# Blood cell count analyses and erythrocyte morphometry in New Zealand white rabbits

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### ABSTRACT

The total and differential leukocyte count, erythrocyte and thrombocyte count, the hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width in male and female New Zealand white rabbits were studied. Furthermore, the basic red blood cell morphometric characteristics were analyzed. The complete blood count values were within the reference intervals for this rabbit breed. Comparing the results regarding the gender, significantly higher hemoglobin concentrations were detected in female rabbits (134.86 vs. 127.5 g/L), whereas in males the anisocytosis was more prominent (RDW 16.09 vs. 15.53). The results of mathematical analysis of the morphometry of erythrocytes revealed statistically different parameters in males and females. Erythrocytes of females had a larger area (30.81 *vs*. 27.6 μm²), greater maximal (3.54 vs. 3.34 μm) and minimal radius (2.61 vs. 2.47 μm), greater convex area (31.95 vs. 29.03 μm²) and greater length (6.73 vs. 6.33 μm) and cell elongation factor (1.14 vs. 1.10) than the erythrocytes of males. Though the average cell volume was not significantly different, morphometry data showed that the erythrocytes in males were smaller and with lesser prominence than in females, which is congruent with higher RDW and lower hemoglobin concentration in the male rabbits' blood, and presumably the higher presence of microcytes in the blood of male rabbits. In conclusion, erythrocyte morphometry may provide valuable data about the changes in erythrocyte morphology and could complement the results obtained by standard hematology methods.

**Key words**: rabbits, blood cell count, erythrocyte morphometry

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**Introduction**

Rabbits are often used as live models in scientific research where changes in blood count occur, since they handle multiple blood sampling well, their surface veins are pronounced, and they are still and suitable for manipulation in delicate procedures (MADER, 2003). Haematological diagnosis is becoming more immanent in veterinary medicine, especially in the detection of health disorders in pet rabbits, where being familiar with referent values is extremely important (HEWITT et al., 1989). Referent haematological values in laboratory lagomorphs, the European rabbit included, have been described in literature on many occasions (LEPITZKI and WOOLF, 1991). However, rabbits display unpredictable oscillations in haematological values, which have resulted in different reference values being published by different authors. These differences may be the result of applying different analytical techniques in different laboratories (HARCOURT-BROWN, 2002). Examination of the literature in order to gain reference haematological data for this species has shown great oscillations with regard to different physiological conditions, such as breed, age, pregnancy, nutrition, environment and daily variations in rhythm (FOX and LAIRD, 1970; BARTOLOTTI et al., 1989). Rabbit diseases, stress, and the administration of cortical steroids rarely cause an increase in total leukocyte count. On the contrary, they more frequently cause a change in the share of specific leukocyte forms due to their re-distribution (TOTH and KRUEGER, 1989; JENKINS, 2006). A feature of many rabbit diseases is an altered ratio of heterophils to lymphocytes, as well as a reduction of blood cell characteristics. The ratio of heterophils to lymphocytes has been proposed as a method which can indicate whether or not rabbits are healthy (McLAUGHIN and FISH, 1994), but this ratio changes as they get older, come under stress, and in all conditions involving higher concentrations of cortisole in the blood (TOTH and JANUARY, 1990; MOORE, 2000).

A complete blood count is a good indicator of general health, as stress and numerous illnesses can modify haematological parameters, especially with regard to erythrocyte and lymphocyte counts (HINTON et al., 1982). A complete blood count is undisputedly the most important diagnostic method available to veterinarians, along with proper anamnesis and a physical examination of the animal. However, when taking into consideration the stipulated oscillations of haematological parameters, one should be very careful when interpreting altered results.

