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Original article

Natural immunity factors in Polish mixed breed rabbits

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Abstract

Mixed-breed rabbits in Poland are widely used for diagnostic and scientific research and as utility animals, therefore there is a need to know their immunological status, as well as their haematological status. In this study natural immunity factors were analyzed in Polish mixed-breed rabbits and Polish mixed-breed rabbits with addition of blood of meet-breed, considering the impact of sex and season of the year (spring, summer, autumn, winter) using measurement of non-specific cellular and humoral immunity parameters in peripheral blood. The study has revealed that there is a variety between the two commonly used mixed-breed types of rabbits, especially when sex and season is concerned, which is crucial for using these animals in experiments.

Key words: mixed breed rabbits, natural immunity, acquired immunity, sex, season

Introduction

Studies regarding immunological factors of blood in rabbits are few as compared to those dealing with analogical parameters in other laboratory animals (mice, rats). Observations related to natural immunity factors, measured with phagocytosis process, namely **non-specific cellular immunity** and **humoral immunity** parameters, in Polish mixed-breed rabbits, are very limited (Deptuła et al. 1995, Deptuła et al. 2002, Deptuła et al. 2005, Nowaczyk et al. 2005, Deptuła et al. 2008). Furthermore, when assessing such parameters in rabbits, the impact of the season of the year and sex of the animals have not been analysed, and the importance of such factors has been evidenced in reference to haematological factors (Pintor and Grassini 1957, Fox and Laird 1970, Nowaczyk et al. 2005, Burnett et al. 2006, Chineke et al. 2006, Black et al. 2009, Cetin et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Yaqub et al. 2013), although Nowaczyk et al. (2005) recorded, in Polish mixed-breed rabbits, the influence of the year season on the polimorphonuclear (PMN) cells adherence capacity and lysozyme (LZM) volume in serum. It must be mentioned that natural immunity factors are so important that their role has been evidenced in bacterial and viral infections in rabbits (Tew et al. 1971, Tokarz-Deptuła 1998, Hukowska-Szematowicz 2006, Niedźwiedzka 2008, Tokarz-Deptuła 2009) and when assessing various

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substances, including drugs (Prokopowicz 1972, Prokopowicz et al. 1972, Prokopowicz and Ziobro 1972, Szmigielski 1972, Rausch and Moore 1975, Garbuliński et al., 1985, Dębowy et al. 1988, Obmińska-Domoradzka 1992, Świtała 1992, Jasińska et al. 1993, Obmińska-Domoradzka et al. 1993, Szeniawska et al. 1993, Wessely-Szponder et al. 2012).

Despite general breeding of mixed-breed rabbits in Poland, used for diagnostic and scientific research and as utility animals, there are actually hardly any studies on natural immunity factors measured with non-specific cellular and humoral immunity parameters in such animals, dealing with developing reference values for them. Such a condition became the reason for undertaking a study leading to the development of reference values for such factors, namely non-specific cellular immunity factors (adherence and PMN cells absorption capacity, potential killing capacity assessed with spontaneous, stimulated and spectrophotometric nitro-blue tetrazolium (NBT) reduction test, stimulation index, spontaneous and stimulated granulocyte metabolic activity ratio), and humoral immunity factors [oxygen-dependent killing capacity assessed with myeloperoxidase (MPO) activity and oxygen-independent killing capacity, measured with lysozyme (LZM) volume and activity] in peripheral blood of Polish mixed-breed rabbits and Polish mixed-breed rabbits with addition of blood of meet-breed, considering the impact of sex and season of the year (spring, summer, autumn, winter).

