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Haematological parameters of Hungarian, farmed ostriches

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Abstract

The analysis of haematological parameters is of great importance from a diagnostic point of view and is important on a global scale as well. The study aimed to evaluate the effects of the month, farm, bird age, and sex on blood haematology values and to make a reference range for diagnostic purposes for ostriches. Blood samples were taken during the slaughter of the birds in October and November, 2021 and in May and June, 2022. In total, 22 females and 33 males aged between 8 and 60 months from three farms in eastern Hungary were included in the evaluation. Temperature and humidity data were also collected in the indicated months. The month, farm, and age of birds strongly influenced the haematology values. The white blood cell count (WBC) was the highest in June and the mean corpuscular haemoglobin (MCH) was the lowest in May. Farm "B" differed from the two other farms in the mean cell volume (MCV), expressing the lowest mean cell value. For the 8–10- and 15–17-month-old birds, the red blood cell count (RBC), haemoglobin (HGB), and haematocrit (HCT) showed lower values compared to the 11–13- and 48–60-month-old birds. The white blood cell count (WBC), mid-cell count (MID#), and granulocyte count (GRAN#) were the highest for the 8–10- and 11–13-month old age groups; the mean cell volume (MCV) was the highest for the 48–60-month-olds. Differences could not be explained by the weather conditions. Contrary to the literature, birds on farms using probiotics did not always show higher haematological values. To draw more precise conclusions, diagnostic and serum chemical analyses should also be performed in the future.

Keywords: age, blood, farms, Hungary, month, ratites, sex
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Introduction

The species (*Struthio camelus*) was domesticated in the 1800s. With a live weight of 150 kg and standing 2-m tall, the ostrich is the biggest bird in the world (Brassó *et al*., 2020). The red-, blue- and black-necked subspecies can be distinguished within the species (Engelbrecht, 2013). Ostrich meat is considered a premium product in the markets of South Africa, the USA, Australia, Spain, Poland, and the Middle East. In addition to the meat, eggs, skin, fat, and feathers are also used. Eggs are primarily used for hatching purposes (Brassó *et al*., 2021).

The high individual value and long life expectancy of individuals of the species (180–240 months) justify the evaluation of blood composition for diagnostic purposes (Campbell *et al*., 1986). The haematological properties of blood are influenced by the climate, month, management conditions (Mushi *et al*., 1999; Durgun *et al*., 2005), species, subspecies, genotype, health status, stress condition, and sex of the analysed animals and also by the composition of the feed provided for the birds (Sabino *et al*., 2011; Soltan *et al*., 2014). The ostrich has been present in Hungary for over three decades, but we do not have any information on the blood haematology values of domesticated birds. Therefore, evaluating as many factors as possible under local conditions is necessary. By examining the

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haematological values, we can learn about the adaptability of domestic birds and increase their performance (Aikins-Wilson *et al*., 2012).

The study aimed to determine the haematological values of ostriches of different ages and sexes in other months and farm conditions. By examining blood composition, we aimed to create reference values for individuals kept under domestic conditions for diagnostic purposes. The analysis is unique at the Hungarian level, and no international literature deals with the combined effect of the four factors on haematological parameters that are examined here (month, farm, age, sex).

Materials and Methods

The slaughter process was conducted according to the 1998/XXVII legislation on the protection and mild treatment of slaughter animals.

The samples originated from three farms in Eastern Hungary (Farm1, Farm2, Farm3). The names of the farms are not disclosed for data protection reasons. The effect of the months (October, November, May, and June) was also analysed according to the weather conditions (Table 1). The chosen months represent the standard slaughter periods for ostriches in Hungary. The weather stations nearest to the evaluated farms were selected for appropriate data. Two of the stations were local, belonging to the cities (Farm1 and Farm3). The third station was located closest to Farm2 and was ~23 km away. All the farms were in the lowlands with small differences in their meters-above-sea levels. October and November were the mildest on Farm2, whereas May and June were ~1 ˚C colder on Farm3 than Farm1 and Farm2. No weather extremes (heat or cold stress) were detected in the analysed months.

