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PHYSIOLOGICAL VALUES OF SOME BLOOD INDICATORS IN SELECTED DWARF RABBIT BREEDS

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Abstract: The aim of the present study was to evaluate the effect of breed on haematological and biochemical indicators in 3 dwarf rabbit breeds. In the experiment, 30 sexually intact dwarf rabbit females aged 6 mo were used. With the sole exception of white blood cells and haematocrit value, breed had the most significant effect on the majority of haematological indicators monitored. The red blood cell count was higher in the Dwarf Lop compared to the Netherland Dwarf (+1.91×10^{12} cells/L; P<0.05) and also the Teddy Dwarf (+1.32×10^{12} cells/L; P<0.05). For haemoglobin concentration, a higher value was found in the Netherland Dwarf than in the Teddy Dwarf (+39.29 g/L; P<0.05) and the Dwarf Lop (+26.36 g/L; P<0.05). For erythrocytic indicators, the highest values of mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were found in the Netherland Dwarf. The breed had a significant effect on the urea and potassium values. A higher value of urea was recorded in the Dwarf Lop compared to the Teddy Dwarf (+1.56 mmol/L; P<0.05). For potassium, a higher value was found in the Netherland Dwarf compared to the Teddy Dwarf (+0.85 mmol/L; P<0.05). In addition, a significantly positive correlation (P<0.05) was found between the live weight of dwarf females and values of haematocrit (0.49), albumin (0.54), alanine aminotransferase (0.51), and aspartate aminotransferase (0.41), while a significantly negative correlation (P<0.05) was found between their live weight and values of triacylglycerols (–0.44), alkaline phosphatase (–0.38) and inorganic phosphorus (–0.52).

Key Words: dwarf rabbit, breeds, haematological indicators, biochemical indicators, plasma, normal values.

INTRODUCTION

Recently, keeping rabbits as household pets has greatly expanded (Harcourt-Brown, 2002; Oxley *et al.,* 2015). There are roughly 180 breeds of domestic rabbits. For hobby breeding, mainly dwarf rabbits are reared (Snook *et al.,* 2013), with the Dwarf Lop and the Netherland Dwarf being the most popular (González-Redondo and Contreras-Chacón, 2012). This interest in the rearing of pet rabbits is also reflected in the veterinary field (Sweet *et al.,* 2013).

Regarding the clinical examination of rabbits, Wesche (2014) recommends evaluating the influence of the particular breed in addition to assessing the results of haematological and biochemical examinations. However, the great interest of breeders in pet rabbits is not accompanied by similarly intense study of their clinical pathology. Most of the reference data on blood indicators in rabbit come mainly from laboratory research which does not include dwarf rabbits. This can make diagnosis in pet rabbits more difficult (Murray, 2006). Although the physiology and clinical pathology of laboratory rabbits has been studied extensively, studies in this area in pet rabbits are rare (Meredith, 2014). Recently, a considerable breed effect on blood indicators was found in popularly reared Czech medium-sized rabbit breeds (Martinec *et al.,* 2012). We assumed that this effect might also be present in dwarf rabbits.

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In general, the blood indicators of rabbits have been intensively researched, mainly with regard to the effect of nutrition (Ironkwe and Oruwari, 2011; Trebušak *et al.,* 2014), age (Jeklová *et al.,* 2009; Kisch, 2010), gender (Özkan *et al.,* 2012), welfare (Ondruska *et al.,* 2011) and genetic diseases (Supuka *et al.,* 2014).

So far, little attention has been paid to the relevance of particular rabbit breeds. Nevertheless, some studies presented interesting findings within selected medium-sized breeds (Tůmová *et al.,* 2013). The influence of breed on the blood indicators has also been demonstrated in relation to the breeding method used (Burnett *et al.,* 2006; Abdel-Azeem *et al.,* 2010). None of these studies, however, has evaluated the impact of the breed in dwarf rabbits. Therefore, the aim of this study was to evaluate the effect of breed on haematological and biochemical indicators in selected dwarf rabbit breeds. In addition, the values of blood indicators we found in the specific dwarf breeds can be used for more accurate assessment of health of these rabbits in common clinical practice. Taking into consideration the respective age and sex of rabbits, these values could be used as reference levels.