Materials and Methods

Animals and scheme of the experiment

The study was performed on 200 mixed-race rabbits and 200 mixed-race rabbits with addition of blood of meet breed, coming from a licensed breeding farm under continuous veterinary and zootechnical supervision (Annon 1987), weighting from 3.2 to 4.2 kg, aged 6-8 months, females and males in four seasons of the year – spring, summer, autumn and winter. During the experiment, the animals stayed at the vivarium of the Department of Microbiology and Department of Immunology, Faculty of Biology, University of Szczecin, where zootechnical parameters abide by the standards recommended in Poland developed in line with the European Union Directive as regards temperature and humidity, as well as lighting and size of cages for animals (Anon 2006). After transportation to the Department vivarium, the animals were provided with a two-week adaptation period. The animals were fed all-mash rabbit feed (16% Królik z Motycza), at volume of 0.15-0.20 kg/day, and had unlimited access to water.

Blood drawing was carried out twice (every seven days) in four seasons. Blood for tests was drawn by establishing a port from the marginal vein of the ear, in 24-hour intervals, for three consecutive days, at 8:00 AM, namely at hours 0, 24 and 48 h from commencement of the study.

Methods used in the experiment

In blood, parameters of natural immunity measured by parameters of non-specific cellular and humoral immunity were investigated, according to the methods descried before (Niedźwiedzka-Rystwej and Deptuła 2010). All the results were subjected to statistical analysis with Student t test in the Statistica software version 6.0 (StatSoft, Poland) and are shown in Tables 1-4.

Results

When assessing the details in the area of values of PMN cell adherence capacity, it must be stated that they remained within the range from 25.47 to 41.65%, spectrophotometric NBT test: from 3.31 to 7.32 x 10^9/l, and stimulation index: from 1.56 to 2.23 (Table 1). In turn, in the case of PMN cell absorption index, it was recorded that its values remained within the range of from 4.90 to 6.70. As regards the percentage of absorbing cells, it was recorded that the values ranged from 73.57 to 83.79%. However, in the case of spontaneous NBT test values, it was determined that they remained within the range of from 7.32 to 13.32, stimulated NBT test: from 15.72 to 22.86, and spontaneous test of metabolic activity of granulocytes: from 0.26 to 0.50, while spontaneous test of metabolic activity of granulocytes: from 0.40 to 0.87.

Detailed analysis of the impact of the seasons on the studied elements of peripheral blood in rabbits without differentiation of sex (Table 1) revealed statistically significant differences between the values obtained in spring and summer which refered to the PMN cell adherence capacity, spectrophotometric and spontaneous, as well as stimulated NBT test, and stimulation index; between spring and autumn – to adherence capacity and PMN cell absorption index, NBT and stimulated granulocyte metabolic activity test, as well as stimulation index; between spring and winter – to PMN cell absorption capacity index, spectrophotometric, spontaneous and stimulated NBT test, as well as stimulated granulocyte metabolic activity test. The assessment revealed differences between:

Table 1. Values of natural immunity (parameters of non-specific cell mediated immunity) in mixed breed Polish rabbits.

