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*Original article*

# Hematological parameters in Polish mixed breed rabbits with addition of meat breed blood in the annual cycle

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## Abstract

In the paper we studied haematologic values,such as haemoglobin concentration, haematocrit value, thrombocytes, leucocytes: lymphocytes, neutrophils, basophils, eosinophils and monocytes in the pheral blood in Polish mixed-breeds with addition of meat breed blood in order to obtain the reference values which are until now not available for this animals. In studying this indices we took into consideration the impact of the season (spring, summer, autumn, winter), and sex of the animals. The studies have shown a high impact of the season of the year on those rabbits, but only in spring and summer. Moreover we observed that the sex has mean impact on the studied values of haematological parameters in those rabbits. According to our knowledge, this is the first paper on haematologic values in this widely used group of rabbits, so they may serve as reference values.

**Key words**: rabbits, haematological values, reference values

## Introduction

Among animals used for research in Poland, beside mice and rats, rabbits tend to be important, due to the ability of drawing more blood and the possible usage in many fields of studies. Apart from being a pivotal laboratory animal, rabbit is also a highly valued breeding animal, popular for meat and fur. For the latter, especially mixed-breed rabbits with addition of meat breed blood is used, mainly Belgian Hare. This common use of those animals causes the need for monitoring their health, also by the assessment of blood factor parameters, for which there are no standards in Poland.

Haematological studies performed in Poland leading to the development of reference values in Polish mixed-breed rabbits are rather few (Buczma et al. 1997, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005, Niedźwiedzka-Rystwej and Deptuła 2010). Moreover, even fewer of them refer to the assessment of the impact of environmental elements, such as the season of the year (Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005) and physiological factors, such as sex (Niedźwiedzka-Rystwej and Deptuła 2010) on haematological image in such animals. Also in Polish literature (Knorr 1980, Katkiewicz 1989, Brylińska and Kwiatkowska 1996, Deptuła et al. 2003, Winnicka 2011, Szarek et al. 2013), despite

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having values of haematological parameters in rabbits, there is no assessment of season or sex. On the other hand, the worldwide research have been performed on rabbits of different races (New Zealand, Agora, Czech and Sylvilagus Bachmani Riparius, cottontail, Soviet Chinchilla, grey giant, albino) and many unspecified mixed-breed rabbits, but only some of them have analysed the impact of the season of the year (Pintor and Grassini 1957, Black et al. 2009, Çetin et al. 2009, Abdel-Azeem et al. 2010, Yaqub et al. 2013), sex (Fox and Laird 1970, Kabata et al. 1991, Aleman et al. 2000, Kim et al. 2002, Burnett et al. 2006, Chineke et al. 2006, Black et al. 2009, Çetin et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Yaqub et al. 2013), age (Bortolotti et al. 1989, Chineke et al. 2006, Olayemi and Nottidge 2007, Archetti et al. 2008, Jeklova et al. 2009, Yaqub et al. 2013), and race of rabbits (Cazabon et al. 2000, Rohilla et al. 2000, Burnett et al. 2006, Chineke et al. 2006, Martinec et al. 2012, Yaqub et al. 2013). Also books (Feldman et al. 2000, Harkness et al. 2007) regarding reference values for haematological parameters in rabbits, present such values only for the New Zealand race, and do not account for environmental or physiological factors.

The aim of the study was to develop reference values for haemoglobin concentration, haematocrit value, volume of thrombocytes, leucocytes: lymphocytes, neutrophils, basophils, eosinophils and monocytes in peripheral blood in Polish mixed-breeds with addition of meat breed blood, considering the impact of the season (spring, summer, autumn, winter) and sex of the animals.

## Materials and Methods

The experiment was conducted on 200 Polish mixed-breed rabbits with an addition of blood of meat breeds, from a licenced farm (Annon 1987). The animals were 6-8 months old, weighing 3.2-4.2 kg, both females and males. During the experiment, the animals were kept at the vivarium of the Department of Microbiology and Department of Immunology of the Faculty of Biology at the University of Szczecin, where zoo-technical parameters were in line with the recommended Polish standards developed in line with the European Union Directive as regards temperature and humidity, as well as lighting and size of cages for animals (Annon 2010). After transportation to the Department vivarium, the animals were provided with a two-week adaptation period. The animals were fed with all-mash rabbit feed (16% Królik z Motycza), at amount of 0.15-0.20 kg/day, and had unlimited access to water.