MATERIALS AND METHODS

The experimental procedures were approved by the Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences (UVPS) Brno (no. 15/2015/2230 / FVHE).

Animals

In the experiment, 30 clinically healthy sexually intact dwarf rabbit females aged 6 mo were used, from the following breeds: Dwarf Lop (n=10), Netherland Dwarf (n=10) and Teddy Dwarf (n=10). According to the Czech Standard of Perfection for rabbits, all the animals used displayed important exterior traits that are typical of an exhibition rabbit within these breeds. Rabbits were taken from a common hobby breeding stock focused on exhibition activities according to the regulations of the Czech Small Animal Breeders' Association.

From the age of 4 mo, the females were kept individually in outdoor cages (65×60×45 cm, wide×high×deep) sheltered against unfavourable weather conditions. Within the housing area, natural ventilation was ensured (from 0.1 to 0.2 m/s). The air temperature was in the range 15-17°C. The average air humidity ranged from 65-70%. All females were kept under identical conditions.

Rabbits were fed identical pelleted feed mixture designed for nutrition of dwarf rabbits (Berkel-Futter Light 6 008, Coesfeld, Germany). According to the manufacturer's recommendations, the females received daily amounts of 20-30 g of feed per kg of live weight (LW) offered regularly once a day in the evening at 8 p.m. In addition, the rabbits were given hay at a dose of 30 g/d 3 times a week. Rabbits received hay composed of different plants; its composition was mainly based on Kentucky bluegrass (*Poa pratensis*), cock's-foot (*Dactylis glomerata*), timothy-grass (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and perennial ryegrass (*Lolium perenne*) and did not vary throughout the experimental period. Concerning chemical composition of the diet, we determined the content of crude protein, ether extract, starch, crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), gross energy, ash and selected minerals. Crude protein was determined by Kjeldahl method using Buchi analyser (Centec Automatika, Czech Republic). Ether extract was determined by Soxhlet method. Crude fibre, ADF, NDF and ADL were determined by ANKOM 220 fibre Analyzer (O.K. Servis BioPro, Czech Republic). Starch was determined using the Automatic Digital Polarimetr P3002RS (Krüss, Germany). Gross energy was determined by the Kalorimetr AC500 (LECO, s.r.o., Czech Republic). Digestible energy was calculated according to Villamide *et al.* (2009). Ash was determined by weighing the sample after incineration at 550°C under prescribed conditions. The calcium, sodium, copper, zinc and manganese contents were determined by atomic absorption spectrometer 240 AA (Agilent Technologies, USA). The total phosphorus content was determined spectrophotometrically. An acidic solution of ash was treated with molybdovanadate reagent and the absorbance of the solution was measured at 430 nm (Helios Alpha UV-Vis spectrophotometer, Thermo Fisher Scientific Inc.). The presented values for the content of iron, iodine, selenium, biotin and vitamins A, D_3 and E in pelleted feed mixture were stated by the manufacturer. Ingredients and chemical composition of the diet are presented in Table 1. Chemical composition of the meadow hay is presented in Table 2. During the entire experiment, rabbits had free access to drinking water.

The animals were treated identically and vaccinated at 2 mo of age against myxomatosis and rabbit haemorrhagic disease. Vaccines MXT (Dyntec, Czech Republic) and Pestorin (1 mL/rabbit; Bioveta, Czech Republic) were used. Six weeks before the study, the animals were treated against endoparasites by Panacur (Fenbendazol, 10 mg/kg of LW; MSD Animal Health, Netherlands).

Blood sampling

One day before blood sampling, all the females were given their last feed ration consisting of pelleted feed and meadow hay at 8 p.m. The meadow hay was available during the night. In the morning before blood sampling, the females were not fed pelleted concentrated feed. Females were weighed and their overall health was evaluated and rectal temperature measured. Blood sampling was carried out between 9-10 a.m. In view of the short length and small size of ears of dwarf rabbits, blood samples (2 mL) were collected from the *vena saphaena lateralis* using a 23-gauge sterile needle. Blood samples were relocated to designated sample tubes with heparin and transported to the laboratory to be analysed.