Parameters			Values of studied parameters											
			spring			summer			autumn			winter		
			female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)
PMN cell adherence capacity (%)		$\bar{x}$	35.92	34.12	35.02^{b1}	25.47	23.71	24.59	41.65	41.27	41.46^{b2b4b6}	32.42	39.86	36.14^{b5}
		SD±	2.38	2.04	2.15	2.03	2.57	2.58	2.33	2.54	2.14	2.08	2.32	2.17
Absorbing capacity	absorption index (l.b)	$\bar{x}$	6.13	6.70	6.42^{b2b3}	6.48	6.46	6.47^{b4b5}	5.33	5.52	5.43	4.90	4.90	4.90
		SD ±	0.21	0.16	0.75	0.86	0.81	0.82	0.89	0.85	0.89	0.49	0.73	0.57
	% of absorbing cells (%)	$\bar{x}$	82.77	83.79	83.28	73.57	76.59	75.08	76.66	78.33	77.49	73.84	71.93	72.89
		SD ±	7.96	7.26	8.01	14.75	11.51	12.45	11.68	9.83	10.45	15.67	14.88	14.96
NBT reduction test	spektrophotometric (10^9/l)	$\bar{x}$	5.38	4.87	5.13^{b1}	4.14	3.31	3.73	5.63	5.94	5.79^{b4}	6.68	7.32	7.00^{b3b5b6}
		SD ±	0.63	0.75	0.76	0.89	0.96	0.98	0.73	0.65	0.64	0.45	0.59	0.56
	spontaneous (l.b)	$\bar{x}$	7.32	8.82	8.07	13.32^{a}	9.86	11.59^{b1b4b5}	9.69^{a}	8.38	9.04	11.04^{a}	9.60	10.32^{b3b6}
		SD ±	0.67	0.53	0.58	0.38	0.32	0.36	0.53	0.66	0.61	0.38	0.39	0.38
	stimulated (l.b)	$\bar{x}$	15.99	15.72	15.86	21.47^{a}	19.29	20.38^{b1}	21.25	21.27	21.26^{b2}	22.39	22.86	22.63^{b3}
		SD ±	0.86	0.53	0.67	0.76	0.80	0.79	0.63	2.31	1.97	0.98	0.76	0.75
	stimulation index (l.b)	$\bar{x}$	1.59	1.83	1.71^{b2}	1.78	2.23^{a}	2.01^{b1b4b5}	1.70	1.71	1.71	1.56	1.71	1.64
		SD ±	0.27	0.36	0.32	0.63	0.78	0.88	0.62	0.62	0.62	0.42	0.47	0.48
WAMG	spontaneous (l.b)	$\bar{x}$	0.39	0.50	0.45	0.35	0.26	0.31	0.38	0.33	0.36	0.33	0.32	0.32
		SD ±	0.03	0.04	0.04	0.09	0.04	0.05	0.03	0.02	0.03	0.04	0.07	0.06
	stimulated (l.b)	$\bar{x}$	0.40	0.44	0.42	0.56	0.52	0.54	0.87^{a}	0.84	0.85^{b2b4}	0.68	0.78	0.73^{b3b5}
		SD ±	0.02	0.03	0.02	0.08	0.04	0.06	0.08	0.09	0.07	0.08	0.09	0.08

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, ^{a} – statistically significant difference between males and females, ^{b} – statistically significant difference between seasons (together), ^{b1} – statistically significant difference between spring and summer; ^{b2} - statistically significant difference between spring and autumn; ^{b3} – statistically significant difference between spring and winter; ^{b4} – statistically significant difference between summer and autumn; ^{b5} – statistically significant difference between summer and winter; ^{b6} – statistically significant difference between autumn and winter.

Table 2. Values of natural immunity (parameters of non-specific humoral mediated immunity) in mixed breed Polish rabbits.

Parameters		Values of studied parameters											
		spring			summer			autumn			winter		
		female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)
Myeloperoxidase activity (MPO) (l.b.)	$\bar{x}$	1.90	1.75	1.83^{b2}	2.21	2.12	2.17^{b4}	1.25	1.26	1.26	1.71	1.83	1.77^{b5b6}
	SD ±	0.29	0.22	0.25	0.34	0.34	0.39	0.26	0.28	0.27	0.43	0.36	0.40
Lysozyme concentration (mg/l)	$\bar{x}$	2.36	2.21	2.29^{b2}	2.62^{a}	2.06	2.34^{b4}	1.60^{a}	1.12	1.36	4.06^{a}	3.04	3.55^{b3b5b6}
	SD ±	0.37	0.38	0.33	0.27	0.33	0.30	0.14	0.15	0.14	0.28	0.24	0.26
Lysozyme activity index (l.b.)	$\bar{x}$	0.0016	0.0018	0.0017	0.0037	0.0077^{a}	0.0057^{b1b4b5}	0.0012	0.0011	0.0012	0.0025	0.0019	0.0022^{b6}
	SD ±	0.0004	0.0007	0.0006	0.0005	0.0006	0.0005	0.0004	0.0004	0.0004	0.0002	0.0003	0.003

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, ^{a} – statistically significant difference between males and females, ^{b} – statistically significant difference between seasons (together), ^{b1} – statistically significant difference between spring and summer; ^{b2}– statistically significant difference between spring and autumn; ^{b3} – statistically significant difference between spring and winter; ^{b4} – statistically significant difference between summer and autumn; ^{b5} – statistically significant difference between summer and winter; ^{b6} – statistically significant difference between autumn and winter.

