

# Leukocyte Intracellular Killing Test and selected serum proteins in Polish mixed-breed rabbits<sup>1)</sup>

BEATA HUKOWSKA-SZEMATOWICZ, BEATA TOKARZ-DEPTUŁA,  
PAULINA NIEDŹWIEDZKA-RYSTWEJ, MATEUSZ ADAMIAK,  
ALICJA TRZECIAK-RYCZEK, WIESŁAW DEPTUŁA\*

Department of Immunology, \*Department of Microbiology, Faculty of Biology,  
University of Szczecin, Z. Felczaka 3c, 71-412 Szczecin

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Hukowska-Szematowicz B., Tokarz-Deptuła B., Niedźwiedzka-Rystwej P.,  
Adamiak M., Trzeciak-Ryczek A., Deptuła W.

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### Summary

The study involved an analysis aimed at developing standards regarding leukocytes' IKT, number of serum IgG and total volume of serum immunoglobulin, as well as total protein volume in the blood serum of rabbits, considering the impact of their sex and season of the year on the parameters analyzed. The study was performed on a representative group of Polish mixed-breed rabbits and Polish mixed-breed rabbits with the addition of blood of meat-breeds at the age of 6-8 months. The obtained results of the study in typical Polish mixed-breed rabbits and Polish mixed-breed rabbits with the addition of blood of meat-breeds are similar, as in typical Polish mixed-breed rabbits IKT values amounted to 0.65-0.70; serum IgG ranged from 14.1 to 17.2; total Ig in ZST units amounted to 20.16-31.36, and total protein in serum: 54.14-62.86. In turn, in Polish mixed-breed rabbits with the addition of blood of meat-breeds, IKT values amounted to 0.56-0.65; IgG: 13.1-16.9; total Ig in ZST units: 21.36-31.14, and total protein in serum 48.49-59.16. In the case of both typical Polish mixed-breed rabbits and Polish mixed-breed rabbits with the addition of blood of meat-breeds, little impact of sex was observed on the values of the parameters analyzed, opposite to the season of the year. The latter parameter, in typical Polish mixed-breed rabbits, affected the values of serum IgG values, while to a slightly smaller extent total serum Ig in ZST units, whereas in the case of Polish mixed-breed rabbits with addition of blood of meat-breeds, the season equally strongly affected both serum IgG and total serum Ig in ZST units.

**Keywords:** Polish mixed-breed rabbits, Leukocyte Intracellular Killing Test, serum IgG, total protein

Among laboratory animals, rabbits are generally used for scientific and diagnostic research. Such research is very often carried out in Poland on mixed-breed rabbits, and these are also animals popularly bred for their use as meat. Despite these facts, literature lacks data on reference values in the area of immunological factors in such animals, including mixed-breed rabbits, which are necessary to assess and interpret the results obtained in various studies. In Poland the values of intracellular killing test (IKT) of leucocytes (8, 9), volumes of serum immunoglobulin (Ig) of G class (7, 9, 10) and total volume of serum Ig, expressed in ZST units (7, 9, 11, 23, 24), as well as total protein in serum (11, 23, 24), have been recorded (Tab. 1) for mixed-breed rabbits considering age (7, 11) and season (22, 23, 24). However, no impact of such properties was

assessed on the values of the parameters analyzed. Moreover, in foreign studies analogical observations are even more moderately represented (Tab. 2) and refer exclusively to one study on serum IgG in rabbits of unspecified breed and sex (21), along with several papers referring to the volume of serum total protein in rabbits of New Zealand (5, 25), Riparian Brush (3), Angora (6) and Florida breeds (16, 20), as well as mixed-breeds: Baladi Red, Chinchilla Giganta, French Giant, Papillona, Simenwar and Egyptian breeds (1), and breeds originating from Trinidad and Tobago (5), and Italy (2), of various age and sex. Nevertheless, similarly as in Polish studies, no impact of age and sex on such parameters has been assessed. It must be stated, however, that while analyzing the hematological factors in Polish mixed-breed rabbits, the impact of age and sex (7) as well as season (22) has been evidenced, which was also confirmed in foreign experiments on

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Tab. 1. Leukocyte intracellular killing test (IKT) and selected blood serum proteins in rabbits in the studies by Polish authors