Analysis of haematological and biochemical indicators

Blood samples for analyses were delivered to laboratories within 2.5 h after collection and promptly assayed. In haematological analysis, the red and white blood cell counts were determined manually using the haemocytometer, the Hayem solution and the Türck solution, respectively, as a diluting fluid. Haematocrit value was determined by the micro-haematocrit method in the micro-centrifuge at 16000 rpm for 3 min. Haemoglobin concentration was determined using the cyanohaemoglobin method with Drabkin solution (Lassen and Weiser, 2004). Then, the following erythrocytic indicators were determined: mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration.

Table 1: Ingredient and chemical composition of the pelleted diet.

Item	Pelleted diet
Ingredient of diet (g/kg DM)	
Alfalfa meal	417.00
Wheat bran	226.00
Malt sprouts	151.00
Barley	85.00
Oat bran	60.00
Sugar beet pulp	29.00
Molasses	19.00
Monocalcium phosphate	1.00
Calcium carbonate	8.50
Sodium chloride	3.50
Chemical composition of diet (g/kg DM)	
Crude protein	160.50
Ether extract	26.80
Starch	151.90
Crude fibre	173.20
Acid detergent fibre	233.60
Neutral detergent fibre	420.00
Acid detergent lignin	53.00
Ash	86.20
Ca	11.20
P	5.70
Na	2.00
Cu (mg/kg)	20.00
I (mg/kg)	0.40
Fe (mg/kg)	25.00
Zn (mg/kg)	96.10
Mn (mg/kg)	72.40
Se (mg/kg)	0.20
Biotin (mg/kg)	100.00
Vitamin A (IU)	13500.00
Vitamin D_3 (IU)	800.00
Vitamin E (mg/kg)	50.00
GE (MJ/kg)	18.30
DE (MJ/kg)	9.84

DM, dry matter; GE, gross energy; DE, digestible energy.

Half of the blood sample volume (approx. 1 mL) was centrifuged for 15 min at 3000 rpm at the temperature 22°C. Biochemical indicators were determined in blood plasma, after centrifuging heparin-stabilised blood. Samples were analysed using a DPC Konelab 20i Analyzer® (Thermo Fisher Scientific, Finland). The following biochemical indicators were determined: total protein (detection limit (DL) 0.50 g/L; coefficient of variance (CV) 0.91%), albumin (DL 2.00 g/L; CV 1.12%), glucose (DL 0.06 mmol/L; CV 0.67%), creatinine (DL 18.00 µmol/L; CV 1.30%), urea (DL 0.30 mmol/L; CV 2.33%), cholesterol (DL 0.08 mmol/L; CV 0.61%), triacylglycerols (DL 0.02 mmol/L; CV 0.69%), calcium (DL 0.02 mmol/L; CV 1.50%), inorganic phosphate (DL 0.065 mmol/L; CV 1.12%), sodium (DL 20.00 mmol/L), potassium (DL 0.20 mmol/L), chloride (DL 25.00 mmol/L) and activities

Table 2: Chemical composition of the meadow hay.

Item	Hay
Chemical composition of hay (g/kg DM)	
Crude protein	84.20
Ether extract	14.30
Crude fibre	329.40
Acid detergent fibre	375.30
Neutral detergent fibre	588.90
Acid detergent lignin	64.50
Ash	76.50
Ca	6.820
P	2.20
GE (MJ/kg)	17.90

DM, dry matter; GE, gross energy.

of aspartate aminotransferase (DL 1.80 U/L; CV 1.38%), alanine aminotransferase (DL 4.10 U/L; CV 1.60%) and alkaline phosphatase (DL 1.80 U/L; CV 0.67%).

Statistical analysis

Statistical analyses were performed using the STATISTICA CZ version 10 software (StatSoft Inc., 2011). Arithmetic mean and its 95% confidence interval were determined. A Shapiro-Wilk test was used to test normal distribution of the data within respective breeds. Normality was found in these indicators: LW, rectal temperature, red blood cells count, white blood cells count, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, glucose, triacylglycerols, alanine aminotransferase, alkaline phosphatase, inorganic phosphate and sodium.

One-way ANOVA was used to determine differences in indicators showing the normal distribution and non-significant correlation with LW. The differences for these indicators were tested according to the following statistical model:

$$Y_{ij}=\mu+a_i+e_{ij}$$

where Y_{ij} is variance associated with parameter a, μ is the overall mean, a_i is a breed effect and e_{ij} is the error term. Differences among breeds were analysed for significance using Tukey's test.