Table 4. Values of natural immunity (parameters of non-specific humoral immunity) in mixed breed Polish rabbits with addition of blood of meet breed.

Parameters		Values of studied parameters											
		spring			summer			autumn			winter		
		female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)
Myeloperoxidase activity (MPO) (l.b.)	$\bar{x}$	1.63	1.51	1.60	1.53	1.54	1.54	2.10	2.31	2.20^{b2b4}	1.89	2.05	1.97^{b5}
	SD ±	0.31	0.21	0.29	0.37	0.40	0.44	0.36	0.35	0.39	0.20	0.20	0.28
Lysozyme concentration (mg/l)	$\bar{x}$	0.48	0.47	0.48^{b1b3}	0.22	0.26	0.24	0.60	0.56	0.58^{b4b6}	0.16	0.26	0.22
	SD ±	0.10	0.26	0.20	0.07	0.22	0.17	0.48	0.40	0.44	0.07	0.17	0.14
Lysozyme activity index (l.b.)	$\bar{x}$	0.00245	0.00285	0.00254	0.0029	0.0046^{a}	0.0038^{b5}	0.0035	0.0044^{b6}	0.00392^{b2}	0.0023	0.0032	0.0027
	SD ±	0.0019	0.00494	0.00273	0.00049	0.00172	0.00129	0.00024	0.000278	0.00026	0.000162	0.00019	0.00018

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, ^{a} – statistically significant difference between males and females, ^{b} – statistically significant difference between seasons (together), ^{b1} – statistically significant difference between spring and summer; ^{b2}– statistically significant difference between spring and autumn; ^{b3} – statistically significant difference between spring and winter; ^{b4} – statistically significant difference between summer and autumn; ^{b5} – statistically significant difference between summer and winter; ^{b6} – statistically significant difference between autumn and winter.

Table 3. Values of natural immunity (parameters of non-specific cell immunity) in mixed breed Polish rabbits with addition of blood of meet breed.

Parameters			Values of studied parameters											
			spring			summer			autumn			winter		
			female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)
PMN cell adherence capacity (%)		$\bar{x}$	29.46	23.97	28.22	30.07	29.43	29.73	46.12[a]	30.45	38.88[b2b4]	42.04	44.28	43.21[b3b5]
		SD ±	7.71	7.60	7.91	7.68	7.63	7.12	12.57	8.87	6.13	14.22	14.97	14.50
Absorbing capacity	absorption index (l.b)	$\bar{x}$	6.17	5.83	6.04	5.56	5.50	5.52	6.12	5.85	5.99	5.86	6.49	6.16
		SD ±	0.76	0.69	0.44	0.49	0.46	0.47	0.61	0.38	0.53	0.64	0.64	0.71
	% of absorbing cells (%)	$\bar{x}$	62.25	66.29	63.74	74.77	76.62	75.76[b1]	74.76	80.81	77.79[b2]	76.90	74.29	75.66[b3]
		SD ±	11.14	12.93	9.09	14.46	14.30	14.35	16.01	14.03	15.92	16.15	15.45	17.93
NBT reduction test	spektrophoto-metric (10^9/l)	$\bar{x}$	4.89	4.61	4.05	3.92	4.22	4.08	4.51	5.39[a]	4.94[b2b4]	5.20	4.50	4.86[b3b5]
		SD ±	0.59	0.44	0.39	0.46	0.80	0.65	0.38	0.44	0.44	0.59	0.43	0.43
	spontaneous (l.b)	$\bar{x}$	10.92	12.71	11.58[b3]	10.05	10.58	10.3[b5]5	9.07	9.17	9.12	8.25	8.61	8.42
		SD ±	1.44	1.36	1.98	1.76	1.10	1.06	1.30	1.03	1.08	1.30	1.23	1.27
	stimulated (l.b)	$\bar{x}$	21.17	20.29	20.84	22.80	23.68	23.26	22.53	22.54	22.53	23.16	22.24	22.72
		SD ±	2.21	2.93	2.46	3.11	3.69	3.44	1.84	1.86	1.84	1.97	2.97	2.53
	stimulation index (l.b)	$\bar{x}$	2.82	2.86	2.86	2.34	2.29	2.32	2.52	2.49	2.51	2.78	2.63	2.71
		SD ±	0.63	0.50	0.86	0.59	0.49	0.54	0.44	0.49	0.46	0.54	0.47	0.51
WAMG	spontaneous (l.b)	$\bar{x}$	0.35	0.37	0.43[b2]	0.32	0.38	0.35	0.23	0.30	0.26	0.36	0.33	0.34
		SD ±	0.08	0.13	0.11	0.11	0.14	0.13	0.07	0.08	0.08	0.10	0.11	0.11
	stimulated (l.b)	$\bar{x}$	0.98	0.89	0.95[b2]	0.72	0.85	0.79	0.56	0.76	0.66	0.95	0.68	0.84
		SD ±	0.18	0.20	0.19	0.22	0.27	0.25	0.14	0.26	0.23	0.23	0.15	0.24