Morphometry is a quantitative description of geometrical structures in all dimensions (BAAK, 1985; VAN DIEST and BAAK, 1991). It provides a numerical objectification of the most subtle modifications unavailable to visual estimation, and as such has clinical and research applications that are becoming more numerous, especially in cytology and histopathology (OBERHOLZER et al., 1991; NAFE, 1991; RUSSACK, 1994). A typical image analysis system consists of a microscope, a high-quality video camera and colour

monitor, and a microcomputer. The light microscopic image is converted to an analogue electronic signal by the video camera. This signal is then digitized by an imaging board in the microcomputer, resulting in a matrix of picture elements called pixels (BARTELS and THOMPSON, 1994). Whereas the human eye can distinguish 30 to 40 shades of gray, 256 shades can be distinguished by the each pixel (KISNER, 1988). Morphometry is the simplest form of image cytometry and refers to the evaluation of cells or tissue by measurement of various cellular features, in a two-dimensional view. Human medicine has already accepted and uses morphometry in the diagnosis and prognosis of malignant diseases, because it enables analysis of changes to the entire cell, cytoplasm changes and changes in the nucleus and nucleus structures (DALTON, 1992; RUSSACK, 1994). Abnormal and insufficient white blood cell function is most often reflected in modified cell morphology, and mathematical analysis of morphometrical cell characteristics is very useful for its estimation (BINS, 1985). Furthermore, changes in morphometrical erythrocyte indicators have been detected in certain human (ALEXANDRATOU et al., 1999; MANJUNATHA and SINGH, 2000) and dog ailments (BEREZINA et al., 2001).

Since rabbits are often used for research, and considering the detected differences in reference values of haematological indicators for rabbits in literature, the aim of this paper is to determine the values of haematological parameters in private breeding. Moreover, due to the growing application of the analysis of morphometrical blood cell indicators in diagnostics and the prognosis of different illnesses, erythrocyte morphometry in rabbits was carried out. The results of the conducted research were compared with respect to gender for the purpose of determining possible differences between male and female rabbits.

**Materials and methods**

The research was carried out on 26 New Zealand white rabbits (*Oryctolagus cuniculus*). When acquired, the rabbits were 90 to 100 days old and had a Health Certificate issued by the competent Veterinary Inspectorate. The rabbits were then put in a test room at the Immunology Laboratory at Veterina d.o.o. Rakov Potok (Kalinovca, Croatia), in controlled temperature conditions of 18 - 22 ºC and humidity between 60 - 70%, with natural ventilation and light. Monitoring of the mentioned parameters was carried out on a daily basis and recorded in a control book as an integral part of standard operating procedure. The rabbits were placed in $35 \times 40 \times 40$ cm cages for individual animals made of stainless steel, with a wire floor and self-irrigating flooring. The rabbits were fed once a day with 100 g of standard rabbit food KUN II F17% (Cibus d.o.o., Lepoglava, Croatia), and were given water *ad libitum* from the water supply network via an automatic drinking trough. During blood sampling, the rabbits were between 120 and 130 days old, and weighed 3 to 3.5 kilograms. Blood samples from twelve male and fourteen female rabbits were collected by puncturing the saphene vein (v. saphena

magna) over a period of two days, always at the same time, between 8 and 9 a.m. Three millilitres of blood was collected from each rabbit in a sterile glass test-tube with sub-pressure (K3E, BD Vacutainer, Plymouth, UK). The samples were then stored at +4 °C and processed in the laboratory on the same day. A smear was made from each sample, which was then coloured according to the Pappenheim method and monitored under a light microscope immersion lens with 1000x enlargement (Olympus BX 41) in order to determine the differential count of specific types of leukocytes. One hundred leukocytes were counted on each blood smear. The total count of leukocytes and erythrocytes, the haemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and total thrombocyte count were performed using a haematological meter (SERONO - 9120 Baker System).

The red cell morphometry was carried out on a personal computer with an "SFORM" supporting system provided by VAMSTEC, Zagreb, Croatia. The system consists of a «Pulinix» high-resolution colour camera which digitalises and transfers the image from an Olympus BH2 light microscope onto a personal computer. The morphometric analysis was carried out on standard-coloured smears according to the Pappenheim method. Cytoplasm margins were designated interactively, along with corrections by hand. At least 100 cells per concoction were analyzed. The following parameters were determined: area, perimeter, convexity, minimal and maximal axis length, length and breadth of red cells, and the form factor and elongation factor of the cell.

The statistical analysis of the results was carried out with the STATISTICA 7.1. computer programme (StatSoft Inc. USA, 2004). For each continuous variable, a distribution form was determined, and the significant differences between means were checked by Student's $t$-test.