Normality was not found in these indicators: haematocrit value, mean corpuscular haemoglobin concentration, total protein, albumin, urea, creatinine, cholesterol, aspartate aminotransferase, calcium, chloride and potassium. A non-parametric Kruskal-Wallis ANOVA was used to determine differences in blood indicators showing the non-normal distribution of data within breeds and non-significant correlation with LW. Differences among breeds were analysed for significance using multiple comparisons of mean ranks.

Regarding a significant correlation between the LW and blood indicators, an ANCOVA was used to determine differences among breeds, while LW was used as a covariate. For data showing non-normal distribution, logarithmic or square root transformations were used for ANCOVA. The differences for these indicators were tested according to the following statistical model:

$$Y_{ij}=\mu+a_i+B(c_i-m_{ij})+e_{ij}$$

where Y_{ij} is the *jth* observation under the *ith* categorical group, μ is the overall mean, a_i is a breed effect of the *ith* level, B is a regression coefficient for *ith* covariate LW, c_i is the *jth* observation of the covariate LW under the *ith* group, m_{ij} is the mean of *ith* covariate LW and e_{ij} is the error term. Differences were considered significant at $P<0.05$.

RESULTS

Means of the LW, rectal temperature and selected haematological indicators (red blood cells, haematocrit value, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and white blood cells) within all evaluated dwarf rabbit breeds are presented in Table 3. At the age of 6 mo, the Dwarf Lop females displayed a considerably higher LW when compared to the Teddy Dwarf females (+595.5 g; $P<0.05$) and the Netherland Dwarf females (+373.2 g; $P<0.05$). We observed similar rectal temperature among females of the studied dwarf rabbit breeds.

With the exception of the white blood cells and haematocrit value, the breed had the significant effect on majority of the monitored haematological indicators. The count of red blood cells was higher in the Dwarf Lop as compared to the Netherland Dwarf (+1.91×10^{12} cells/L; $P<0.05$) and the Teddy Dwarf (+1.32×10^{12} cells/L; $P<0.05$). Concerning haemoglobin concentration, its higher value was found in the Netherland Dwarf than in the Teddy Dwarf (+39.29 g/L;

Table 3: Live weight, rectal temperature and physiological values for some haematological indicators (mean and its 95% confidence interval) in dwarf rabbit breeds.

	Breed					
	Dwarf Lop (n=10)		Teddy Dwarf (n=10)		Netherland Dwarf (n=10)	
Item	Mean	Confidence interval	Mean	Confidence interval	Mean	Confidence interval
Live weight (g)	1360.9[b]	1218.4-1503.4	795.4[a]	703.8-887.0	987.7[a]	855.3-1120.1
Rectal temperature (°C)	38.3	37.9-38.9	38.9	38.4-39.2	38.8	38.3-39.1
RBC ($\times 10^{12}$/L)	5.77[b]	4.87-6.68	4.45[a]	3.57-5.33	3.86[a]	3.30-4.42
HCT (L/L)	0.357	0.328-0.386	0.326	0.295-0.358	0.370	0.350-0.390
HGB (g/L)	100.54[a]	91.43-109.65	87.61[a]	74.88-100.33	126.90[b]	110.52-143.28
MCV (fl)	64.76[a]	52.28-77.25	77.35[a]	63.91-90.78	99.04[b]	85.30-112.78
MCH (pg)	17.93[a]	15.71-20.15	20.50[a]	17.37-23.62	33.54[b]	28.96-38.14
MCHC (g/L)	285.22[ab]	250.98-319.46	271.89[a]	232.13-311.65	342.54[b]	304.58-380.50
WBC ($\times 10^{9}$/L)	4.95	4.04-5.86	4.10	3.27-4.94	5.45	4.55-6.34

RBC, red blood cells; HCT, haematocrit; HGB, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells.

[a,b]Means within a row with different superscript letters differ ($P<0.05$).