Samples were collected during four seasons of the year (spring, summer, autumn, winter) twice, by placing a port, for three consecutive days, namely at 0, 24 and 48 h from the commencement of the study, each time at 8 am, from marginal vein of the ear (every seven days).

The blood was analysed for haemoglobin concentration, haematocrit value, as well as volume of thrombocytes, leucocytes: lymphocytes, neutrophils, basophils, eosinophils and monocytes according to commonly known and used standards. The results of haematological tests were subject to the statistical analysis with t-Student test with Statistica 6.0 software. It was confirmed with Shapiro-Wilk's test that the population is normally distributed. Results have been presented in Table 1.

## Results

The analysis of the seasons' of the year impact on the blood parameters in Polish mixed-breed rabbits with an addition of blood of meat breeds, without considering the sex (Table 1) revealed that an increase was recorded in females exclusively in summer and autumn, while in males in summer and winter, whereas in females this only referred to the number of eosinophils and neutrophils, while in males – to the number of neutrophils and thrombocytes. The season of the year has big impact, as it affects six (number of basophils, eosinophils, monocytes, and to a lesser degree of lymphocytes, haemoglobin concentration and number of neutrophils) out of ten analysed haematological factors, principally in spring and summer. Sex of the animals affects three seasons: summer, autumn and winter, but to a small extent and only in number of eosinophils (in females), neutrophils (in males and females), and thrombocytes (in males).

## Discussion

The results obtained for haematological parameters in Polish mixed-breed rabbits with an addition of blood of meat breeds (Table 1), have been compared to the results of studies obtained by Polish authors performing observations also on Polish mixed-breed rabbits (Knorr 1989, Brylińska and Kwiatkowska 1996, Buczma et al. 1997, Deptuła et al. 2003, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005, Niedźwiedzka-Rystwej and Deptuła 2010, Winnicka 2011, Szarek et al. 2013), and to a lower extend to the results by other authors regarding rabbits other than Polish mixed-breed rabbits, the results of which studies were presented in the same units as

Table 1. Haematological parameters in mix-breed Polish rabbits with addition of blood of meat breed blood in annual cycle considering sex.