Literature	Number of animals	Age of animals (months)	Sex of animals	Season (month) of the study	IKT (a. no.)	Serum proteins		
						IgG (g/l)	Total Ig volume in ZST units	Total protein (g/l)
13	40	3-11	males	no data	not analysed	not analysed	10.6-28.1	43.0-67.0
9	40	3-5	males and females	no data	not analysed	10.8	20.3	not analysed
10	80	3-4.5	no data	no data	0.65	not analysed	not analysed	not analysed
11	no data	3	no data	no data	0.64	10.8-14.9	8.8-75.0	not analysed
12	200	3-5	males and females	no data	not analysed	14.30	not analysed	not analysed
22	80	none tested	no data	April (W) July (L) October (J) December (Z)	not analysed	not analysed	W-74.5 L-75.0 J-72.2 Z-68.3	W-68.0 L-63.0 J-61.0 Z-62.0
24	78	none tested	no data	April (W) July (L) October, November (J) December, March (Z)	not analysed	not analysed	W-74.5 L-75.0 J-none Z-68.3	W-68.0 L-none J-61.0 Z-none
23	80	no data	no data	(W) (L) (J) (Z)	not analysed	not analysed	W-74.5 L-75.0 J-72.2 Z-68.3	W-68.0 L-63.0 J-61.0 Z-62.3

Explanations: W – spring; L – summer; J – autumn; Z – winter; a. no. – absolute number

Tab. 2. Selected blood serum proteins in rabbits in the studies by foreign authors

Literature	Number of animals	Age of animals (months)	Sex of animals	Rabbit breed and/or mixed-breed type	Season (month) of the study	Serum proteins	
						IgG (g/l)	Total protein (g/l)
1	no data	6-9	no data	Mixed-breeds Baladi Red, Chinchilla Giganta, French Giant, Papillona, Simenwar and Egyptian	no data	not analysed	48.9-58.9
4	366	1-1.5-2	no data	Mixed-breeds from Italy	no data	not analysed	42.0-55.0
5	265	22	males and females	Riparian Brush	March-May (W) June-August (L) September-November (J) December-February (Z)	not analysed	51.0-74.0
6	24	no data	females	New Zealand	no data	not analysed	14.0-84.0
7	70	young and adult	males and females	Mixed-breeds from Trinidad & Tobago and rabbits of New Zealand breed	September, October, November (J), December (Z)	not analysed	70.3-70.4
8	45	no data	males and females	Angora	April (W), July (L), October (J), January (Z)	not analysed	W-43.0 L-43.5 J-43.1 Z-49.4
14	no data	no data	no data	no data	no data	not analysed	55.0-75.0
15	70	no data	no data	Florida	March-April (W) July-August (L) September-October (J) December-January (Z)	not analysed	W-42.0 L-103.0 J-89.0 Z-110.0
19	6	no data	no data	Florida	no data	not analysed	70.0
20	no data	no data	no data	no data	no data	5.0-20.0	not analysed
25	40	10-12	males and females	New Zealand	no data	not analysed	62.5-69.9

Explanations: as in Tab. 1.

rabbits of New Zealand breed, where the relationship of the age of animals (4, 17, 19), season of the year (26) and breed (18, 28) was observed.

There is no data regarding reference values for immunological factors in Polish mixed-breed rabbits

and Polish mixed-breed rabbits with the addition of blood of meat-breeds; animals very often used for experimental studies, although also bred as utility animals, which became the objective of these observations aimed at developing standards regarding

leucocyte IKT, serum IgG and total volume of serum immunoglobulin, as well as total protein in serum of such animals, considering the impact of their sex and season of the year on the parameters analyzed.

### Material and methods

The study involved 400 Polish mixed-breed rabbits (200 Polish mixed-breed rabbits, 200 Polish mixed-breed rabbits with the addition of blood of meat-breeds) of both sexes, weighing 3.2-4.2 kg, aged 6-8 months, labeled as conventional animals originating from a licensed farm remaining under complete zoo-technical and veterinary supervision (15), in the course of four seasons of the year (spring, summer, autumn, winter). During the study, the rabbits remained at the vivarium, where the conditions of the rooms in the aspect of temperature, humidity and lighting conformed to national standards developed in line with the European Union Directive on temperature and humidity, as well as lighting and size of animal cages (12). After transportation to the Department vivarium, the animals were provided with a two-week adaptation period. The animals stayed individually in metal cages designed for such animals, and were fed with full-portion rabbit feed LSK, manufactured in Miłosławiec near Poznań, in the quantity 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 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.