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, [a] – statistically significant difference between males and females, [b] – statistically significant difference between seasons (together), [b1] – statistically significant difference between spring and summer; [b2] statistically significant difference between spring and autumn; [b3] statistically significant difference between spring and winter; [b4] – statistically significant difference between summer and autumn; [b5] statistically significant difference between summer and winter; [b6] – statistically significant difference between autumn and winter.

summer and autumn in adherence and PMN cell absorption capacity, spectrophotometric and spontaneous NBT test, and stimulation index, as well as granulocyte metabolic activity test; summer and winter in PMN cell adherence and absorption capacity, spectrophotometric and spontaneous NBT test, and stimulation index, as well as stimulated granulocyte metabolic activity test; autumn and winter in the area of PMN cell adherence capacity, and spectrophotometric and spontaneous NBT test. Investigations dealing with the impact of the seasons on the factors of natural immunity considering sex of the animals (Table 1) revealed that the seasons affected males and females in a different way, as in females, statistically significant values were recorded in summer, autumn and winter, while in males exclusively in summer, and in females they referred to spontaneous NBT test and stimulated granulocyte metabolic activity test, while in males exclusively to stimulation index.

The analysis of the results obtained in the area of natural immunity factors measured with phagocytosis process as regards *non-specific humoral* immunity factors in Polish mixed-breed rabbits (Table 2) revealed that MPO activity values remained within the range of from 1.25 to 2.21 (Table 2). However, LZM concentration values remained within the range of from 1.12 to 4.06 mg/l, and LZM activity from 0.0011 to 0.0077 (Table 2).

Detailed analysis of the impact of the seasons on the elements of peripheral blood in mixed-breed rabbits without considering the sex (Table 2) revealed that statistically significant differences between the values obtained in spring and summer were recorded in LZM activity, while between spring and autumn, in MPO activity and LZM concentration; whereas between spring and winter, exclusively as regards LZM concentration. The assessment also revealed differences between summer and autumn, and summer and winter in all three parameters analysed, namely MPO activity and LZM concentration and activity. Investigations on the impact of the seasons on the parameters considering sex of the animals (Table 3) showed that the seasons affected males and females in a different way, as in females, statistically significant values were recorded in summer, autumn and winter, while in males exclusively in winter, and these in females referred to LZM concentration and activity, while in males, exclusively to LZM concentration.