| Parameters | | Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 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) |
| Haemoglobin concentration [mmol/L] | $\bar{x}$ | 10.98 | 9.58 | 9.65 | 11.66 | 11.63 | **11.64**$^{b1}$ | 11.75 | 11.54 | **11.64**$^{b2}$ | 12.15 | 11.61 | **11.89**$^{b3}$ |
| | SD ± | 0.40 | 0.88 | 0.64 | 1.00 | 1.33 | 1.17 | 1.53 | 1.87 | 1.71 | 1.22 | 1.47 | 1.36 |
| Haematocrit [L/L] | $\bar{x}$ | 0.34 | 0.36 | 0.36 | 0.36 | 0.35 | 0.36 | 0.37 | 0.35 | 0.36 | 0.38 | 0.36 | 0.37 |
| | SD ± | 0.027 | 0.028 | 0.034 | 0.034 | 0.031 | 0.038 | 0.038 | 0.038 | 0.034 | 0.034 | 0.029 | 0.033 |
| Thrombocytes [$10^9$/L] | $\bar{x}$ | 490.83 | 498.8 | 179.54 | 396.73 | **519.76**$^{a}$ | 416.40 | 445.13 | 442.00 | 443.63 | 419.53 | 428.08 | 423.50 |
| | SD ± | 139.34 | 120.15 | 174.21 | 91.32 | 84.80 | 101.34 | 157.26 | 149.89 | 152.75 | 104.45 | 116.00 | 109.03 |
| Leukocytes [$10^9$/L] | $\bar{x}$ | 5.16 | 5.36 | 5.23 | 5.22 | 5.69 | 5.93 | 5.43 | 5.24 | 5.34 | 5.24 | 5.59 | 5.82 |
| | SD ± | 0.84 | 0.26 | 0.67 | 0.65 | 0.79 | 0.75 | 0.69 | 0.72 | 0.64 | 0.92 | 0.70 | 0.88 |
| Lymphocytes [$10^9$/L] | $\bar{x}$ | 7.20 | 6.50 | **7.00**$^{b1b2bb3}$ | 6.10 | 6.20 | 6.20 | 5.30 | 5.70 | 5.50 | 6.10 | 5.80 | 5.90 |
| | SD ± | 0.60 | 0.70 | 0.64 | 0.60 | 0.70 | 0.60 | 0.90 | 1.10 | 1.00 | 0.80 | 0.80 | 0.80 |
| Neutrophils [$10^9$/L] | $\bar{x}$ | 3.40 | 3.50 | 3.00 | 3.40 | 3.30 | 3.30 | **4.20**$^{a}$ | 3.60 | **4.00**$^{b1b4b6}$ | 2.80 | **3.30**$^{a}$ | 3.00 |
| | SD ± | 0.60 | 0.60 | 0.60 | 0.60 | 0.50 | 0.53 | 0.50 | 0.50 | 0.50 | 0.60 | 0.60 | 0.50 |
| Basophils [$10^9$/L] | $\bar{x}$ | 0.12 | 0.11 | 0.12 | 0.18 | 0.19 | **0.18**$^{b1}$ | 0.23 | 0.23 | **0.23**$^{b2}$ | 0.30 | 0.27 | **0.28**$^{b3b5}$ |
| | SD ± | 0.01 | 0.01 | 0.04 | 0.06 | 0.05 | 0.05 | 0.07 | 0.06 | 0.07 | 0.07 | 0.06 | 0.06 |
| Eosinophils [$10^9$/L] | $\bar{x}$ | 0.05 | 0.05 | 0.04 | **0.14**$^{a}$ | 0.11 | **0.01**$^{b1}$ | **0.22**$^{a}$ | 0.13 | **0.17**$^{b2b4}$ | 0.16 | 0.19 | **0.17**$^{b5}$ |
| | SD ± | 0.02 | 0.09 | 0.14 | 0.04 | 0.03 | 0.03 | 0.04 | 0.02 | 0.04 | 0.02 | 0.03 | 0.02 |
| Monocytes [$10^9$/L] | $\bar{x}$ | 0.17 | 0.13 | 0.16 | 0.19 | 0.19 | 0.19 | 0.31 | 0.28 | **0.30**$^{b2b4}$ | 0.35 | 0.36 | **0.36**$^{b3b5}$ |
| | SD ± | 0.03 | 0.02 | 0.03 | 0.07 | 0.06 | 0.06 | 0.03 | 0.06 | 0.05 | 0.08 | 0.09 | 0.08 |

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, **a** – statistically significant difference between male and female, **b** – statistically significant difference between seasons (together), where **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 summer and winter; **b6** – statistically significant difference between autumn and winter

in the present study (Pintor and Grassini 1957, Fox and Laird 1970, Bortolotti et al. 1989, Kabata et al. 1991, Aleman et al. 2000, Cazabon et al. 2000, Rohilla et al. 2000, Kim et al. 2002, Burnett et al. 2006, Chineke et al. 2006, Olayemi and Nottidge 2007, Archetti et al. 2008, Black et al. 2009, Çetin et al. 2009, Jeklova et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Martinec et al. 2012, Özkan et al. 2012, Yaqub et al. 2013).