Table 1 Mean temperature and humidity related to the examined months and farms

Month/farm/climatic parameter	**Farm1**		**Farm2**		**Farm3**	
	T (˚C)	**H (%)**	**T (˚C)**	**H (%)**	**T (˚C)**	**H (%)**
October 2021	8.60	69.00	9.52	72.10	9.20	68.60
November 2021	4.50	93.70	5.18	86.83	4.70	88.70
May 2022	17.40	60.40	17.42	60.06	16.00	54.10
June 2022	22.30	58.40	22.15	58.37	21.30	55.40

T (˚C) – temperature; H (%) – humidity; Hungarian Meteorological Service, 2021–2022

Farm1 had more than 100 breeding animals kept in trios and harems and produced 30–40 slaughter birds annually. The farm had self-incubated and self-raised chicks, which were sold at the age of 4 w. The chicks were kept in batteries from day old to 4 w of age in nursing rooms of 26–30 ˚C, with 20 chicks per battery. From the age of one month, they were housed in a barn at above 15 ˚C with a run. Until the age of 8 w, they were given an *ad libitum* starter diet and chopped green alfalfa in a ratio of 2:1. The starter feed consisted of a 50% chicken and 50% turkey starter diet. From the age of 8 w to 8 m, the juveniles were fed *ad libitum* turkey grower feed and chopped green alfalfa. In winter, the chopped alfalfa was replaced with alfalfa hay. From the age of 8 d until slaughter and the onset of the laying season, they received a finisher diet of 1.5 kg/day/bird, supplemented with 1 kg of chopped alfalfa. Probiotics were also added to their water until a few weeks of age.

Farm2 kept 60 breeding animals in trios. The farm produced chicks for slaughter purposes, which were slaughtered and processed at a processing plant in Hungary at the age of 10–18 m, depending on the market demand and the birds' growth rate. The chicks were kept in a battery inside the barn until they were 6 w old. From the age of 3 w, an outside run was available in addition to the barn. From the seventh week, they were kept in a 300-m^2 pen, with a stocking density of 10 m^2/bird, in mixed-sex groups. Chicks received an ostrich starter diet until the age of 8 w, then a mixture of starter and grower diets from 9–11 w. They were fed a grower diet from 12–24 w, and a finisher diet from the age of 25 weeks until slaughter. Before the age of 2 w, the feed was supplemented with a mixture of dried, chopped nettle and lemongrass leaves. Till the age of 11 w, they were given probiotics and joint protectors dissolved in drinking water. Chopped alfalfa was also added to their feed from the age of 12 w.

Farm3 purchased the chicks at the age of 4 w and fattened them until they reached optimal slaughter weight (90–100 kg). The birds were kept in a building at 21 °C on deep litter. The building also included a 50-m^2 enclosure, where they were released at an outside temperature of over 15 °C. The slaughter birds were placed in a 600-m^2 pen, at 40 birds per pen, in mixed-sex groups. A barn closed on four sides was available for the ostriches. The fattening was carried out using a semi-intensive, grain-based technology. The feed consisted of commercial turkey feed, maize, wheat, and alfalfa pellets. Until 8 m of age, the birds were fed *ad libitum* commercial fodder, turkey feed, and alfalfa pellets. After 8 m, 1 kg of commercial fodder, 25 dkg of turkey feed and 25 dkg of alfalfa pellets were provided per day. Birds did not receive vitamin and mineral or probiotic supplements at any age.

In total, 55 blood samples were collected from the slaughtered birds from 8–60m. The ostriches showed no symptoms or signs of illness, so they were considered healthy. Birds from 8–17 m were juveniles (females: n = 20, males: n = 32), as they had not yet started laying eggs. An 8 m-old bird had to be euthanized due to a complete joint sprain (*luxation*). The 48 m-old females (n = 2) and the 60 m-old male already in production were culled due to their inadequate performance. The genotype of birds was not known and the breeds/subspecies have not been distinguished yet, so results refer only to the species in general. However, breeders plan to have their individuals identified by molecular methods to know which genotype/subspecies they belong to. Financial and practical constraints have retarded this process.

The following blood parameters were measured:
- WBC (10^9L) – White blood cell count
- LYM# (10^9L) – Lymphocyte count
- MID# (10^9L) – Mid cell count
- GRAN# (10^9L) – Granulocyte count
- RBC (10^{12}L) – Red blood cell count
- HGB (g/dL) – Haemoglobin amount
- HCT (%) – Haematocrit value
- MCV (fL) – Mean corpuscular volume
- MCH (pg) – Mean corpuscular haemoglobin
- MCHC (g/L) – Mean corpuscular haemoglobin concentration