In blood drawn from animals, the following was determined: leucocyte intracellular killing capacity (IKT), volume of serum IgG, total volume of serum Ig, and total protein in serum.

**Leucocyte Intracellular Killing Test (IKT)** was performed according to Quie method (27) in two phases. In Phase I: the *Staphylococcus aureus* 209 P strain, proliferated in the broth base, was centrifugated (2000 revolutions 10 minutes) and rinsed (by centrifugation of 2000 revolutions for 10 minutes) twice with Hanks' fluid. After centrifugation, the bacterial cells' sediment was suspended in 1 ml of Hanks' fluid, preparing the so-called "input suspension". From this suspension, further dilutions were made in the Hanks's fluid to obtain the density of  $3 \times 10^8$  cells in 1 ml (acc. to McFarland's scale). From this sample, by the method of surface inoculation, 0.1 ml of bacterial suspension was inoculated onto the agar medium, and 24 h later the number of colonies cultivated was marked. The number of live bacteria in the sample was calculated according to the formula: *Number of bacterial cultures on the glass*  $\times$  *dilution*  $\times$  10. Bacterial suspension of the density of  $3 \times 10^8$  cells in 1 ml also served to make the "proper marking" in Phase II. In this phase of the study, serum of a healthy rabbit was diluted with Hanks' fluid at the ratio of 1 : 5. Heparinized blood was placed in the thermostat (37°C) for 60 minutes. The skin of leucocytes was collected and suspended in 1 ml of Hanks's fluid and then centrifugated (1800 revolutions for 10 min) and lead to obtain  $5 \times 10^6$  cells/ml. The prepared leucocyte suspension in the quantity of 0.5 ml was placed

in a sterile tube, and then the following were consecutively added: 0.4 ml of diluted serum (1 : 5), and 0.1 ml of bacterial suspension of the  $3 \times 10^8$ /ml density. Such prepared material was incubated at the temperature of 37°C for 20 minutes, with several shaking and centrifugation (500 to 1000 revolutions for 10 minutes). The supernatant was removed and 1 ml of Parker's fluid was added (containing 500 units of gentamycin in 100 ml of fluid). From this sample 0.16 ml of material was sampled (the remaining volume was placed for 2 hours to the thermostat – 37°C), which was then entered to 5 ml of Hanks' fluid, and centrifugated (1000 rpm for 10 minutes). The supernatant was removed and the sediment was suspended in 4 ml of sterile distilled water, agitating vigorously. From these suspensions dilutions were made (using sterile distilled water) of 1 : 100 and 1 : 200, from which 0.5 ml each were inoculated onto regular agar medium. Samples incubated for two hours, after mixing with 5 ml of Hanks's fluid, were centrifugated as described above. The sediment was suspended in 4 ml of sterile distilled water, and then diluted 1 : 10 and 1 : 20. From these dilutions (as above), surface inoculations were made onto glasses with agar medium. After 24 hours of incubation (37°C) on all media, the *Staphylococcus aureus* 209P cultures were calculated. The value of *leukocyte intracellular killing Test (IKT)* was presented as IKT index calculated from the formula:

$$\text{IKT Index} = \frac{\text{Number of colonies proliferated after 120 min (average from dilutions 1 : 10 + 1 : 20)}}{\text{Number of colonies proliferated after 20 min (average from dilutions 1 : 100 + 1 : 200)}}$$

**The concentration of serum immunoglobulin class G (IgG)** was marked with the platelet method according to the IgG standards by ICN (USA-Kit for the quantitative determination of rabbit IgG), in line with the manufacturer's procedure.

**Marking of total number of immunoglobulins (Ig)** was done using Mc Ewan method (13). Immunoglobulins in the blood serum of rabbits were precipitated using zinc sulphate ( $\text{ZnSO}_4$ ), which caused turbidity, and its measurement was made using a spectrophotometer. For each of the tested samples of blood serum, two markings were made: first was the tested sample (0.1 ml of serum + 6 ml  $\text{ZnSO}_4$ ), second was a control sample (0.1 ml of serum + 6 ml distilled  $\text{H}_2\text{O}$ ). After one hour, extinction of tested samples was read against the control samples, at the wave length of 500 nm. Immunoglobulin concentration in the tested samples was read from the analytical curve made from a series of barium chloride ( $\text{BaCl}_2$ ) solutions, which allowed to express immunoglobulin concentration in turbidimetric units (ZST units).