### Results

*Haematological indicators.* The measured values of the erythrocyte and leukocyte count, the haemoglobin concentration in the blood, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and the thrombocyte count for male and female rabbits are illustrated in Table 1. Almost all the mentioned parameters were similar in the blood of the male and female rabbits, except haemoglobin concentration, which was significantly higher ($P<0.05$) in female rabbits. In contrast, the mean RDW value in male rabbits was significantly higher ($P<0.01$) when compared to RDW values in females (Table 1). The differential count of specific forms of leukocytes in male and female rabbits is illustrated in Table 2. Lymphocytes are behind heterophils as far as their count is concerned (Table 2). The mean value of the relative

lymphocyte count in male rabbits was 38.67 %, expressed as an absolute lymphocyte count to the amount of 3.66 ×10$^9$/L. The relative lymphocyte count in female rabbits was 46.14 %, or 3.95 ×10$^9$/L expressed as an absolute lymphocyte count. A very low percentage of monocytes were encountered in blood smears. Heterophils were the most represented populations of leukocytes in rabbit blood smears. Their relative count in male rabbits was 60.25 % with an absolute count of 5.02 × 10$^9$/L. The relative count of heterophils in female rabbits was 53 % and the absolute count of 4.66 ×10$^9$/L. Eozinophils in male rabbits were between 0 and 2 %, and in female rabbits between 0 to 3 %. Bazophils were the least represented group of leukocytes in the blood smears. Their relative count in male and female rabbits was 0 to 1 %.

Table 1. Haematological parameters in rabbits

| Haematological parameter | Male rabbits (n = 12) | | Female rabbits (n = 14) | |
|---|---|---|---|---|
| | Range | Mean ± SD | Range | Mean ± SD |
| Leukocytes (10$^9$/L) | 4.2 - 12.3 | 8.33 ± 2.48 | 4.4 - 13.2 | 8.81 ± 3.03 |
| Erythrocytes (10$^{12}$/L) | 4.08 - 6.96 | 5.86 ± 0.79 | 4.89 - 6.85 | 6.21 ± 0.52 |
| Haemoglobin (g/L) | 104 - 140 | 127.5 ± 11.99 | 123 - 151 | 134.86 ± 8.53* |
| PCV (L/L) | 0.29 - 0.44 | 0.38 ± 0.04 | 0.36 - 0.44 | 0.41 ± 0.03 |
| MCV (fL) | 61.4 - 70.3 | 65.56 ± 3.14 | 60.2 - 72.8 | 65.66 ± 2.91 |
| MCH (pg) | 19.7 - 26 | 21.93 ± 1.91 | 19.2 - 25.2 | 21.79 ± 1.36 |
| MCHC (g/L) | 309 - 371 | 334.33 ± 16.01 | 316 - 346 | 331.78 ± 8.33 |
| RDW | 15.3 - 17.2 | 16.09 ± 0.55 | 14 - 16.4 | 15.53 ± 0.65** |
| Trombocytes (10$^9$/L) | 390 - 821 | 529.75 ± 125.85 | 353 - 703 | 499.93 ± 94.0 |

Values are expressed as a mean value ± standard deviation. Significance of differences with regard to gender at the level of: *P<0.05; **P<0.01.

Table 2. Differential leukocyte count in blood of rabbits

| Blood cells | Male rabbits (n = 12) | | Female rabbits (n = 14) | |
|---|---|---|---|---|
| | Range | Mean ± SD | Range | Mean ± SD |
| Lymphocytes (%) | 16 - 70 | 38.67 ± 19.55 | 20 - 69 | 46.14 ± 18.52 |
| Monocytes (%) | 0 - 3 | 0.75 ± 1.21 | 0 - 3 | 0.5 ± 0.94 |
| Heterophils (%) | 27 - 94 | 60.25 ± 20.52 | 27 - 79 | 53 ± 18.51 |
| Eosinophils (%) | 0 - 2 | 0.25 ± 0.62 | 0 - 3 | 0.21 ± 0.8 |
| Basophils (%) | 0 - 1 | 0.08 ± 0.29 | 0 - 1 | 0.14 ± 0.36 |