The blood samples were collected at slaughter via the jugular vein. The blood was drained into a 10-ml blood tube (BD, Belliver Industrial Estate, Plymouth, UK) containing anticoagulant (EDTA). Until examination, the samples were stored in a container with ice packs. The evaluations took place within a few hours of sample collection. The blood samples were analysed using the URIT-3000 Vet Plus automatic haematology analyser (URIT Medical Electronic Co., Ltd, P.R. China). When preparing the samples, 20 µL of blood was prediluted to 1 ml with Dia-Diluent-D dilution liquid (dilution buffer) and then gently mixed by hand. Dia-Lyse-Diff D-CF solution (Diagon Ltd., Hungary) was used to determine the values of HGB and WBC. Each sample was measured in three replicates. The WBC, RBC, PLT, HGB, and MCV values were obtained during the direct analysis. LYM#, MID# and GRAN#, HCT, MCH, and MCHC numerical values were generated using the following formulae:

$$\text{LYM\# } (10^9\text{L}) = \text{LYM\%*WBC/100} \quad (1)$$
$$\text{MID\# } (10^9\text{L}) = \text{MID\%*WBC/100} \quad (2)$$
$$\text{GRAN\# } (10^9\text{L}) = \text{GRAN\%*WBC/100} \quad (3)$$
$$\text{HCT } (\%) = \text{RBC*MCV/10} \quad (4)$$
$$\text{MCH (pg)} = \text{HGB/RBC} \quad (5)$$
$$\text{MCHC (g/L)} = \text{100*HGB/HCT} \quad (6)$$

The effects of month, farm, age, and sex on blood haematology parameters were evaluated using a multifactor analysis of variance. The examined values were included in the model as dependent variables and the month, the farm, the age, and the sex of the birds were included as fixed effects. Results are presented as means and standard error of the mean. The statistical evaluations were performed using the IBM SPSS Statistics 23.0 program. The difference between the mean values was analysed using Tukey's test at a confidence level of 95% ($P \leq 0.05$).

Results and Discussion

The WBC, MID#, GRAN#, MCV, MCH, and MCHC values differed substantially by month (Table 2). In June, the WBC was the highest. The MID# was higher in June than in October and November, but values in May did not differ from the other months. The GRAN# had the lowest value in October and the highest value in June. The MCV had the lowest values in May and June and the highest values in October and November. The MCH and MCHC were the lowest in May and the highest in November. Minimal differences were found between the monthly temperature and humidity data on the analysed farms (Table 1). Overall, Farm1 was the most humid and Farm2 had the highest average temperature. November 2021 was the most humid month, whereas June 2022 had the lowest humidity. The average monthly temperature was the highest in June and the lowest in November. In June, the average daily temperature was between 17 and 30 °C, but values above 28 °C were typical only after the sample-collection period at the end of June. In this respect, there was no cold or heat stress in the examined periods. Some samples were collected in October and November, 2021, and others in May and June, 2022. In October, the samples were obtained only from Farm1.

No literature is available regarding the effect of month on the ostrich blood haematological parameters. In native Nigerian hens, Pewan *et al*. (2019) observed that in the late rainy season (July–September), due to optimal temperature and humidity, the birds' feed intake was higher than in the early dry, late dry, and early rainy seasons. Therefore their HGB, MCV, and MCHC values were also higher. Dry-hot weather means stress for the birds, such that their haematological values are lower (Donkoh, 1989). May and June are drier and warmer in Hungary than in October and November. Although the HGB content did not change, substantially lower MCH and MCHC values were measured in spring and early summer than in the winter period. Heat stress is known to upset the homeostasis of birds, thereby reducing the number of red blood cells (Akinyemi & Adewole, 2021) and slowing down protein synthesis (Jacob, 1995). Heat stress negatively affects the lymphocytes and decreases the LYM# (Lucas & Marcos, 2013). Despite June being the hottest month in our study and in Hungary, there were no extremes in the climatic parameters (28–32 °C) in this month that would have caused heat stress (Tumová *et al*., 2014), such that haematological values did not change. Cold stress has the opposite effect on haematology values as heat stress, i.e., cold stress increases HGB and LYM# in the blood (Aarif & Mahapatra, 2013). Even though the thermal optimum of the ostrich is 20–25 °C, indicating that the weather in June is the most favourable for the species (Schou *et al*., 2021), neither the monthly changes of HGB nor LYM# showed the presence of cold stress.