**Titering of total protein in blood serum** is based on the color reaction of protein in an alkaline environment (29). 0.05 ml serum was added to 4 ml biuret reagent and aspired, while the whole was incubated at room temperature for 30 minutes. After incubation, extinction value was read against a blind sample (biuret reagent) at wave length 500 nm. Protein content was read from template curve preparation which involved the use of control serums with titered protein content.

The results of the study in the area of determined immunological factors in the animals analyzed were presented as average values and standard deviations in Tables 3 and 4, and were subjected to statistical analysis with t-Student test at  $p = 0.05$ .

### Results and discussion

When analyzing the results obtained in the area of parameters observed, namely leukocyte IKT, serum IgG, total Ig in ZST units, and total protein in serum (Tab. 3), it must be stated that the values obtained in Polish mixed-breed rabbits can be compared to the results of studies obtained by other Polish authors (Tab. 1) carried out on analogical Polish mixed-breed rabbits, as well as foreign studies (Tab. 2) which referred to one publication regarding serum IgG in rabbits of unspecified breed and sex (20) and several studies (1-6, 14, 16, 20, 25) related to total protein in serum, carried out on rabbits of various breeds and various mixed-breed rabbits.

When assessing leukocyte IKT results in Polish mixed-breed rabbits, it is stated that they remained within the range of from 0.65-0.70 (Tab. 3), and are almost identical with previously obtained results (Tab. 1, literature items 8, 9) in such Polish mixed-breeds. The range of serum IgG in analogical mixed-breed rabbits varied from 14.1 to 17.2 g/l, which conforms to the results obtained in studies on mixed-breed females and males aged 3-5 and 3 months (Tab. 1, literature items 9, 10), and is slightly lower than the result obtained for Polish mixed-breed rabbits – 10.8 g/l aged 3-5 months (Tab. 1, literature item 7). Conversely, when comparing the results obtained for serum IgG performed on Polish mixed-breed rabbits with the results of foreign studies (on rabbits with unspecified breed and sex) (Tab. 2, item 21), it must be stated that the values obtained in the presently analyzed rabbits were comparable, as they ranged from 5.0 to 20.0 g/l. Also, when comparing total volume of serum immunoglobulin Ig expressed in ZST units in

Tab. 3. Leukocyte intracellular killing test (IKT) and selected serum proteins in Polish mixed-breed rabbits

Parameters analysed	Parameter values in the various seasons of the year												
	Spring			Summer			Autumn			Winter			
	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (25)	
Intracellular killing test (a. no.)	$\bar{x}$	0.68	0.72	0.70	0.62	0.68	0.65	0.62	0.72 <sup>a</sup>	0.67	0.61	0.73	0.67
	$\pm$ SD	0.05	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.06	0.06
IgG (g/l)	$\bar{x}$	15.2	15.3	15.3	17.1	17.2	17.2 <sup>b1,b3</sup>	16.2	15.1	15.9	13.1	14.8	14.1
	$\pm$ SD	1.22	1.23	1.22	1.13	1.30	1.21	1.30	1.21	1.25	1.22	1.20	1.21
Total Ig volume in ZST units	$\bar{x}$	26.16	26.41	26.28	20.73	19.52	20.16	29.23	24.91	26.74	31.09	31.58	31.36 <sup>b3</sup>
	$\pm$ SD	4.39	4.99	4.12	6.02	6.68	6.34	6.27	5.54	8.65	8.04	9.15	9.63
Total protein (g/l)	$\bar{x}$	54.74	53.42	54.14	57.67	60.60	59.08	61.04	64.83	62.86	52.51	56.99	54.98
	$\pm$ SD	6.96	5.07	6.17	6.45	6.05	6.40	8.95	9.14	9.96	8.59	7.36	7.21

Explanations: () – numbers in parentheses are the numbers of animals;  $\bar{x}$  – average value;  $\pm$  SD – standard deviation; a – statistically significant difference between females and males; b – statistically significant differences between the values in particular seasons (without considering sex – total): 1 – statistically significant difference between spring and summer, 2 – difference between summer and autumn, 3 – difference between summer and winter, 4 – difference between spring and winter

Tab. 4. Intracellular killing test (IKT) and selected serum proteins in Polish mixed-breed rabbits with addition of meat-breed rabbits' blood