Non-specific cellular and humoral immunity factors in Polish mixed-breed rabbits with addition of blood of meet breeds

Values of PMN cell adherence capacity in Polish mixed-breed rabbits with addition of blood of meet breeds remained within the range of 23.97 to 46.12%, and for spectrophotometric NBT test from 3.92 to 5.39 x 10^9/l (Table 3). In turn, in the case of PMN cell absorption index, it was recorded that its values remained within the range of 5.50 to 6.49. For the percentage of absorbing cells, it was recorded that the values of the parameter remained within the range of 62.25 to 80.81%, and for stimulation index from 2.29 to 2.86. However, in the case of spontaneous NBT test, it was determined that the values remained within the range of 8.25 to 12.71, for stimulated NBT test from 20.29 to 23.26, and for spontaneous test of metabolic activity of granulocytes from 0.23 to 0.43, while for spontaneous test of metabolic activity of granulocytes from 0.56 to 0.98.

Detailed analysis of the impact of the seasons on the studied elements of peripheral blood in the rabbits without differentiation of sex (Table 3) showed that statistically significant differences between the values obtained in spring and summer refered to the percentage of absorbing cells; between spring and autumn, to adherence capacity and percentage of absorbing cells, as well as spectrophotometric NBT, and spontaneous and stimulated granulocyte metabolic activity test; between spring and winter, to PMN cells adherence capacity and percentage of absorbing cells, as well as spectrophotometric and spontaneous NBT test. Differences between summer and autumn were determined in the adherence capacity and spectrophotometric NBT test, whereas between summer and winter, in PMN cell adherence capacity, and spectrophotometric and spontaneous NBT test. In turn there were no statistically significant differences between autumn and winter. Investigations dealing with the impact of the seasons on the analysed natural immunity factors considering animal sex (Table 3), revealed that the seasons in males and females have similar impact, as both in males and females, they were recorded in autumn, however, exclusively in females, in the area of PMN cell adherence capacity, while in males, as regards spectrophotometric NBT test.

While when assessing the results obtained in the aspect of natural immunity factors, as regards phagocytosis process assessed with the *parameters of non-specific humoral immunity* in Polish mixed-breed rabbits with addition of blood of meet breeds (Table 4), it must be stated that the values of MPO activity remained within the range of 1.51 to 2.20. However, LZM concentration values presently remained within the range of 0.16 to 0.60 mg/l, and LZM activity from 0.0023 to 0.0046.

The investigations revealed the impact of the seasons on the analysed elements of peripheral blood in rabbits without considering the sex (Table 4) and stat-

istically significant differences between the values obtained in spring and summer were recorded in the area of LZM concentration, while between spring and autumn, in MPO and LZM activity; between spring and winter, exclusively as regards LZM concentration; between summer and autumn in MPO activity and LZM concentration; between summer and winter in MPO and LZM activity, and between autumn and winter in LZM concentration. In turn, when analysing the impact of the seasons considering the sex of the animals (Table 4), it was found that the seasons only affected males, where changes were recorded in summer and considered only LZM activity.

Discussion

Non-specific cellular and humoral immunity factors in Polish mixed-breed rabbits

When analysing the results obtained for natural immunity factors measured with the process of phagocytosis, namely *non-specific cellular immunity* parameters in Polish mixed-breed rabbits (Table 1), it must be stated that the values obtained can be compared to the results obtained previously on mixed-breed rabbits. The values of PMN cell adherence capacity, spectrophotometric NBT test and stimulation index are similar to the results obtained previously in Polish mixed-breed rabbits, while the results of PMN cell absorption index, bring them closer to the results obtained by Deptuła et al. (1995, 2002, 2005, Nowaczyk et al. 2005, Deptuła et al. 2008), although also lower as well as higher values were previously recorded. The values of percentage of absorbing cells were similar to those obtained previously, although lower values were also obtained in other studies. However, the results of spontaneous NBT test values, stimulated NBT test, and spontaneous test of metabolic activity of granulocytes, as well as spontaneous test of metabolic activity of granulocytes conform to previous results, although in previous studies by Deptuła's team (Deptuła et al. 1995, Deptuła et al. 2002, Deptuła et al. 2005, 2008, Nowaczyk et al. 2005) also higher values were recorded.