Table 2 Blood haematology values of ostriches in the examined months

Haematology parameters/months	October (n = 12)	November (n = 19)	May (n = 10)	June (n = 14)
WBC (10^9L)	98.19±8.92^{a}	111.29±5.05^{a}	108.77±6.67^{a}	120.52±6.09^{b}
LYM# (10^9L)	39.56±2.08	38.83±1.18	41.83±1.56	41.44±1.42
MID# (10^9L)	11.48±0.94^{a}	12.08±0.53^{a}	13.00±0.71^{a,b}	13.92±0.64^{b}
GRAN# (10^9L)	47.15±7.08^{a}	56.49±4.00^{a,b}	53.93±5.29^{a,b}	65.16±4.83^{b}
RBC (10^{12}L)	1.08±0.18	1.20±0.10	1.46±0.14	1.40±0.13
HGB (g/dL)	17.53±1.59	19.57±0.89	18.65±1.19	18.71±1.08
HCT (%)	24.00±3.69	26.08±2.09	30.97±2.76	29.36±2.52
MCV (fL)	225.10±3.55^{b}	218.97±2.00^{b}	214.09±2.65^{a}	211.46±2.42^{a}
MCH (pg)	176.37±27.77^{a,b}	185.34±15.71^{b}	135.28±20.76^{a}	148.64±18.95^{a,b}
MCHC (g/L)	77.43±11.47^{a,b}	83.96±6.49^{b}	62.77±8.57^{a}	69.74±7.82^{a,b}

$^{a, b}$ letters indicate monthly significant differences for a parameter (P <0.05), WBC (10^9L) – White blood cell count; LYM# (10^9L) – Lymphocyte count; MID# (10^9L) – Mid cell count; GRAN# (10^9L) – Granulocyte count; RBC (10^{12}L) – Red blood cell count; HGB (g/dL) – Haemoglobin amount; HCT (%) – Haematocrit value; MCV (fL) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/L) – Mean corpuscular haemoglobin concentration

Marked differences between farms were found only in the MCV values (Table 3). MCV showed the highest value on Farm1. Our previous examinations (Brassó *et al*., 2023) on the microbiology of Farm1 and Farm2 indicated no significant difference between farms. Viruses were not analysed, but the detected bacterial pathogens were *Salmonella spp*. and *Staphylococcus spp* and, except for *Enterococcus faecalis*, they were in the same quantity on both farms. However, the investigation did not cover the slaughterhouse and the processing plant. The differences are therefore not well-founded from a microbiological point of view. In most cases, the chemical composition of the feed of birds was not evaluated for financial reasons, only the type of feedstuffs, so no conclusions can be drawn.

White blood cells (including lymphocytes, heterophil, eosinophil, and basophilic granulocytes, as well as monocytes) are immune cells that, arriving at the site of infection and inflammation, protect the body from xenobiotic substances and infections (Guyton & Hall, 2006; Davis *et al*., 2008). Stress reduces the number of lymphocytes, thus decreasing disease resistance (Gross & Siegel, 1983). The white blood cell (WBC) count can vary within relatively wide limits (97–121 10^9/L) (Mushi *et al*., 1999; Davis *et al*., 2008; Minka & Ayo, 2008). Although there is no literature comparing farms, blood haematology research has been carried out in several different countries, which can also be treated as different housing environments. The WBC values of ostriches nursed in the Tel Aviv Zoo and kept in Barcelona were between 19.5 × 10^9L and 21.0 × 10^9L (Palomeque *et al*., 1991), whereas in Botswana, the WBC was 5.0 × 10^9L (Mushi *et al*., 1999), and in Nigeria was 2.0 × 10^9L (Minka & Ayo, 2008). The literature data differed substantially, but at the same time, reported remarkably lower values compared to our results. The alterations may have resulted from the differences in geographical location and husbandry technology, although the latter is not known.

Table 3 Blood haematology values of ostriches from the three different examined farms

Haematology parameters/farms	Farm1 (n = 12)	Farm2 (n = 34)	Farm3 (n = 9)
WBC (10^9L)	98.19±8.92	116.98±3.86	104.44±7.45
LYM# (10^9L)	39.56±2.08	36.19±1.04	39.68±2.01
MID# (10^9L)	11.48±0.94	11.54±0.15	11.40±0.29
GRAN# (10^9L)	47.15±7.08	61.38±3.07	51.70±5.92
RBC (10^{12}L)	1.08±0.18	1.39±0.80	1.10±0.15
HGB (g/dL)	17.53±1.59	19.38±0.69	17.69±1.33
HCT (%)	24.00±3.69	29.42±1.59	24.18±3.08
MCV (fL)	225.10±3.55^b	213.49±1.54^a	220.98±2.96^b
MCH (pg)	176.37±27.77	153.09±12.03	190.88±23.21
MCHC (g/L)	77.43±11.47	71.06±4.97	85.91±9.58