$P<0.05$) and the Dwarf Lop (+26.36 g/L; $P<0.05$). As for erythrocytic indicators, highest values of mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were found in the Netherland Dwarf. The Netherland Dwarf displayed a higher mean corpuscular volume value as compared to those of the Dwarf Lop (+34.28 fl; $P<0.05$) and the Teddy Dwarf (+21.69 fl; $P<0.05$). In addition, the Netherland Dwarf showed a higher mean corpuscular haemoglobin value than the Dwarf Lop (+15.61 pg; $P<0.05$) and the Teddy Dwarf (+13.04 pg; $P<0.05$). Regarding mean corpuscular haemoglobin concentration, the Netherland Dwarf showed its higher value compared to the Teddy Dwarf (+70.65 g/L; $P<0.05$).

Concerning blood plasma, mean values for selected biochemical indicators (total protein, albumin, glucose, creatinine, urea, cholesterol, triacylglycerols, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, calcium, phosphorus, sodium, potassium and chlorine) in respective breeds are presented in Table 4. The breed had a significant effect on values of urea and potassium ($P<0.05$). Regarding urea, its higher value was recorded in the

Table 4: Physiological values for some biochemical indicators (mean and its 95% confidence interval) in dwarf rabbit breeds.

	Breed					
	Dwarf Lop (n=10)		Teddy Dwarf (n=10)		Netherland Dwarf (n=10)	
Indicator	Mean	Confidence interval	Mean	Confidence interval	Mean	Confidence interval
Total protein (g/L)	64.07	60.05-68.10	63.63	60.62-66.64	67.20	63.10-71.31
Albumin (g/L)	33.95	30.18-37.70	28.26	20.49-36.02	34.62	31.95-37.29
Glucose (mmol/L)	6.64	6.17-7.01	6.43	6.12-6.74	6.51	5.69-7.34
Creatinine (µmol/L)	97.41	87.17-107.65	87.29	63.36-111.22	113.19	77.64-148.74
Urea (mmol/L)	6.91[b]	5.94-7.89	5.35[a]	4.77-5.93	6.80[ab]	4.86-8.75
Cholesterol (mmol/L)	1.01	0.57-1.45	1.19	1.01-1.38	1.25	0.96-1.54
Triacylglycerols (mmol/L)	0.87	0.70-1.04	1.57	1.06-2.09	1.09	0.82-1.36
Alanine (U/L)	86.98	52.90-121.06	46.89	40.40-53.38	64.34	50.59-78.10
Aspartate (U/L)	45.36	18.37-72.35	31.02	21.94-40.09	49.67	31.07-68.27
Alkaline (U/L)	69.63	52.93-86.34	93.48	82.14-104.82	81.10	63.75-98.44
Ca (mmol/L)	3.43	3.25-3.61	3.35	3.24-3.46	3.50	2.93-4.07
P (mmol/L)	1.34	0.93-1.75	1.69	1.29-2.10	1.43	1.00-1.86
Na (mmol/L)	137.18	133.54-140.82	137.20	134.96-139.44	136.91	133.84-139.98
K (mmol/L)	5.59[ab]	5.13-6.05	5.04[a]	4.59-5.50	5.89[b]	5.29-6.50
Cl (mmol/L)	106.24	103.46-109.00	104.89	102.99-106.79	104.15	101.80-106.50

[a,b]Means within a row with different superscript letters differ ($P<0.05$).

Table 5: Correlation coefficients between the live weight and blood indicators in dwarf rabbits.

Indicator	LW
Haematological indicators	
Red blood cells	0.13
Haematocrit value	0.49*
Haemoglobin concentration	0.25
Mean corpuscular volume	0.18
Mean corpuscular haemoglobin	0.23
Mean corpuscular haemoglobin concentration	0.02
White blood cells	0.29
Biochemical indicators	
Total protein	0.08
Albumin	0.54*
Glucose	–0.27
Creatinine	0.22
Urea	0.06
Cholesterol	–0.15
Triacylglycerols	–0.44*
Alanine	0.51*
Aspartate	0.41*
Alkaline	–0.38*
Calcium	0.02
Phosphorus	–0.52*
Sodium	–0.06
Potassium	0.21
Chlorine	0.08

*(P<0.05).

Dwarf Lop compared to the Teddy Dwarf (+1.56 mmol/L; P<0.05). Concerning potassium, its higher value was found in the Netherland Dwarf compared to the Teddy Dwarf (+0.85 mmol/L; P<0.05).

