Journal of Animal and Feed Sciences, 34, 3, 2025, xxx–xxx, https://doi.org/10.22358/jafs/200131/2025
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ARTICLE IN PRESS

Seasonal effects on haematological and biochemical blood parameters in cold-blooded mares

A. Czech[1], I. Sembratowicz[1,*] and E. Wnuk[2]

[1] University of Life Sciences in Lublin, Faculty of Animal Sciences and Bioeconomy, Department of Biochemistry and Toxicology, Akademicka 13, 20-950 Lublin, Poland
[2] University of Life Sciences in Lublin, Faculty of Animal Sciences and Bioeconomy, Department of Horse Breeding and Use, Akademicka 13, Lublin 20-950, Poland

KEY WORDS: biochemistry, blood, haematology, mares, seasons

Received: 2 October 2024
Revised: 20 December 2024
Accepted: 15 January 2025

* Corresponding author:
e-mail: iwona.sembratowicz@up.lublin.pl

ABSTRACT. The feeding regime and physical activity of horses are modified according to day length and weather conditions, which vary between individual seasons of the year. These changes may be reflected in peripheral blood parameters, key indicators of equine health and welfare. This study aimed to determine the effect of season on changes in haematological and biochemical blood parameters in cold-blooded mares. The experiment involved 22 cold-blooded mares aged 9 ± 3 years. Blood samples were collected from each mare to analyse haematological parameters, i.e., haematocrit (Ht), haemoglobin (HB), red blood cells (RBC), white blood cells (WBC) with a leukogram, and biochemical parameters, including glucose, total protein, urea, creatinine, total cholesterol (TCH), triacylglycerols (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and concentrations of P, Ca, Mg, Cu, Fe, and Zn. Blood was sampled in February (winter), May (spring), August (summer), and November (autumn). The analyses demonstrated that the values of most blood parameters tested varied between seasons, except for RBC and WBC counts, and urea level. The lowest Ht and HB levels, as well as the lowest percentage of granulocytes, were recorded in spring, whereas several biochemical markers, including total cholesterol, TG and HDL had lower values in autumn. Therefore, seasonal variations of the analysed blood parameters should be considered when diagnosing equine health and disease.

Introduction

Evaluation of haematological and biochemical blood parameters is an essential element of diagnosing the health condition of horses. It also allows the assessment of their nutritional status, detection of overtraining and suitability for specific type of work (Dmoch et al., 2008; Burlikowska et al., 2015). These parameters are influenced by many endogenous factors, including age, sex, breed, physiological state, and specific use of horses (Czech et al., 2019; Udeh et al., 2021). Additionally, external stress factors related to transport, training, racing, or maintenance are also relevant in this aspect (Massányi et al., 2022). In addition to the aforementioned factors, some data indicate that, despite domestication, horses retain the ability to respond to changes in the surrounding environment. Their diet and physical activity are modified according to environmental conditions, which vary throughout the year. In the warmer months, horses spend more time in paddocks or pastures, feeding on fresh forage,

Table 1. Average values of temperature (°C), humidity (%) and precipitation (mm) in 2023 (data provided by the Institute of Meteorology and Water Management, National Research Institute for the Lublin Province)

Item	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Temperature	2.2	0.5	4.3	8.1	12.7	17.1	19.5	20.3	17.4	10.9	3.3	0.8
Humidity	90	81	75	74	66	72	71	75	78	85	87	90
Precipitation	68.7	37.3	36.5	62.5	71.6	38.4	87.7	68.6	33.2	73.9	51.2	43.6

while in autumn and winter, they are mainly kept in stables with a diet based on hay.

However, when grazing on pasture, they are more exposed to injuries and insect bites, which can not only cause allergies but also be carriers of other diseases (Schaffartzik et al., 2012). All these factors can be reflected in the values of blood parameters assayed in horses and other animals. Studies on the seasonal effects on haematological and biochemical blood parameters in equine blood are limited and have mostly focused on warm-blooded horses or small horse breeds, such as ponies (Dmoch et al., 2008; Satué et al., 2014; Shawaf et al., 2018; Ono et al., 2021). Meanwhile, cold-blooded horses constitute the majority of the equine population in Poland, accounting for approximately 60% of all bred horses. These horses are valued for their good utility features and multi-faceted use in agritourism farms and agriculture (Mroczkowski et al., 2010). Understanding seasonal variations in haematological and biochemical blood parameters in cold-blooded horses could assist veterinarians and breeders in optimising horses' management and care.