Values are expressed as a mean value ± standard deviation

Table 3. Morphometric characteristics of erythrocytes in rabbits

| Morphologic characteristics | Male rabbits n = 12 ($n_1$ = 1200) | Female rabbits n = 14 ($n_1$ = 1400) |
|---|---|---|
| Cell area ($\mu m^2$) | 27.6 ± 1.88 | 30.81 ± 1.29* |
| Cell perimeter ($\mu m$) | 20.54 ± 0.99 | 21.20 ± 0.24 |
| Maximum axis length ($\mu m$) | 3.34 ± 0.13 | 3.54 ± 0.06* |
| Minimum axis length ($\mu m$) | 2.47 ± 0.09 | 2.61 ± 0.02* |
| Convex area ($\mu m^2$) | 29.03 ± 2.05 | 31.95 ± 0.60* |
| Cell length ($\mu m$) | 6.33 ± 0.24 | 6.73 ± 0.12* |
| Cell breadth ($\mu m$) | 5.74 ± 0.21 | 5.94 ± 0.07 |
| Cell form factor | 0.83 ± 0.05 | 0.86 ± 0.03 |
| Cell elongation factor | 1.10 ± 0.01 | 1.14 ± 0.03* |

Values are expressed as a mean value ± standard deviation. Significance of differences with regard to gender at the level: *$P<0.05$. $n_1$ = total number of analysed erythrocytes

*Erythrocyte morphometry.* A total of 2,600 erythrocytes were analysed in 26 smears of the peripheral blood of rabbits. The results of the basic morphometric characteristics of the erythrocytes in male and female rabbits are illustrated in Table 3. The area of erythrocytes in female rabbits was significantly higher ($P<0.05$) than the area of erythrocytes in male rabbits, and averagely amounted to 30.81 $\mu m^2$, while the same average in male rabbits amounted to 27.6 $\mu m^2$. Furthermore, female rabbits had significantly greater ($P<0.05$) maximal and minimal axis length (3.54; 2.61 $\mu m$) when compared to the minimal and maximal axis length in male rabbits (3.34; 2.47 $\mu m$). Moreover, female rabbits had a greater degree ($P<0.05$) of erythrocyte protrusion or convexity, and the mean value was 31.95 $\mu m^2$ compared to 29.3 $\mu m^2$ in male rabbits. The erythrocytes in female rabbits were significantly ($P<0.05$) longer (6.73 $\mu m$) than erythrocytes in male rabbits (6.33 $\mu m$), which is congruent with the significantly higher ($P<0.05$) cell elongation factor of 1.14 in female rabbits in relation to the elongation factor in male rabbits of 1.10. The erythrocyte perimeter, the cell breadth and form factor did not significantly differ between male and female rabbits. The cell form factor was equal for both genders and amounted to 0.86 in females and 0.83 in male rabbits (Table 3).

### Discussion

In spite of the well known variability of haematological indicators in rabbits with regard to breed-related and individual differences, the measured values of the complete blood count in this research occur within the range of previously published values for rabbits (CARPENTER, 2005). The values of the total erythrocyte count in blood were at the