As shown in Table 5, a significantly positive correlation was found between the LW of dwarf rabbit females and the values of haematocrit (P<0.05), albumin (P<0.05), alanine aminotransferase (P<0.05), and aspartate aminotransferase (P<0.05), while a significantly negative correlation was found between their LW and the values of triacylglycerols (P<0.05), alkaline phosphatase (P<0.05) and phosphorus (P<0.05).

DISCUSSION

The average LW of 6-mo old females of dwarf rabbit breeds in our study are in agreement with LW values published for these breeds in Czech Breed Standards (Zadina, 2003). Concerning the health status of rabbits in our study, all of them had rectal temperature values within the physiological range (Harcourt-Brown, 2002) and no clinical signs of any diseases were found in any of the rabbits before blood sampling.

The rabbits were given the pelleted feed as well as the meadow hay in the evening before blood collection in the morning the next day. With respect to the gastrointestinal physiology, the rabbits had free access to meadow hay during the night before blood collection. We assume that by using this technique, the blood indicators we evaluated were minimally affected and that the intake of hay during the night before sampling was sufficient to cover the energy metabolism requirements of the rabbits.

Haematological examination

The results of our study confirmed the assumption of the influence of breed on the values of some haematological indicators. Wesche (2014) generally draws attention to the great variability of reference values for haematology indicators in rabbits.

In our study, we found the significant effect of a breed on the red blood cells, which is consistent with the results found by Burnett *et al.* (2006) and Abdel-Azeem *et al.* (2010) within large- and medium-sized breeds. Values of red blood cells in the Dwarf Lop in our study are consistent with the physiological data from the previous studies of Jeklová *et al.* (2009) in 5-mo old New Zealand White rabbits (5.4 cells$\times10^{12}$/L) and also Tokarz-Deptuła *et al.* (2014) in medium-sized rabbit females (5.5 cells$\times10^{12}$/L). On the other hand, the lowest value of this blood indicator in the Netherland Dwarf in our study is similar to that published by Chineke *et al.* (2006) in 5-6-mo old medium-sized rabbits under tropical humid conditions (3.5 cells$\times10^{12}$/L).

Haematocrit values found in our study fall within the physiological range for rabbits stated by Hewitt *et al.* (1989), who found the range 0.27-0.47 L/L in their study. In addition, our findings are consistent with the normal range for pet rabbits 0.3-0.4 L/L, published by Harcourt-Brown (2002). Haemoglobin concentrations in our study were significantly different among breeds. Breed effect was also confirmed by Abdel-Azeem *et al.* (2010) within 4 pure breeds and their crossbreds and by Martinec *et al.* (2012) within 7 medium-sized Czech national breeds. In our study, we found a positive significant correlation between the LW and the haematocrit value.

Values of mean corpuscular volume observed in the Dwarf Lop and the Teddy Dwarf females in our study fall in the physiological range published by Hewitt *et al.* (1989), who found its range as 58.0-79.6 fl. These values are slightly higher than those found by Archetti *et al.* (2008) and Poljičak-Milas *et al.* (2009) in non-pregnant and non-lactating New Zealand White females (50.0-61.0 and 60.2-72.8 fl, respectively). In our experiment, we found a considerably increased value for mean corpuscular volume in the Netherland Dwarf breed (99.0 fl). Also Martinec *et al.* (2012) found significant differences in the value of mean corpuscular volume within seven medium-sized breeds (range of 73.8-83.0 fl). However, the values of mean corpuscular volume in our study displayed a wider range than that of Martinec *et al.* (2012), which could indicate the specific effect of dwarf breeds on this haematological indicator.