Bearing this in mind, the study aimed to analyse the impact of seasons on haematological and biochemical blood parameters in cold-blooded mares.

Material and methods

The study was approved by the Animal Welfare Group at the Faculty of Animal Sciences and Bioeconomy of the University of Life Sciences in Lublin, Poland (No. ZdsDZ/3/2024). Animal care and experimental procedures were in accordance with the regulations of the European Animal Research Association under Directive 2010/63/EU(2010) on the protection of animals used for scientific purposes.

The experiment was conducted on 22 non-pregnant cold-blooded mares (Polish Coldblood Horse) aged 9 ± 3 years, kept at a single horse rearing centre in the Lublin Province (south-eastern Poland). Blood samples for analyses were collected by a veterinarian from each mare four times a year, in the middle of a representative month for each season, i.e., February (winter), May (spring), August (summer), and November (autumn). Table 1 presents the average temperatures, humidity, and precipitation recorded in individual months.

All mares were clinically healthy and showed no behavioural abnormalities. In addition, they were not engaged in any physical work. For at least 6 months prior to the experiment, all mares were housed in the same stable facilities with free access to a running yard. Each corner of the running yard was equipped with a trough, an automatic drinker, a feeder for bulk roughage (hay or meadow green forage depending on the stage of the experiment) and a salt lick.

During spring and summer, mares grazed on pastures for 12 h a day, with unrestricted access to green forage and water. They spent the remaining time in the stable, where they were provided with 5 kg of meadow hay per night. In the autumn and winter, horses spent 2–3 h per day in paddocks and the rest of the time in the stable. During this period, they were fed at 08:00, 13:00 and 18:00, receiving a total of 3 kg of oats and 2 kg of fodder beets, as well as hay *ad libitum*. To prevent gastrointestinal disorders, dietary and management adjustments between the seasonal periods were introduced gradually. The animals were fed in accordance with the guidelines of the NRC (2009), with unlimited access to water throughout the year, and locally sourced meadow green forage/hay, oats and fodder beets.

Blood was collected from the external jugular vein from all mares as part of routine preventive examinations, under conditions of minimal stress. Sampling was conducted in the morning between 6:00 and 7:00, before morning feeding to eliminate potential circadian variability. Blood was drawn into heparinised 10-ml test tubes.

Haematocrit (Ht) and haemoglobin (HB) concentrations, red blood cell (RBC) count, white blood cell (WBC) count, and the percentage composition of white blood cells (leukogram), including granulocytes (GRA), lymphocytes (LYM), and MID (mid-size cells), were determined in whole blood samples using an Abacus Junior Vet automatic haematology analyser.

Blood plasma samples were used for spectrophotometric analyses of selected biochemical parameters, i.e., glucose (Liquick Cor-GLUCOSE 60, catalogue No. 2-201), total protein (TP; Liquick Cor-TOTAL PROTEIN 60, catalogue No. 2-236), urea (Liquick Cor-UREA 60, catalogue No. 2-206), creatinine (CREAT; Liquick Cor-CREATININE 60, catalogue No. 2-233), total cholesterol (CHOL; Liquick Cor-CHOL 60, catalogue No. 2-204), triacylglycerols (TG; Liquick Cor-TG 30, catalogue No. 2-262), and HDL cholesterol (HDL-C; Liquick Cor-HDL, catalogue No. 2-053). Analyses were conducted using monotests supplied by Cormay (Lublin, Poland). Low-density lipoprotein (LDL) cholesterol concentration was calculated using the following formula: LDL(mmol/l) = TCH–HDL-TG/2.2