bottom level of the range stipulated for adult New Zealand white rabbits (BARTOLOTTI et al., 1989; KABATA et al., 1991), but since the erythrocyte count in rabbits increases until the age of six months, when it reaches the value characteristic for adult rabbits (MOORE, 2000; JENKINS, 2006), it is our opinion that the total erythrocyte count was physiological for the age of the tested rabbits. As far as differences in the values of haematological gender-specific indicators are concerned, the literature offers differing data. Some authors find higher values for the total erythrocyte count, packed cell volume and haemoglobin concentration in male rabbits (MOORE, 2000; JENKINS, 2006), while other authors do not find such differences, or they do not think of them as strictly defined (SCHERMER, 1967). In this research, female rabbits had a significantly higher concentration of haemoglobin in their blood, but it occurred within the physiological limits for both male and female rabbits. The cause of the measured lower haemoglobin concentration in male rabbits was probably the age of the tested rabbits, where haemoglobin values had not yet reached those found in sexually mature rabbits, as well as the fact that the male rabbits were somewhat heavier, and some portion of iron was possibly spent on myoglobin synthesis. The values of the calculated erythrocyte constants (MCV, MCH and MCHC) reflected the reference values for rabbits (BARTOLOTTI et al., 1989; KABATA et al., 1991; HARCOURT-BROWN and BAKER, 2001), while the values of erythrocyte distribution width (RDW) occurred at the upper level of the proposed ranges (HEWITT et al., 1989; KABATA et al., 1991; HARCOURT-BROWN, 2002). Anisocytosis in rabbits is considered as a physiological finding (JENKINS, 2006), and poikilocytosis also frequently occurs because of echinocyte occurrence (SCHERMER, 1967; MOORE, 2000). A significantly higher RDW was found in the male rabbits than in the female rabbits, and it can therefore be concluded that there was a case of pronounced anisocytosis of erythrocytes in male rabbits.

The total leukocyte count in the rabbits shows rhythmic variations on a daily basis, with the lowest values during the early afternoon and evening (FOX and LAIRD, 1970). Moreover, the total leukocyte count varies in relation to the age of the rabbits. The highest total leukocyte count was recorded in three-month-old rabbits, and then in one year old mature rabbits. The first spike is the lymphocyte count growth result, and the other was the heterophils count growth results (HARCOURT-BROWN, 2002a). Furthermore, differences in leukocytes were recorded with regard to the feeding rhythm (SCHERMER, 1967), breed, gender and season (McLAUGHLIN and FISH, 1994). Pursuant to the aforementioned, quite a wide physiological leukocyte count range of 5-12.5 $\times 10^9$/L (KABATA et al., 1991; CARPENTER, 2005; JENKINS, 2006) was determined for the rabbits. The total leukocyte count in our research occurred within the proposed range and there were no significant differences recorded with regard to gender. The proposed ranges for the share of specific forms of leukocytes are also very wide, from 30 - 80 % for lymphocytes, and 20 - 75 % for heterophils, while the share of eosinophils, as well as the share of monocytes was smaller, from 0 - 4 %, though the range of the basophils share was somewhat higher,

from 0 - 7 % (KABATA et al., 1991; JENKINS, 2006). The share of specific leukocyte forms in the tested rabbits occurred within the proposed ranges. Taking into consideration the extremely wide physiological limits, the ratio of heterophils to lymphocytes (H/L) might be a better indicator of health. The heterophil to lymphocyte ratio changes with age and when expressed as a percentage amounts to 33:60 in two-month-old rabbits, and changes up to 45:45 for one-year-old rabbits (JENKINS, 2006). The H/L ratio in the tested female rabbits was 53:46 and roughly coincides with the values for older rabbits, but in male rabbits this ratio had changed in favour of the heterophils and amounted on average to 60:38. It was determined that the H/L ratio also changes during illness in rabbits, when they are under stress and when there is a higher concentration of cortisole in the blood, due to a redistribution of cells (HINTON et al., 1982; McLAUGHLIN and FISH, 1994). Since the tested rabbits were in good health and under constant veterinary care, the determined high relation of heterophils to lymphocytes in male rabbits was probably the result of a stressful reaction to the blood sampling. Similar changes in the H/L ratio caused by stress during the manipulation of animals are also found in other research (JACOBSON et al., 1978; WARD, 2006). Although the animals were treated with maximum care during blood sampling, the male rabbits struggle against the manipulation which resulted in a change of the H/L ratio, being also the case with other animal species (POLJIČAK-MILAS et al., 2004). Rabbit's erythrocytes are biconcave disks with an average radius of 6.7 - 6.9 μm, and an average breadth of 2.15 - 2.4 μm (MOORE, 2000; JENKINS, 2006). The range of radii in erythrocytes is 5.0 - 7.8 μm, which points to anisocytosis with microcytes in some cases only a quarter of the average cell diameter (HARCOURT-BROWN, 2002a). The results of the mathematical analysis of erythrocyte morphometric parameters showed a very similar average in erythrocyte length (6.33 μm in male rabbits and 6.73 μm in female rabbits), though the cell breadth was much greater than the one outlined in the literature, and amounted to 5.74 μm in male rabbits and 5.49 μm in female rabbits. However, when measuring cell breadth, morphometry perceives the cell as a square, and in addition, enables measurement of area convexity and cell elongation. Mathematical analysis of morphometric parameters determined statistically significant differences in erythrocytes in male and female rabbits. Erythrocytes in female rabbits had a larger area, greater maximal and minimal axis length, a greater convex area and higher length and cell elongation factors. Although the average erythrocyte volume (MCV) did not significantly differ, morphometric data show that the measured erythrocytes in male rabbits were smaller and of a lower protrusion degree than those in female rabbits. This was probably a case of a higher mycrocyte percentage in the blood of the male rabbits, which concurs with the measured statistically significant higher RDW values and lower concentration of haemoglobin in the blood of the male rabbits.

