	19.15	11.00	20.92	24.83	5.33	0.51	0	-
M	22.63	18.40	16.68	12.48	14.78	12.49	11.02	15.05	10.37	0.27*	0.06*	-
S.D.	8.56	7.04	4.72	2.98	5.03	5.16	5.66	5.28	5.03	0.16	0.9	-
S.E.	3.83	3.15	2.11	1.33	2.25	2.31	2.53	2.36	2.25	0.07	0.4	-

* Statistical significance of the difference between the mean values (M) of the test and control group of chickens ($P<0.001$).

Heterophils. Changes in heterophil count in the blood of investigated chickens are presented in Table 6. The reported figures indicate a decreased number of heterophils in the test group of chickens throughout the test period. However, differences between test and control groups were not significant on the first and third days of the test. Statistically significant differences at the level of 0.001 were noted on the 5th and 7th days of the test group contamination. No heterophils were found in blood of the only surviving bird on the tenth day after contamination.

Eosinophils. Changes in eosinophil count in blood of investigated chickens are presented in Table 7. The reported figures indicate a decreased number of eosinophils in the test group of chickens throughout the test period. Statistically significant differences were noted on the third ($P<0.025$) and fifth ($P<0.005$) days after radionucleid application. On the seventh day after contamination eosinophils were found only in one animal; on the tenth day no eosinophils were found in blood of the only surviving bird.

Table 7. Eosinophil leukocyte count in chicken blood

	Days after application of ^{32}P											
	Control group						Test group					
No	0.	1.	3.	5.	7.	10.	0.	1.	3.	5.	7.	10.
1	1.40	2.30	1.55	1.44	0.42	0.86	0.97	1.67	0.06	0.20	0	0
2	0.46	0.58	0.68	1.63	-	0.54	1.17	0.24	0.61	0.13	0.12	-
3	-	0.90	0.39	1.55	2.66	1.13	0.69	0.56	0	0	0	-
4	1.23	2.72	2.32	3.9	2.60	0.81	1.65	1.25	0.42	0.10	0	-
5	0.59	1.80	1.84	1.51	0.68	0.88	1.50	1.98	0.37	0.16	0	-
M	0.92	1.66	1.36	2.00	1.59	0.84	1.20	1.14	0.29*	0.12*	0.02	-
S.D.	0,46	0.81	0.72	0.95	1.04	0.19	0.39	0.66	0.23	0.07	0.05	-
S.E.	0.21	0.36	0.32	0.48	0.52	0.08	0.17	0.29	0.10	0.03	0.02	-

* Statistical significance of difference between mean values (M) of test and control groups of chickens ($P<0.05$).

Pathomorphological examination. Pathoanatomic examination. Results of pathoanatomic examination of dead animals of the test group revealed a general anaemic condition and dotted bleeding sites on the heart and mucous membrane of intestines and stomach. Also, contaminated chickens presented marked changes on parenchymal organs manifested in spleen atrophy, nephrosis, fatty liver degeneration and myocard degeneration.

Pathohistological examination. The findings of pathohistological examination of organs and tissues showed changes in the liver, duodenum, spleen, kidney, cloacal bursa, lungs, pancreas, adrenal gland and arterioles of all dead animals in the test group. The changes in the livers included parenchymal bleeding with marked full-bloodness and dilatation of hepatic sinusoids in which fibrinoid protein deposits and scattered vacuolar; degeneration with occasional focal hepatic lesions were observed. In duodenum, examination revealed marked congestion with bleeding and intensive epithelial desquamation and occasionally marked intestinal villus atrophy. Spleen presented the highest degree of white pulp atrophy associated with congestion and bleeding, as well as densely disseminated foci of necrosis with basophil coccoid bodies (bacteria) in their centres. Heart examination revealed myofibrillic degeneration and marked full-bloodness with occasionally intensive subepicardial bleeding. Examination of kidneys revealed bleeding and tubulonephrosis with tube dilatation and glomerual shrinkage. Examination of cloacal bursa revealed atrophy of bursal folds and follicles, numerous epithelial invaginations involving peripheral follicles, marked diffuse depletion of lymphoid follicular sheath and medulla cells, including the presence of macrophages and exposure of epitheloid and reticular base and interstitial hyperplasia. Lungs examination revealed necrotic foci, bleeding, abundant mass of

erythrocytes and fibroid substance inside tertiary bronchi and atria and peribronchial and perivascular fibroplasia. Examination of the pancreas revealed full-bloodness with disseminated vacuolar degeneration of acinus cells and partial necrosis of islet cells. In adrenal gland, full-bloodness and atrophy of adrenal medulla was observed. Finally, in arterioles of all organs of dead chickens in the test group, and particularly those of the spleen and lungs, extensive degenerative changes of walls were noted (thickened entodhelium and intima), very often accompanied by endoarteritis, degeneration of tunica media, lumen restriction and perivascular fibroplasia. Additionally, rupture of arteriolar wall was often observed.