Parameters analysed	Parameter values in the various seasons of the year												
	Spring			Summer			Autumn			Winter			
	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (50)	Females (25)	Males (25)	Total (25)	
Intracellular killing test	$\bar{x}$	0.65	0.61	0.63	0.58	0.59	0.58	0.41	0.70 <sup>a</sup>	0.56	0.69	0.60	0.65
	$\pm$ SD	0.07	0.06	0.06	0.08	0.09	0.08	0.04	0.08	0.06	0.09	0.08	0.08
IgG (g/l)	$\bar{x}$	12.1	14.1	13.1	17.1	16.8	16.9 <sup>b1,b3</sup>	15.1	16.1	15.6	13.3	14.8	14.1
	$\pm$ SD	1.21	1.41	1.36	1.56	1.41	1.50	1.52	1.54	1.43	1.42	1.10	1.01
Total Ig volume in ZST units	$\bar{x}$	19.59	24.40	21.36	29.07	29.68	29.40 <sup>b1</sup>	26.35	26.94	26.68	32.23	29.76	31.14 <sup>b4</sup>
	$\pm$ SD	4.65	6.54	4.92	6.17	5.33	5.72	4.88	4.74	4.78	6.83	7.04	6.48
Total protein (g/l)	$\bar{x}$	53.46	59.78	55.79	59.78	58.62	59.16	49.72	54.96	52.34	49.15	47.76	48.49
	$\pm$ SD	9.01	9.53	9.45	9.17	7.21	8.18	9.62	7.61	8.82	9.97	9.77	9.79

Explanations: as in Tab. 3.

Polish mixed-breed rabbits, which ranged from 20.16 to 31.36, it was parallel with previous studies (because they ranged from 10.6 to 28.1) (Tab. 1, items 7, 11), and lower (because they ranged from 68.3 to 75.0 ZST units) (Tab. 1, item 9, 22, 23, 24) than the values obtained in the present study.

In turn, total protein in serum of typical Polish mixed-breed rabbits in the present study remained within the range of 54.14-62.86 g/l (Tab. 3), which conforms with previous results, also obtained in typical Polish mixed-breed rabbits (Tab. 1, items 11, 22, 23, 24). As compared to the values obtained in foreign studies, these results are both lower and higher, which seems to be related to the rabbit breed or type of mixed-breeds (Tab. 2, literature items 1, 2, 3, 4, 5, 6, 14, 16, 20, 25).

To reiterate, the values obtained in the present study in rabbits, regarding leukocyte IKT, serum IgG, total serum Ig expressed in ZST units, and total protein in serum, it must be stated that both sex and the season affect the values to a small extent. In the case of sex, it was determined that differences between males and females are observed in autumn in the area of leukocyte IKT (Tab. 3), whereas the analysis of the impact of the season (without considering the sexes) on the parameters analyzed revealed that statistically significant differences only refer to the volume of serum IgG between summer and winter, and between spring and summer, as well as in the area of total Ig expressed in ZST units between summer and winter. It must be stated that no impact of the season was detected on leukocyte IKT values and on total protein values in the analyzed rabbits. Therefore to conclude on the image of changes regarding the impact of sex on four analyzed immunological factors, it must be stated that this property has no impact on the parameters analyzed (except for leukocyte IKT values). In turn, the analysis of the impact of the season on four analogical immunological factors analyzed in rabbits without differentiating among sexes indicated that the season affects the values of serum IgG most, as two statistically significant changes to its values were recorded, while to a smaller extent on Ig in ZST units, where one statistically significant change was observed.

When analyzing the results regarding four analyzed factors in Polish mixed-breed rabbits with the addition of blood of meat-breeds (Tab. 4), it must be stated that they are difficult to compare to other study results due to the fact that there are no studies on such mixed-breed rabbits. However, an attempt can be made to compare such values with the values obtained in Polish mixed-breed rabbits (Tab. 1), or various foreign mixed-breed and various breed rabbits (Tab. 2). When assessing the results for leukocyte IKT in Polish mixed-breed rabbits with addition of the blood of meat-breed rabbits, it must be stated that they remain within the range of 0.56-0.65, which conforms to the values recorded for this param-