Human medicine has accepted morphometry as a method which provides higher reliability and reproducibility of cytological and histopathologycal diagnoses, tumour

re-classification and correlation of morphometric data (BAAK, 1985). Moreover, morphometric characteristics of erythrocytes are considered to be diagnostic indicators of coronary insufficiency in humans (ALEXANDRATOU et al., 1999). Changes in morphometric erythrocyte parameters during hemorrhagic shock have been found in dogs, and are considered to be the results of oxidative stress (BEREZINA et al., 2001). We may conclude that the morphometric analysis of erythrocytes in our research has provided valuable data on changes in erythrocytes and complemented findings obtained by classical haematological methods.

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**SAŽETAK**

Istražen je ukupan i diferencijalni broj leukocita, broj eritrocita, koncentracija hemoglobina, hematokrit, srednji volumen eritrocita, srednja količina hemoglobina, srednja koncentracija hemoglobina po eritrocitu, raspodjela eritrocita po volumenu i ukupan broj trombocita u mužjaka i ženki novozelandskoga bijeloga kunića. Nadalje, učinjena je analiza osnovnih morfometrijskih karakteristika eritrocita. Vrijednosti pokazatelja kompletne krvne slike bile su unutar granica referentnih vrijednosti za tu pasminu kunića. Raspodjela rezultata prema spolu kunića, pokazala je da su ženke imale značajno veću koncentraciju hemoglobina u krvi od mužjaka (134,86/127,5 g/L), dok su mužjaci imali jače izraženu anizocitozu (RDW 16,09/15,53). Rezultati matematičke analize morfometrijskih pokazatelja eritrocita pokazali su statistički značajne razlike između eritrocita mužjaka i ženki. Eritrociti ženki imali su veću površinu (30,81/27,6 $\mu m^2$), veći maksimalni (3,54/3,34 $\mu m$) i minimalni polumjer (2,61/2,47 $\mu m$), veći konveksitet površine (31,95/29,03 $\mu m^2$) te veću duljinu (6,73/6,33 $\mu m$) i faktor izduženosti stanice (1,14/1,10). Iako se prosječni volumen stanica nije značajno razlikovao, morfometrijski podatci govore da su eritrociti u mužjaka bili manji i slabije ispupčeni od onih u ženki, što se podudara s većom RDW vrijednosti i nalazom niže koncentracije hemoglobina u krvi mužjaka, te se vjerojatno radilo o većem postotku mikrocita u krvi kunića muškog spola. Zaključeno je da morfometrija eritrocita može pružiti vrijedne podatke o promjenama izgleda eritrocita i upotpuniti nalaze dobivene klasičnim hematološkim metodama.

Ključne riječi: kunići, diferencijalna krvna slika, eritrociti, morfometrija

White blood cells are created inside the bone marrow, the spongy tissue inside bones. There is an estimate as to what is a typical white blood cell count and what is too high or too low. A high WBC count is one sign that there is an infection, inflammatory disease, or an inflammatory process taking place somewhere in the body.ï»¿ï»¿ There are several different conditions that could cause a higher than normal or a lower than normal WBC count, but it is important to keep in mind that this test is not specific enough to diagnose any particular disease. In some cases, although not always, people who h