Discussion

Clinical examination. Clinical examination of all contaminated animals revealed symptoms of radiation sickness, which began to appear on the sixth day after application of radioactive phosphorus ^{32}P. The same symptoms were observed by ĐUKIĆ et al. (1964), who contaminated hens with the same quantity of ^{32}P. Unlike in our experiment, contaminated hens survived the 10th day after contamination. The difference between the results in these two experiments is probably due to differences in age and breed of the test animals.

Haematological examination. Erythrocytes and haematocrit. Erythrocyte count and haematocrit values were lower in the test group throughout the trial period. A statistically significant fall in values of the erythrocyte count was recorded from the fifth day, and in haematocrit values from the 7th day after contamination. A possible reason for the decrease of these two indicators is either bleeding (OUMEISH, 2002) or significant reduction in or complete cessation of erythrocyte production (COGGLE, 1983), or a combination of these two causes. Previous investigations have shown that the impact of lethal radiation doses upon erythrocyte percursor cells was the main cause of decrease of these parameters. Precisely, elevated radiation doses cause significant damage to bone marrow and can lead to its total "breakdown" (ADELSTEIN and DEALY, 1965). Bone marrow is equally sensitive to beta-radionucleids which, after having penetrated a body, deposit mostly in bones (JARPLID, 1978; GILLETT et al., 1987). However, in our experiment, considering that a significant fall in erythrocyte count was registered as early as the 5th day after contamination, the most probable reason for such a decrease in these three indicators was the bleeding, which occurred because of increased vascular permeability (PARK et al., 2000) as demonstrated by subsequent dissection and pathohistological examination. Similar findings have been reported by ĐUKIĆ et al. (1964), and KRALJEVIĆ et al. (1988).

Thrombocytes. Similar to the above described indicators, a statistically significant reduction in thrombocyte count in the test group was registered from the 5th day after contamination. One of the reasons for such an early decrease of thrombocyte count in peripheral circulation could be either decresed hematopoetic activity after radiation

exposure (PECAUT et al., 2002) or serious damage to bone marrow, i.e. its parent cells - mononuclealthromboplasts (ODELL, 1971). Another reason, which also may be considered in this experiment, is the bleeding which occurs as a consequence of exposure to a lethal radiation dose (OUMEISH, 2002). A more drastic decrease in thrombocytes count than in erythrocytes count is most probably due to a shorter thrombocyte life cycle which, in the case of chickens, is about 8 days (SWENSON, 1993) as compared to the erythrocyte life cycle, which lasts 28 - 35 days (SMITH et al., 2000). Similar findings have been reported for hens by KRALJEVIĆ et al. (1988) for chickens. Our results additionally correspond to those obtained by LUCAS and DENINGTON (1957), who also conducted their research on chickens, as well as to results reported by authors who conducted their research on dogs (GILLETT et al., 1987) and pigs (MILOŠEVIĆ et al., 1984).

Total leukocyte count. Results indicated a statistically significant decrease in total leukocyte count in the trial group from the very first day after contamination. These results correspond to those obtained by other authors, who also recorded leukopoenia in poultry the first day after internal contamination with radioactive phosphorus ^{32}P (ĐUKIC et al., 1964). Similar results were obtained by LUCAS and DENINGTON (1957) after having exposed chickens and hens to sub-lethal doses of X-radiation, and by KRALJEVIĆ et al. (1988) who, in their experiment conducted on chickens contaminated with 166,6 MBq/kg of a body mass of ^{32}P, noted a fall in total leukocyte count on the third day after contamination.

Lymphocytes. Similar to total leukocyte count, the lymphocyte count in blood of contaminated chickens presented a statistically significant decrease (to 45% of initial value) on the very first day after contamination. From the 3rd to the 7th day this value was reduced to 4.7% of the initial count, while on the 10th day no lymphocytes were found in the only surviving animal. The fall in lymphocyte count was therefore rapid and drastic. In view of the fact that lymphocytes, besides parent cells of the bone marrow, are the body cells most sensitive to radiation (JACOBS, 1998) such a result was expected. And identical result was achieved by KRALJEVIĆ et al. (1988) in tests conducted on chickens, and by LUCAS and DENINGTON (1957) on chickens and laying hens exposed to various X-ray doses. The higher the dose, the earlier the occurrence and the longer the period of decrease in lymphocyte count. The correlation between radiation dose and lymphocyte decrease rate in the peripheral circulation has also been confirmed by PECAUT (2002).