eter in Polish mixed-breed rabbits (Tab. 1, literature items 8, 9). As regards serum IgG in such rabbits, the values remained within the range of 13.1-16.9, and also correlate to the values obtained for Polish mixed-breed rabbits (Tab. 1, items 7, 9, 10). In turn, in foreign studies performed on rabbits of unspecified breed, the values ranged from 5.0 to 20.0 (Tab. 2, literature item 21). In the case of total Ig in ZST units, in mixed-breed rabbits with the addition of meat-breed rabbits' blood, the values ranged from 21.36-31.14. Comparing the obtained results with results for Polish mixed-breed rabbits (Tab. 1), it should be noted that they were somewhat similar (Tab. 1, literature items 7, 11), but also completely different, because they amounted to 75.0 ZST units (Tab. 1, literature items 9). In the case of total protein in serum of Polish mixed-breed rabbits with the addition of blood of meat-breeds, the range of values amounted to 48.49-59.1 g/l, and was similar to the values recorded for typical Polish mixed-breed rabbits (Tab. 1, literature items 11, 22, 23, 24) and the values obtained in the present study in typical Polish mixed-breed rabbits (Tab. 3), as well as similar to the values recorded in rabbits of various mixed breeds, and of various breeds, obtained in foreign studies (Tab. 2, items 1, 2, 3, 4, 5, 6, 14, 16, 20, 25).

When analyzing the values obtained in the Polish mixed-breed rabbits with the addition of blood of meat-breeds regarding leukocyte IKT, serum IgG, total serum Ig expressed in ZST units, and total protein in serum, it must be stated that they change to a small degree depending on the sex and the season of the year, as observed in the present study in typical mixed-breed rabbits (Tab. 3). In the case of sex, it was determined that the factors analyzed pointed to differences between males and females as regards to IKT (Tab. 4), but only in autumn. In turn, animal sex did not affect the values of such parameters as serum IgG, total volume of serum Ig expressed in ZST units, and total protein. Detailed analysis of the behavior of four factors analyzed in particular seasons of the year (without differentiating among the sexes) revealed that statistically significant differences occur in the area of IgG between spring on the one hand and summer and winter on the other, as well as in the case of total Ig expressed in ZST units, between spring and summer, and spring and winter. It must be pointed out that no impact of the season was observed on the values of intracellular killing by leukocytes and on total protein values. To conclude on the changes regarding the impact of sex on four analyzed immunological factors, it can actually be stated that this parameter has no impact on the analyzed factors. In turn, the analysis of the seasons of the year on the studied factors in Polish mixed-breed rabbits with the addition of blood of meat-breeds revealed that the season most strongly affects the values of serum IgG and total serum Ig in ZST units, as these parameters recorded two statistically significant changes each.

To conclude on the obtained results of the study in typical Polish mixed-breed rabbits and Polish mixed-breed rabbits with addition of blood of meat-breeds, it needs be stated that the results are similar, as in typical Polish mixed-breed rabbits IKT values amounted to 0.65-0.70; serum IgG ranged from 14.1 to 17.2; total Ig in ZST units amounted to 20.16-31.36, and total protein in serum: 54.14-62.86 (Tab. 3). Conversely, in Polish mixed-breed rabbits with the addition of blood of meat-breeds, IKT values amounted to 0.56-0.65; IgG: 13.1-16.9; total Ig in ZST units: 21.36-31.14, and total protein in serum 48.49-59.16 (Tab. 4).

Additionally, in the case of both typical Polish mixed-breed rabbits and Polish mixed-breed rabbits with the addition of blood of meat-breeds, little impact of sex was observed on the values of the parameters analyzed, opposite to the season of the year. The latter parameter in typical Polish mixed-breed rabbits affected the values of serum IgG values, while to a slightly smaller extent total serum Ig in ZST units, whereas in the case of Polish mixed-breed rabbits with the addition of blood of meat-breeds, the season equally strongly affected both serum IgG and total serum Ig in ZST units.

It must also be stated that due to the fact that the values of the parameters analyzed for Polish mixed-breed rabbits and Polish mixed-breed rabbits with the addition of blood of meat-breeds were obtained on a large and equal animal sample, and are similar to results obtained in previous studies (Tab. 1 and 2), they should constitute reference values for Polish mixed-breed rabbits.

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Corresponding author: Beata Hukowska-Szematowicz, PhD, Z. Felczaka 3c, 71-412 Szczecin, Poland; e-mail: beatahsz@gmail.com