$^{a,\,b}$ letters indicate significant differences for a parameter by farm (P <0.05), WBC (10^9L) – White blood cell count; LYM# (10^9L) – Lymphocyte count; MID# (10^9L) – Mid cell count; GRAN# (10^9L) – Granulocyte count; RBC (10^{12}L) – Red blood cell count; HGB (g/dL) – Haemoglobin amount; HCT (%) – Haematocrit value; MCV (fL) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/L) – Mean corpuscular haemoglobin concentration

WBC, MID#, and GRAN# were the highest in ostriches of 8–13 m and were the lowest in birds of 15–17 m (Table 4). Except for the MCV values, the 48–60 m values did not differ from other age groups. RBC, HGB, and HCT were the lowest in the 8–10 and 15–17 m old birds; the highest was in the 11–13 m birds. The MCV was the highest in the 48–60 m age group, but the other groups were similar in this respect.

Mushi *et al*. (1999) found the WBC of one- and 10-month-old ostriches to be 3.8 × 10^9/L and for the 11–18 m ostriches to be 5 × 10^9/L; the RBC was 1.8 and 2.1 × 10^{12}/L, the HGB was 10.9 and 16.68 g/dl, and the HCT was 36 and 43.25%, respectively (Mushi *et al*., 1999). The haematological values were higher in the older age group. In our case, the HCT was measured to be substantially lower, whereas the other parameters were much higher. A haematocrit centrifuge, haemoglobin meter, and Natt–Herrick dilution were used for the haematological examinations. The alterations could also stem from differences in the type and sensitivity of the methods. The authors did not provide information on the husbandry technology. According to Campbell (1994), the normal haematocrit value of farmed ostriches is 35–55%; a value below 35% indicates anaemia. Its decrease can occur due to blood loss, haemolysis, feed deficiencies, toxins, and environmental stressors (Harrison and Harrison, 1986; Fair *et al*., 2007). In the current study, there was no information on the parameters listed above.

Aikins-Wilson *et al*. (2012) stated that the WBC of ostriches of 56–64 w of age is 84.3–96.78 × 10^9/L range, which decreases with age. Their results were similar to those of the ostriches of a similar age (15–17 w) in our study, although the trend was different; the MCV was substantially lower, between 81.29 and 105.20 fL (Aikins-Wilson *et al*., 2012), than we experienced. The authors declared that regarding age, the MCV decreased from 56–60 w of age and then increased from 60–64 w of age. The CELL-DYN 1800 automatic analyser was used for the analysis. No information was provided regarding the feeding and husbandry technology of the birds. Higher MCV values - according to our knowledge - can be the result of iron deficiency caused by liver cirrhosis, vitamin B_{12}, or folic acid deficiency but a diagnosis did not support this. For domestic chickens (*Gallus gallus domesticus*) of 1–7 w of age, Onyishi *et al*. (2017) reported increasing HGB, HCT, MCV, and MCH values, whereas the WBC and RBC decreased with age. The authors explained the changes by the different amounts of feed consumed by birds at different ages; the Fe content of feed could also vary. In the current study, even though the different age groups consumed different amounts of feed, with the oldest (adult) group consuming the most feed, the haematology values did not differ from the other age groups. The Fe content of feedstuffs was not known.

Table 4 Blood haematology values of ostriches of different age groups

Haematology parameters/ages (month)	**8–10 (n = 5)**	**11–13 (n = 29)**	**15–17 (n = 18)**	**48–60 (n = 3)**
WBC (10^9L)	121.91±9.62[b]	115.34±4.03[b]	97.37±5.49[a]	105.67±12.91[a,b]
LYM# (10^9L)	40.62±2.25	41.01±0.94	38.58±1.28	40.18±3.01
MID# (10^9L)	13.98±1.02[b]	13.11±0.43[b]	11.17±0.58[a]	12.13±1.37[a,b]
GRAN# (10^9L)	67.31±7.64[b]	59.40±3.20[b]	46.04±4.36[a]	53.37±10.25[a,b]
RBC (10^{12}L)	1.04±0.19[a]	1.51±0.08[b]	1.12±0.11[a]	1.04±0.27[a,b]
HGB (g/dL)	16.64±1.71[a]	20.29±0.72[b]	16.42±0.98[a]	19.35±2.29[a,b]
HCT (%)	21.79±3.98[a]	31.96±1.67[b]	24.24±2.27[a]	23.83±5.34[a,b]
MCV (fL)	211.28±3.83[a]	214.23±1.60[a]	218.28±2.19[a]	231.22±5.14[b]
MCH (pg)	166.58±29.96	148.59±12.55	170.98±17.10	198.96±40.19
MCHC (g/L)	78.41±12.37	68.61±5.18	77.76±7.06	85.42±16.59