When analysing the impact of the season and sex of the animals (Table 1), it must be stated that both the season and sex affected values of the parameters analysed. To conclude on the changes regarding the impact of the seasons on the analysed natural immunity factors measured with phagocytosis process parameters related to non-specific cellular immunity, it can be stated that the season of the year most strongly affects PMN cells adherence capacity, and spectrophotometric and spontaneous NBT test. Slightly smaller variability referred to the absorption and stimulation index, as well as stimulated granulocyte metabolic activity test. Lower number of changes was observed in the stimulated NBT test, whereas the percentage of absorbing cells and spontaneous granulocyte metabolic activity test did not reveal any changes (Table 1). However, while analysing the impact of the seasons on the factors of natural immunity considering sex of the animals (Table 1), it has been evidenced that the seasons affect males and females in a different way, as in females, statistically significant values were recorded in summer, autumn and winter, while in males exclusively in summer, and these in females referred to spontaneous NBT test and stimulated granulocyte metabolic activity test, while in males, exclusively to stimulation index.

In turn, the analysis of the results obtained in the area of natural immunity factors measured with phagocytosis process as regards *non-specific humoral* immunity factors in Polish mixed-breed rabbits (Table 2) has revealed that the values obtained can only be compared to those performed previously in Poland (Deptuła et al. 1995, 2002, 2005, 2008, Nowaczyk et al. 2005). The comparison to other studies (Rausch and Moore 1975) is impossible to perform due to different units used in the data. It must be stated that MPO activity values conform to previous results. However, LZM concentration values, and LZM results are much lower than the values obtained previously, although also in one case they are similar to the values recorded in previous studies (Deptuła et al. 1995, 2002, 2005, 2008, Nowaczyk et al. 2005).

When analysing the impact of the season and sex of the animals (Table 2), it must be stated that both the season and sex affected values of the parameters analysed. To recuperate on the changes regarding the impact of the seasons on such parameters, it can be stated that the season very similarly affects three parameters analysed (Table 2). While analysing the impact of the seasons on the parameters considering sex of the animals (Table 2), it was evidenced that the seasons affected males and females in a different way, as in females, statistically significant values were recorded in summer, autumn and winter, while in males exclusively in winter, and those in females referred to LZM concentration and activity, while in males, exclusively to LZM concentration.

Non-specific cellular and humoral immunity factors in Polish mixed-breed rabbits with addition of blood of meet breeds

The results obtained in the aspect of natural immunity factors, phagocytosis process measured with

the parameters of *non-specific cellular immunity* in Polish mixed-breed rabbits with addition of blood of meet breeds (Table 3), similarly as the results in the area of the same factors obtained in Polish mixed-breed rabbits, can be compared only to the results obtained previously in Poland. It was showed that values of PMN cell adherence capacity, and spectrophotometric NBT test are similar to the results obtained previously in Polish mixed-breed rabbits (Deptuła et al. 1995, 2002, 2005, Nowaczyk et al. 2005, Deptuła et al. 2008). Results of PMN cell absorption index are comparable to the results obtained previously, although also lower and higher values were recorded in previous studies. The values of the percentage of absorbing cells and stimulation index are similar to the results obtained previously. In the case of spontaneous NBT test, stimulated NBT test, and spontaneous test of metabolic activity of granulocytes, and spontaneous test of metabolic activity of granulocytes the data obtained confirm previous studies, although also higher values were recorded previously (Deptuła et al. 1995, 2002, 2005, 2008, Nowaczyk et al. 2005).

The analysis of the impact of the season and sex of the animals (Table 3) showed that both the season and sex affected values of the parameters analysed. The season of the year most strongly affects PMN cells adherence capacity and spectrophotometric NBT test. Slightly smaller activity depending on the season was revealed by such factors as the percentage of absorbing cells, and spontaneous NBT test. One change each was recorded for spontaneous and stimulated granulocyte metabolic activity test, while PMN absorption index, stimulated NBT test and stimulation index did not reveal any changes (Table 3).