Analyses were also performed to determine the activity of alkaline phosphatase (ALP; Liquick Cor-ALP 60, catalogue No. 1-212), alanine aminotransferase (ALT; Liquick Cor-ALAT 60, catalogue No. 1-216), aspartate aminotransferase (AST; Liquick Cor-ASAT 60, catalogue No. 1-214), and lactate dehydrogenase (LDH; Liquick Cor-LDH 30, catalogue No 1-239). Additionally, the concentrations of selected minerals – phosphorus (P; Liquick Cor-PHOSPHORUS 30, catalogue No. 3-243), calcium (Ca; Liquick Cor-CALCIUM ARSENAZO 60, catalogue No. 3-255), magnesium (Mg; Liquick Cor-MG, catalogue No. 3-229), iron (Fe; Liquick Cor-FERRUM, catalogue No. 3-258), zinc (Zn; BioMaxima catalogue No. 99 28 14) and copper (Cu; BioMaxima catalogue No. 99 33 05) were determined using a colorimetric method. All reagents were supplied by Cormay or BioMaxima (Lublin, Poland).

The results for each group and sampling were presented as arithmetic means with standard deviations. The normality of data distribution was determined using the Shapiro-Wilk test. For normally distributed data, a one-way analysis of variance (ANOVA) was performed, with differences between groups and samplings evaluated using Tukey's test. Statistical significance was set at $P \leq 0.05$. All analyses were conducted using Statistica software, version 13.1.

Results

The concentrations of HB and HCT, as well as the percentage of GRA were significantly higher ($P = 0.006$, $P = 0.008$, and $P = 0.003$, respectively) in the blood samples collected during summer, autumn, and winter. Conversely, the percentage of LYM in mare blood samples was significantly higher ($P = 0.002$) in spring compared to other seasons (Table 2).

The values of all biochemical blood parameters tested, including enzyme activities, lipid profile parameters and minerals (except for P, Ca and Mg in the summer) remained within the reference ranges (Winnicka, 2021) (Table 3). Blood plasma glucose levels were significantly ($P = 0.021$) lower in the samples collected in the spring compared to the other seasons. In summer, mares had significantly ($P = 0.028$) higher total plasma protein concentrations than in autumn and winter, whereas blood plasma CREAT was significantly elevated in autumn compared to summer and winter. Additionally, AST, ALT and LDH activities in the blood plasma were significantly higher ($P = 0.029$, $P = 0.031$,

Table 2. Red and white blood cell parameters in cold-blooded mares

Parameter	Spring	Summer	Autumn	Winter	*P*-value[1]	Reference values for cold blood horses[2, 3]	Reference values for horses[4]
Red blood cell parameters							
RBC, 10^{12}/l	6.70 ± 0.96	6.78 ± 0.75	7.15 ± 0.59	6.72 ± 0.55	0.056	5.9–11.73[2]	5.5–10
HB, mmol/l	5.02^{b} ± 0.26	6.15^{a} ± 0.43	6.27^{a} ± 0.33	6.34^{a} ± 0.27	0.006	5.95–10.03[2]	4.96–11.17
HCT, %	21.07^{b} ± 1.26	25.19^{a} ± 1.09	25.44^{a} ± 1.88	25.61^{a} ± 2.01	0.008	25.99–46.93[2]	24–52
White blood cell parameters							
WBC, 10^{9}/l	10.14 ± 1.06	9.79 ±1.44	8.78 ± 1.36	9.20 ± 1.27	0.681	6.31–16.49[2]	5.5–12
GRA, %	53.63^{b} ± 4.59	68.11^{a} ± 1.11	69.23^{a} ± 2.09	66.53^{a} ± 1.71	0.003	35–75[3]	35–75
LYM, %	45.53^{a} ± 2.43	31.21^{b} ± 3.77	30.20^{b} ± 2.98	33.12^{b} ± 3.45	0.002	15–50[3]	15–50
MID, %	0.83 ± 0.06	0.67 ± 0.04	0.60 ± 0.12	0.35 ± 0.08	0.314		