In our study, we found the effect of breed on the value of mean corpuscular haemoglobin and also mean corpuscular haemoglobin concentration. Brockus (2011) states that the intrinsic value of mean corpuscular haemoglobin concentration is a more accurate basic value for erythrocytes. The value of mean corpuscular haemoglobin concentration in the Netherland Dwarf (342.5 g/L) in our study is consistent with those observed by Hewitt *et al.* (1989) in 4-7-mo old New Zealand White rabbits (311.0-370.0 g/L) and also by Özkan *et al.* (2012) in 8-12-mo old New Zealand White females (315.0-368.0 g/L). However, the mean corpuscular haemoglobin concentration values in the Dwarf Lop and the Teddy Dwarf in our study were slightly lower than those reported by the above cited authors. Lower values of mean corpuscular haemoglobin concentration in the Dwarf Lop and especially in the Teddy Dwarf (271.9 g/L) in our study were related to the statistically significantly lower value of haemoglobin in these 2 breeds as compared to the Netherland Dwarf. This finding shows the breed-specific effect on the mean corpuscular haemoglobin concentration, which could be related to the specific course of selective breeding in these 2 dwarf breeds during their development. Moreover, it was recently found in pigs that values of both the haemoglobin and mean corpuscular haemoglobin concentration were significantly influenced by an intrinsic genotype of specific genes (Wang *et al.,* 2012). The influence of breed on the white blood cells was not confirmed in our study, which is consistent with findings by Martinec *et al.* (2012) within 7 medium-sized breeds. These authors found average values of white blood cells from 2.1 to 3.7×10^9/L for both genders of 3-mo old rabbits of these breeds. These values are slightly lower than the range for this blood indicator in dwarf breeds used in our study ($3.3\text{-}6.3\times10^9$/L). The values of white blood cells we found in dwarf rabbits were within the physiological range found by Tokarz-Deptuła *et al.* (2014) in 6-8-mo old Polish mixed-breed females ($4.5\text{-}5.5\times10^9$/L). A slightly higher average value of this indicator was found by Jeklová *et al.* (2009) in 5-mo old specific pathogen-free New Zealand White rabbits of both sexes (6.2×10^9/L). However, considerably higher values of white blood cells compared to the results of our study were found by Özkan *et al.* (2012) in 10-12-mo old New Zealand White females under laboratory conditions (range of $5.8\text{-}20.1\times10^9$/L). According to Jeklová *et al.* (2009), the white blood cells count can be affected by higher antigen exposure in animal housing environments.

Biochemical examination

Regarding the total protein level, we found that it was not influenced by a breed and its values were in the physiological range determined earlier for other rabbit breeds (Burnett *et al.,* 2006; Martinec *et al.,* 2012; Özkan *et al.,* 2012). In our study, we found the range of 20.5-37.7 g/L for albumin in dwarf rabbits, while the effect of breed on this indicator has not been confirmed. Likewise, Martinec *et al.* (2012) found no breed effect on the albumin level within 3-mo old rabbits of the 7 medium-sized breeds. In their study, the average values of albumin were slightly higher (38.0-54.4 g/L) than the values of this blood indicator in our study. The higher albumin level was found also by Burnett *et al.* (2006) in New Zealand White and crossbred females (51.1 g/L). On the other hand, similar albumin values to those found in our study were found by Özkan *et al.* (2012) in 10-12-mo old New Zealand White females (range of 23.0-35.0 g/L). Moreover, in our study, a positive significant correlation between the LW and albumin level was found (+0.54), which is in agreement with the finding of Abdel-Azeem *et al.* (2010) in four pure breeds and their crossbreds (+0.50).

The glucose level in our study (range of 5.7-7.3 mmol/L) fell within the range (4.2-8.2 mmol/L) established for healthy pet rabbits according to Harcourt-Brown and Harcourt-Brown (2012).

The creatinine level in our study (range of 63.4-148.7 µmol/L) is similar to that determined by Özkan *et al.* (2012) in the 8-12-mo old New Zealand White females (60.0-140.0 µmol/L). In the present study, we found no breed effect

or LW effect or any interaction on the creatinine level. Concerning urea level in our study, the breed had a significant effect on its value, while its higher level was found in the Dwarf Lop (6.9 mmol/L) compared to the Teddy Dwarf (5.4 mmol/L). The urea value determined in our study was similar to the values found in some previous studies by Burnett *et al.* (2006) in the New Zealand White and crossbred females (5.7±0.8 mmol/L) and also of Hanna *et al.* (2008) in 3-mo old New Zealand White females (6.0±0.7 mmol/L).