When analysing the impact of the seasons on the analysed natural immunity factors considering animal sex (Table 3), it was evidenced that the seasons in males and females have similar impact, as both in males and females, they were recorded in autumn, while exclusively in females, in PMN cell adherence capacity, while in males, as regards spectrophotometric NBT test.

When analysing the results obtained in the aspect of natural immunity factors, as regards phagocytosis process assessed with the *parameters of non-specific humoral immunity* in Polish mixed-breed rabbits with addition of blood of meet breeds (Table 4), it must be stated that the values of MPO activity confirm the results obtained previously. However, LZM concentration values, and LZM activity values are much lower than those obtained previously (Deptuła et al. 2002, Deptuła et al. 2005, Nowaczyk et al. 2005, Deptuła et al. 2008).

The assessment of the results in these rabbits in the aspect of the impact of the season and sex of the animals (Table 4) revealed that both the season and sex affected values of the parameters analysed. To recuperate on the results, it must be stated that the season very similarly affects all the analysed non-specific humoral immunity factors, as almost the same number of changes were recorded for them, namely four for LZM concentration, three for MPO activity, and two for LZM activity (Table 4). In turn, when analysing the impact of the seasons considering the sex of the animals (Table 4), it was evidenced that the seasons only affected males, in whom changes to LZM activity were recorded in summer.

To conclude on the results of the studies, it must be stated that the values of the analysed natural immunity factors in the area of phagocytosis process, as represented by **non-specific cellular and humoral immunity** factors in peripheral blood and serum in rabbits studied, were slightly higher in mixed-breed rabbits with addition of blood of meet breeds as compared to Polish mixed-breed rabbits. The results obtained on a large and uniform animal sample may serve as reference standards for Polish mixed-breed rabbits, and for mixed-breed rabbits with addition of blood of meet breeds, the more so that they are similar to the results of the studies obtained in healthy Polish mixed-breed rabbits. Moreover, it must be pointed out that the values also correspond with results obtained in control groups of rabbits in many studies that were carried out on mixed-breed rabbits, and on pure blood rabbits in the area of bacterial and viral infections (Tew et al. 1971, Tokarz-Deptuła 1998, Hukowska-Szematowicz 2006, Niedźwiedzka 2008, Tokarz-Deptuła 2009) as well as in studies dealing with administration of other substances, including drugs (Prokopowicz 1972, Prokopowicz et al. 1972, Prokopowicz and Ziobro 1972, Szmigielski 1972, Rausch and Moore 1975, Dębowy et al. 1988, Garbuliński et al. 1985, Obmińska-Domoradzka 1992, Świtała 1992, Jasińska et al. 1993, Obmińska-Domoradzka et al. 1993, Szeniawska et al. 1993, Wessely-Szponder et al. 2012).

It must also be added that the present study revealed that both the season of the year and the sex of rabbits affects the factors analysed, although differently in Polish mixed-breed rabbits and mixed-breeds with addition of blood of meet breeds. And so, in the case of the season, it has been evidenced that in Polish mixed-breed rabbits, it principally affects PMN cell adherence capacity, as well as spectrophotometric and spontaneous NBT test, and MPO activity, as well as LZM concentration and activity; while in Polish mixed-breed rabbits with addition of blood of meet breeds, the season principally affects PMN cell adherence capacity and spectrophotometric NBT test, as

well as MPO activity, and LZM concentration and activity. In the case of animal sex, it was recorded that regardless of the type of mixed-breed rabbit, it principally affects the analysed immunological factors, causing more changes in females. In the case of Polish mixed-breed rabbits, in females, these occurred in summer, autumn and winter, and referred to spontaneous NBT test and stimulated granulocyte metabolic activity test, as well as LZM concentration and activity, while in males, they were only recorded in summer and winter, and referred to stimulation index and LZM concentration. In Polish mixed-breed rabbits with addition of blood of meet breeds, both in males and females, these changes were recorded in autumn and referred to PMN cell adherence in females and spectrophotometric NBT test in males. Moreover in males changes occurred also in summer in LZM activity.

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