[a, b] letters indicate significant differences for a parameter by age category (P <0.05); WBC (10^9L) – White blood cell count; LYM# (10^9L) – Lymphocyte count; MID# (10^9L) – Mid cell count; GRAN# (10^9L) – Granulocyte count; RBC (10^{12}L) – Red blood cell count; HGB (g/dL) – Haemoglobin amount; HCT (%) – Haematocrit value; MCV (fL) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/L) – Mean corpuscular haemoglobin concentration

The analysed blood parameters showed no marked differences between females and males (Table 5). We also evaluated the difference between the sexes (except the 8-m and 48–60-m age groups; age extremes) to moderate the effect of age as much as possible. However, the multifactor analysis of variance did not show a difference even after the modification (P = 0.82), so the results for the entire examined population are presented.

Contrary to our findings, Soltan *et al*. (2014) stated that the RBC (2.1 and 1.9 × 10^6/μl), HGB (17.8 and 16.77 g/dL), and HCT (43 and 41.5%) values were higher for males. For MCV (261.7–252.9 fL and 228.5-234.4 fL) and MCH (99.39 and 60.91–64.64 pg), the values of females were higher than those of males. For females, RBC can take on values between 1.7 and 1.9 × 10^{12} L. For males, the range is 1.78 to 2.1 × 10^{12} L (Levi *et al*., 1989; Bonadiman *et al*., 2009; Soltan *et al*., 2014). Of those authors, Levi *et al*. (1989) and Bonadiman *et al*. (2009) found no marked difference between the sexes. The RBC data in the literature show values that were only a few tenths higher than measured in the current study. The HGB was similar, but the MCV and MCH values were lower, and the HCT was higher. The reason for the differences may be found in the feeding technology and the genotype. However, there is no information provided on these factors.

Table 5 Blood haematology values of female and male ostriches

Haematology parameters/sexes	Females (n = 22)	Males (n = 33)
WBC (10^9L)	111.17 ± 4.91	110.33 ± 4.58
LYM# (10^9L)	40.37 ± 1.15	40.18 ± 1.07
MID# (10^9L)	12.73 ± 0.52	12.58 ± 0.48
GRAN# (10^9L)	56.77 ± 3.89	56.47 ± 3.63
RBC (10^{12}L)	1.33 ± 0.10	1.22 ± 0.09
HGB (g/dL)	18.64 ± 0.87	18.74 ± 0.81
HCT (%)	28.58 ± 2.03	26.32 ± 1.89
MCV (fL)	216.25 ± 1.95	218.24 ± 1.82
MCH (pg)	154.71 ± 15.28	172.47 ± 14.23
MCHC (g/L)	71.09 ± 6.31	78.00 ± 5.89

WBC (10^9L) – White blood cell count; LYM# (10^9L) – Lymphocyte count; MID# (10^9L) – Mid cell count; GRAN# (10^9L) – Granulocyte count; RBC (10^{12}L) – Red blood cell count; HGB (g/dL) – Haemoglobin amount; HCT (%) – Haematocrit value; MCV (fL) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/L) – Mean corpuscular haemoglobin concentration

From the obtained values, our goal was to create a range typical of ostriches living in Hungary to summarize the results and facilitate diagnostic analyses. Table 6 shows the reference values created based on the results presented above.