The obtained results for haemoglobin concentration (Table 1) are slightly higher than the results obtained previously (Buczma et al. 1997, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005, Niedźwiedzka-Rystwej and Deptuła 2010) and are comparable to some results of other studies (Bortolotti et al. 1989, Aleman et al. 2000, Abdel-Azeem et al. 2010), while at the same time slightly lower than the values presented in Polish books (Knorr 1980, Deptuła et al. 2003, Winnicka 2011). Haematocrit value in Polish mixed-breed rabbits with addition of blood of meat breeds is comparable to the results obtained in studies on Polish mixed-breed rabbits (Niedźwiedzka-Rystwej and Deptuła 2010) and the values presented in Polish textbooks on unspecified rabbits (Winnicka 2011, Szarek et al. 2013), as well as foreign studies (Hewitt et al. 1989, Hillyer 1994, Aleman et al. 2000, Cazabon et al. 2000, Kim et al. 2002, Black et al. 2009) regarding New Zealand and Sivilagus Bachmani Riparus rabbits. However, as refers to foreign studies, they principally referred to New Zealand and unspecified mixed-breed rabbits, where sporadically lower values (Abdel-Azeem et al. 2010, Ragab et al. 2013) and higher values (Sabolovic et al. 1977, Harkness et al. 2007, Çetin et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Ragab et al. 2013) were recorded. The number of thrombocytes (Table 1) was comparable with results obtained for Polish mixed-breed rabbits in Polish studies (Niedźwiedzka-Rystwej and Deptuła 2010) and textbooks (Knorr 1980, Brylińska and Kwiatkowska 1996, Winnicka 2011, Szarek et al. 2013), and foreign studies referring to New Zealand and unspecified mixed-breed rabbits (Harkness et al. 2007, Black et al. 2009, Poljičak-Milas et al. 2009, Özkan et al. 2012), although in the former (Polish) studies regarding mixed-breed and unspecified rabbits higher values were recorded (Katkiewicz 1989, Deptuła et al. 2003, Tokarz-Deptuła and Deptuła 2005). In the foreign studies lower values were observed for Sylvialagus Bachmani Riparius rabbits (Black et al. 2009). The numbers of leucocytes (Table 1) are similar to previously recorded in Polish studies (Buczma et al. 1997, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005), and textbooks (Brylińska and Kwiatkowska 1996, Deptuła et al. 2003, Szarek et al. 2013), as well as foreign studies on New Zealand, Soviet Chinchilla, grey giant, albino, and mixed-breed rabbits (Rohilla et al. 2000, Archetti et al. 2008, Jeklova et al. 2009). It must be, however, noticed that in other Polish studies on mixed-breed rabbits and foreign studies on Silvilagus Bachmani Riparius rabbits, also slightly lower values were recorded (Black et al. 2009), while in foreign studies on New Zealand rabbits often higher values were specified (Pintor and Grassini 1957, Kabata et al. 1991, Kim et al. 2002, Özkan et al. 2012). As regards the number of lymphocytes and neutrophils (Table 1), it can be stated that the results confirm prior domestic results presented in publications (Buczma et al. 1997, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005, Niedźwiedzka-Rystwej and Deptuła 2010) and textbooks (Knorr 1980, Katkiewicz 1989, Brylińska and Kwiatkowska 1996, Deptuła et al. 2003, Winnicka 2011, Szarek et al. 2013), as well as foreign studies regarding New Zealand, Agora, Czech and Sylvilagus Bachmani Riparius, cottontail, Soviet Chinchilla, grey giant, albino and unspecified mixed-breed rabbits. Similarly, the ranges for basophils, eosinophils, and monocytes are analogically with prior Polish and foreign studies (Table 1). To recapitulate, when comparing the obtained values of red and white blood cell parameters in Polish mixed-breed rabbits with addition of blood of meat breads to prior results both in mixed-breed rabbits (Buczma et al. 1997, Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005, Niedźwiedzka-Rystwej and Deptuła 2010), and pure bred rabbits, it can be stated that, similarly as in the case of Polish mixed-breed rabbits, in a vast majority, the values are similar to the ones obtained previously, although as regards the number of thrombocytes and leucocytes, in New Zealand and Sylvilagus Bachmani Riparius rabbits slightly lower values were recorded (Black et al. 2009, Niedźwiedzka-Rystwej and Deptuła 2010, Ragab et al. 2013), as well as higher ones (Pintor and Grassini 1957, Sabolovic et al. 1977, Kabata et al. 1991, Kim et al. 2002, Tokarz-Deptuła and Deptuła 2005, Harkness et al. 2007, Çetin et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Ragab et al. 2013).

It can be stated that the season of the year has the greatest impact on the values of basophils, eosinophils and monocytes, as four significant changes were recorded for these parameters, as well as on haemoglobin concentration, number of lymphocytes and neutrophils, where three significant changes were registered (Table 1). It was not shown by prior Polish studies (Nowaczyk et al. 2005, Tokarz-Deptuła and Deptuła 2005), where the impact of the season was only observed on the number of neutrophil granulocytes in Polish mixed-breed rabbits. However, the results of