The cholesterol level in our study (range of 0.6-1.5 mmol/L) was in the physiological range presented by Gillet (1994) and Wesche (2014) for rabbits (0.3-3.0 and 0.1-2.0 mmol/L, respectively). In our study, the breed had no significant effect on triacylglycerols. On the other hand, Martinec *et al.* (2012) found the effect of breed on this indicator within 3-mo old rabbits of medium-sized breeds (0.8-1.3 mmol/L), when they were fed diet with 3.7% of ether extract. Although rabbits in our study were fed diet with slightly lower content of ether extract (2.7%), triacylglycerol values (0.9-1.6 mmol/L) are similar to those found by Martinec *et al.* (2012). In addition, a negative correlation between the LW and triacylglycerols was found in our study (–0.44), although Abdel-Azeem *et al.* (2010) found in fattening rabbits this correlation to be positive (+0.77).

The breed of dwarf rabbits in our study had no effect on alanine aminotransferase activity, although we found a positive correlation between alanine aminotransferase and LW (+0.51); alanine aminotransferase activity in dwarf rabbits in our study was affected more by the intrinsic LW than by their breed. The alanine aminotransferase values we found for the Teddy Dwarf (46.9 U/L) and the Netherland Dwarf (64.3 U/L) are within the physiological range established by Gillet (1994) and Wesche (2014) for rabbits (25.0-65.0 and 27.0-72.0 U/L, respectively), while the higher alanine aminotransferase activity ($P>0.05$) in the Dwarf Lop in our study (87.0 U/L) exceeds the above stated ranges. However, according to Loeb and Quimby (1989), alanine aminotransferase values up to 100.0 U/L are still considered within the physiological range. The aspartate aminotransferase activity values in our study (31.0-50.0 U/L) were not affected by the breed and these values fall within physiological ranges established by Gillet (1994) and Wesche (2014) for rabbits (10.0-98.0 and 10.0-78.0 U/L, respectively). While a positive correlation was found between the aspartate aminotransferase activity and LW in our study. The alkaline phosphatase activity values in our study were not affected by the breed, while a negative correlation between the alkaline phosphatase activity and LW of dwarf rabbits was found. This correlation has not been found in rabbits up to now. A negative genetic correlation between the alkaline phosphatase activity and LW was also found by Yamaki and Mizuma (1982) in mice. Moreover, alkaline phosphatase activity values in our study were similar to those reported by Burnett *et al.* (2006) in crossbred and New Zealand White rabbits (69.0-111.0 U/L).

Regarding the mineral profile of blood plasma in our study, the breed had no significant effect on levels of Ca, P, Na and Cl, while their values generally fall within the physiological range (Loeb and Quimby, 1989; Harcourt-Brown, 2002; Özkan *et al.,* 2012; Wesche, 2014; Bonvehi *et al.,* 2015). Moreover, we found a highly significant negative correlation between the plasma P level and LW of dwarf rabbits. This correlation has not been published in available scientific sources for rabbits yet. Concerning the K level in our study, its value was affected by the breed, which is consistent with the statement made by Campbell (2004). The values of K level in our study (5.0-5.9 mmol/L) were similar to those reported by Burnett *et al.* (2006) in New Zealand White and crossbred rabbits (5.2-5.8 mmol/L). Moreover, values of K concentration in dwarf rabbits in our study were slightly higher than those reported by Wesche (2014) for rabbits (3.3-5.7 mmol/L).

CONCLUSION

Besides white blood cells and haematocrit value, the breed of dwarf rabbits had a significant effect on the level of majority of evaluated haematological indicators. As for biochemical indicators, breed significantly affected the level of urea and potassium. The results of our study have confirmed the assumption that haematological and biochemical blood indicators can differ depending on the dwarf rabbit breed in question.

In addition, we found that the haematocrit value, albumin, alanine aminotransferase and aspartate aminotransferase had a positive correlation with the LW of dwarf rabbits, while the triacylglycerols, alkaline phosphatase, and phosphorus levels had a negative correlation with their LW.

Values for the majority of blood indicators evaluated in dwarf rabbits are in physiological ranges found in previous studies for other rabbit breeds. However, a slightly higher value of mean corpuscular volume in the Netherland Dwarf, slightly lower value of mean corpuscular haemoglobin concentration in the Teddy Dwarf and slightly higher alanine aminotransferase activity in the Dwarf Lop were found in our study.

The findings in our study present new information on the physiology of dwarf rabbits. From the point of view of medicine and rearing, they can be helpful for both veterinarians and breeders.

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