Table 6 Reference ranges of haematology values based on the results of the current study

Haematology parameters	Reference range
WBC (10^9L)	97.37–121.91
LYM# (10^9L)	36.19–41.83
MID# (10^9L)	11.17–13.98
GRAN# (10^9L)	46.04–67.31
RBC (10^{12}L)	1.04–1.51
HGB (g/dL)	16.42–20.29
HCT (%)	21.79–31.96
MCV (fL)	211.28–231.22
MCH (pg)	135.28–198.96
MCHC (g/L)	62.77–85.91

WBC (10^9L) – White blood cell count; LYM# (10^9L) – Lymphocyte count; MID# (10^9L) – Mid cell count; GRAN# (10^9L) – Granulocyte count; RBC (10^{12}L) – Red blood cell count; HGB (g/dL) – Haemoglobin amount; HCT (%) – Haematocrit value; MCV (fL) – Mean corpuscular volume; MCH (pg) – Mean corpuscular haemoglobin; MCHC (g/L) – Mean corpuscular haemoglobin concentration

The international literature reports WBC values with extremely wide limits from 2×10^9L to 96.78×10^9L (Mushi *et al*., 1999; Minka & Ayo, 2008; Aikins-Wilson *et al*., 2012). Compared to the literature, our results were fairly close to each other (Table 6). No literature data were available for LYM#, MID# and GRAN#. According to the literature, RBC values of $1.3–2.1 \times 10^{12}$L (Levi *et al*., 1989; Mushi *et al*., 1999; Aikins-Wilson *et al*., 2012) have been reported, which are similar to the current study. The HGB reported in the literature is 12.23–18.84 g/dl (Mushi *et al*., 1999; Soltan *et al*., 2014; Pewan *et al*., 2019) and is similar to the current study. Nahid *et al*. (2006) reported the average HCT in the blood of Sudanese ostriches to be 43.6%. Based on the literature data, the basic HCT values for the ostrich species are typically 37–48% (Mushi *et al*., 1999; Nahid *et al*., 2006; Wolmarans, 2011). Haematocrit and RBC values lower than the reference values indicate anaemia, which may result from blood loss, autoimmune disease, infection, or a metabolic problem (Sakas, 2002). The birds in the current study were healthy and symptom-free, although iron deficiency could have caused the difference. However, we did not perform a biochemical analysis. Lower HCT and RBC values could be due to the different sample size and the age of the birds used, compared to our study. Regarding the effect of the month, our knowledge is also limited in other species. Our study showed a 10% difference between the lowest and the highest HCT values. MCV can take on values in a large interval (81.29–215.83 fL) (Mushi *et al*., 1999; Aikins-Wilson *et al*., 2012; Soltan *et al*., 2014; Pewan *et al*., 2019), a range greater than we report. MCH varied widely, with literature reporting values of 50.23–96.41 pg, which are much higher than our results (Palomeque *et al*., 1991; Mushi *et al*., 1999; Aikins-Wilson *et al*., 2012; Pewan *et al*., 2019). MCHC values of 30.28–38.56% have been reported (Palomeque *et al*., 1991; Mushi *et al*., 1999; Pewan *et al*., 2019), which is considerably lower than the values we report. Between farms, haematological values vary depending on climate and geographical location. In addition, the differences can be caused by the husbandry and feeding technology, measurement methods, and individual (e.g., hormonal) effects.

Conclusions

It can be concluded that the month, farm, and age of birds substantially affect the blood haematology values of Hungarian ostriches. Even though the white blood cell count and the amount of corpuscular haemoglobin changed during May and June compared to October and November, differences could not be explained by the weather conditions since there were no heat or cold extremes in the evaluated months. Elevated WBC, GRAN#, and MID# count could be a sign of acute infection or inflammation; however, this was not confirmed by symptoms. Comparing the farms, we observed differences in several values (WBC, MCV, MID#, and GRAN#). Neither the maximum geographical distance (177 km) nor the maximum height above sea level (38 m) between the farms was substantial, such that climatic conditions cannot explain the differences. The basic feed on all three farms was chicken or turkey feed, supplemented with forage; on Farm1 and Farm2, the juveniles were also given probiotics for health protection and disease prevention. The indirect beneficial effects of probiotics and prebiotics on immunological processes and blood haematological parameters are known in turkeys. Contrary to the literature, birds on farms using probiotics did not always show higher haematological values. To draw more precise conclusions, a diagnostic and serum chemical analysis, in parallel with sampling, should be performed in the future.

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Author's contributions
LDB collected the data for the study, carried out the statistical analysis and made the original draft of the manuscript; IK provided help with the statistics and the design of the study; JKL helped with the data collection, laboratory work, and the data interpretation; ZSV, PM, and RK made the visualisation and supervision of the manuscript.

Conflict of interest
The authors declare no conflict of interest.

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