foreign studies are unclear and difficult to compare with the present study. Pintor and Grassini (1957) recorded the impact of the season of the year in unspecified rabbits exclusively on the number of reticulocytes, manifested with increase in autumn and decrease in spring. In turn, in the study by Abel-Azeem et al. (2010), the highest values were recorded for haemoglobin concentration and haematocrit in January, which is not confirmed by the present study. However, Çetin et al. (2009) pointed out that as regards the haemoglobin concentration, the lowest values were recorded in July, which is also different from the present results. The number of leucocytes and lymphocytes was the lowest in July and October, while in our study, the highest number of lymphocytes was recorded in spring. In turn, Black et al. (2009) in Sylvilagus Bachmanii Riparius and rabbits, and Yaqub et al. (2013) in unspecified rabbits, also assessed the impact of the season on haematological parameters in such animals, but did not reveal such impact. To recapitulate these results, it must be stated that the, rather important, impact of the season on such parameters, as revealed in Polish mixed-breed rabbits with addition of blood of meat breeds, is not reflected in prior studies, where the season seems to be of little significance for such parameters, as a smaller number of blood parameters increases, as compared to the present study, including reticulocytes, leucocytes, lymphocytes and haemoglobin concentration. In the present study changes were recorded in the number of basophils, eosinophils, monocytes, and to a smaller extent in lymphocytes, haemoglobin concentration and the number of neutrophils. Furthermore, in our study, the parameters increase more frequently in spring and summer, while in prior studies – in autumn and winter.

The assessment of the impact of the sex of rabbits on the analysed haematological parameters in Polish mixed-breed rabbits with addition of blood of meat breeds, shows that the seasons have a different impact on males and females, as in females significant values were recorded in summer and autumn, while in males – in summer and winter, and in females they refer to the number of eosinophils and neutrophils, while in males – to thrombocytes and neutrophils. The results are hardly comparable to prior Polish studies (Niedźwiedzka-Rystwej and Deptuła 2010), which only involved recording the values both in males and females of Polish mixed-breed rabbits. In turn, the results of the studies obtained presently, as compared to the results obtained abroad, are different and difficult to compare with prior results (Fox and Laird 1970, Kabata et al. 1991, Aleman et al. 2000, Kim et al. 2002, Burnett et al. 2006, Chineke et al. 2006, Black et al. 2009, Çetin et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Yaqub et al. 2013). The present study does not confirm the results obtained by Kabata et al. 1991, who recorded statistically significant lower values of haemoglobin concentration in females of New Zealand breed, and higher values in the number of leucocytes and lymphocytes. Similarly, the results obtained by Özkan et al. 2012, who showed differences between the values obtained in males and females of New Zealand breed exclusively as regards haemoglobin concentration, and the number of granulocytes are different than the present ones. Confirmation of the present results, as regards the number of thrombocytes, can be found in the paper by Burnettet al. 2006 , who revealed that males of New Zealand breed have higher values of this parameter, as well as haemoglobin concentration. The study by Çetin et al. 2009 is not comparable with the present results, as it indicated that in females of Angora breed, lower values are recorded for haemoglobin concentration. Moreover, several studies (Fox and Laird 1970, Aleman et al. 2000, Kim et al. 2002, Chineke et al. 2006, Black et al. 2009, Abdel-Azeem et al. 2010), despite the assessment of the impact of sex on the behaviour of red and white blood cell image parameters in rabbits, did not show any significant changes in this respect. To conclude on the impact of the sex of Polish mixed-breed rabbits with addition of blood of meat breeds on haematological parameters of blood, it is hard to make absolute statements on the impact of this factor on the parameters analysed, as similarly to the case of the first group of rabbits (Polish mixed-breed rabbits) the results are very differentiated. The changes were recorded in the rabbits analysed as regards the number of eosinophils, neutrophils and thrombocytes, while other authors observed changes to haemoglobin concentration, as well as the number of lymphocytes, granulocytes and thrombocytes.

Summing up, it may be concluded that the season of the year in Polish mixed-breed rabbits with addition of blood of meat breeds had quite a high impact, especially in spring and summer, which differs from the previous results, where the changes were mostly observed in autumn and winter. As far as sex of the rabbits is concerned, in Polish mixed-breed rabbits with addition of blood of meat breeds the impact was rather small, as it was shown only on three parameters (number of neutrophils, eosinophils and thrombocytes), but in three seasons of the year (summer, autumn and winter).

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