Heterophils, eosinophils. Results indicated a statistically significant fall in eosinophil and heterophil counts from the 3rd resp. 5th day after contamination. The later decrease in the number of eosinophils and heterophils, as compared to lymphocytes, is obviously due to the fact that lymphocytes feature higher radio-sensitivity than eosinophils and heterophils (JACOBS, 1998). Also, in tests conducted on irradiated animals, some authors have even noted an increase in heterophil count on the 1st day after irradiation, with a subsequent fall on the 3rd day after irradiation (LUCAS and DENINGTON, 1957). The observed hypergranulocytoses

were the consequence of sub-lethal radiation doses. However, as we applied a lethal dose and took blood samples 24 h. after contamination, it is possible that the increase of granulocytes in blood, if any, was not noted. There was a similar occurrence in the research conducted by GILLETT et al. (1987), who also did not note hypergranulocytosis. As regards the earlier fall of the number of eosinophils as compared to heterophils, this is most probably due to the "stimulating" effect of radiation on the former, i.e. non-occurrence of a rise in the number of eosinophils immediately after contamination. This has also been confirmed by the results achieved by LUCAS and DENINGTON (1957) who, after having exposed chickens to a radiation dose of 77.4 mC/kg, did not note an increase of eosinophils in blood, but only a rise in number of heterophils. This also corresponds to the results achieved in tests conducted on mammals, where the increase in number of granulocytes after irradiation was mostly due to the increase in number of neutrophils - which are the mammalian counterpart of avian heterophils in chicken blood (JACOBS, 1998).

Pathomorphological examination. Results of pathomorphological examination indicate significant differences between the control and test groups of animals. In the contaminated chicken group, established changes indicate the consequences caused by radioactive radiation. Some of these changes, in particular those involving the spleen and bursa Frbricii, most probably occurred as a result of direct impact of radioactive radiation on these organs. This corresponds to the results reported by CASARET (1980), indicating similar changes. Also, it is generally known that the effects of radioactive radiation on tissues and organs of contaminated or irradiated animals depend on the radiation dose and on the sensitivity of individual tissues and organs (COTRAN et al., 1989). This fact is based on cell activity in a specific tissue (COGGLE, 1983). Analogously, the changes observed in these organs correspond to the lethal quantity of radioactive ^{32}P applied to test chickens. This led to changes in blood vessels in particular, their increased permeability and subsequent transudation into perivascular tissue of the spleen, lungs and liver, which also correspond to the changes described in the relevant literature (CASARET, 1976; COGGLE, 1983).

Finally, we can conclude that radioactive phosphorus ^{32}P, applied at a dose of 333 MBq per kg of body mass to 50-day-old chickens of the Jata heavy breed caused: 1) anaemia, due to the fall in erythrocyte count on the 5th day after contamination, and a fall in haematocrit value on the 7th day after contamination; 2) leukopoenia, due to the fall in lymphocyte count on the 1st day; decreased eosinophil count on the 3rd day, and decreased heterophil on the 5th day after contamination; 3) thrombocytopoenia, on the 5th day after contamination; 4) onset of clinical signs of radiation sickness on the 6th day, and death of all contaminated animals on the 9th and 10th days after contamination. Pathoanatomic examination of dead animals revealed general anaemia, dotted bleeding sites on heart and mucous membrane of digestive tract, as well as changes in parenchymal organs. Pathohistological examination of tissues and organs confirmed the findings of pathoanatomic examinations, which indicated the changes caused by radioactive radiation.

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SAŽETAK

Meso peradi po svojoj kvaliteti i relativno niskoj cijeni, zbog jednostavnog i kratkog proizvodnog ciklusa, zauzima vrlo visoko mjesto u prehrani namirnicama životinjskoga podrijetla. U okolnostima radioaktivne kontaminacije meso peradi, upravo zbog jednostavne i brze proizvodnje, može postati glavni izvor bjelančevina životinjskoga podrijetla. Navedene činjenice, te činjenica da ni do danas nije načinjeno istraživanje o učinku radioaktivnog ^{32}P u tovnih pilića, potaknule su nas da istražimo utjecaj letalne količine ovoga radionuklida u peradi u dobi za klanje. Istraživanje je obavljeno na pilićima teške pasmine »Jata« oba spola, starosti 50 dana i tjelesne mase od 1500 i 2000 g. Pilićima je apliciran radiaoktivni ^{32}P u količini od 333 MBq po kilogramu tjelesne mase. Pokusne su životinje klinički pregledane, a krv za hematološku analizu uzimana je iz krilne vene. Neposredno nakon smrti životinja učinjena je razudba i patohistološka pretraga. Dobiveni rezultati pokazali su da ^{32}P, apliciran pilićima teške pasmine »Jata« 50. dana starosti u količini od 333 MBq/kg tjelesne mase uzrokuje: 1) anemiju koja se očituje padom broja eritrocita 5. dana nakon radioaktivne kontaminacije te padom hematokrita koji se očituje 7. dana nakon radioaktivne kontaminacije; 2) leukopeniju uzrokovanu padom broja limfocita 1. dana nakon radioaktivne kontaminacije, smanjenjem broja eozinofila 3. dana nakon radioaktivne kontaminacije i smanjenjem broja heterofila 5. dana nakon radioaktivne kontaminacije; 3) trombocitopeniju 5. dana nakon radioaktivne kontaminacije; 4) pojavu kliničkih znakova radijacijske bolesti 6. dana i smrt svih kontaminiranih životinja 9. i 10. dana nakon radioaktivne kontaminacije. Patohistološka pretraga tkiva i organa sukladna je patoanatomskom nalazu.

Ključne riječi: pilići, radioaktivni fosfor ^{32}P, hematologija, klinička slika, patomorfološke promjene