RBC – red blood cells, HB – haemoglobin, HCT – haematocrit, WBC – white blood cells, GRA – granulocytes, LYM – lymphocytes, MID – mid-size cells; [1] the overall effect of the season, [2] Miglio et al. (2019), [3] Weiss and Wardrop (2010), [4] Winnicka (2021); [a-c] – mean values within a row for individual parameters with different superscripts are significantly different at $P \leq 0.05$, based on Tukey's post hoc test

Table 3. Biochemical parameters and mineral elements in the blood plasma of cold-blooded mares

Parameter	Spring	Summer	Autumn	Winter	*P*-value[1]	Reference values for cold blood horses[2,3]	Reference values for horses[4]
Glucose, mmol/l	$4.62^{b} \pm 0.21$	$5.43^{a} \pm 0.28$	$5.02^{ab} \pm 0.23$	$5.40^{a} \pm 0.37$	0.021	4–5.8[2]	3.1–6.2
Total protein, g/l	$71.80^{ab} \pm 3.41$	$76.00^{a} \pm 2.48$	$66.50^{b} \pm 2.25$	$67.88^{b} \pm 3.06$	0.028	57–76[2]	60–78
Urea, mmol/l	6.57 ± 1.35	5.53 ± 0.29	6.47 ± 0.83	7.42 ± 1.63	0.381	3.6–11.4[2]	4.15–7.47
Creatinine, µmol/l	$131.1^{ab} \pm 10.27$	$110.1^{c} \pm 8.95$	$145.5^{a} \pm 14.5$	$125.2^{bc} \pm 8.87$	0.003	61.3–133[2]	106.1–167.9
Enzymes, U/l							
AP	$232.2^{b} \pm 25.10$	$209.1^{b} \pm 19.40$	$196.8^{b} \pm 29.87$	$306.4^{a} \pm 13.47$	0.046	59–319[2]	109–315
ALT	$13.56^{a} \pm 1.61$	$10.04^{ab} \pm 1.67$	$8.32^{b} \pm 1.92$	$8.76^{b} \pm 1.37$	0.031	7.9 ± 2.94[3]	3–25
AST	$293.2^{a} \pm 27.43$	$312.0^{a} \pm 36.67$	$210.8^{b} \pm 31.42$	$232.5^{b} \pm 21.51$	0.029	189–456[2]	205–555
LDH	$646.0^{a} \pm 69.51$	$723.9^{a} \pm 58.41$	$531.6^{b} \pm 48.72$	$627.9^{ab} \pm 72.18$	0.049	924.41 ± 224.62[3]	520–1480
Lipid profile parameters							
TCH, mmol/l	$1.67^{ab} \pm 0.19$	$2.02^{a} \pm 0.17$	$1.42^{b} \pm 0.16$	$1.95^{a} \pm 0.15$	0.028		1.3–2.8
HDL, mmol/l	$1.31^{ab} \pm 0.12$	$1.57^{a} \pm 0.15$	$0.98^{c} \pm 0.09$	$1.28^{b} \pm 0.09$	0.041		
LDL, mmol/l	$0.27^{b} \pm 0.14$	$0.33^{b} \pm 0.18$	$0.38^{b} \pm 0.12$	$0.54^{a} \pm 0.17$	0.043		
TG, mmol/l	$0.21^{b} \pm 0.02$	$0.28^{a} \pm 0.08$	$0.11^{c} \pm 0.09$	$0.28^{a} \pm 0.08$	0.016		0.1–0.7
%HDL	78.79 ± 6.72	78.11 ± 8.54	70.24 ± 9.70	66.08 ± 5.69	0.051		
CHOL/HDL	1.28 ± 0.11	1.30 ± 0.15	1.45 ± 0.23	1.52 ± 0.15	0.218		
Mineral components							
P, mmol/l	$0.79^{b} \pm 0.29$	$0.54^{c} \pm 0.06$	$0.72^{b} \pm 0.11$	$1.45^{a} \pm 0.10$	0.002	0.4–1.6[2]	0.65–1.68
Ca, mmol/l	$2.70^{b} \pm 0.13$	$2.28^{c} \pm 0.10$	$3.19^{a} \pm 0.18$	$2.69^{b} \pm 0.10$	0.047	2.7–3.1[2]	2.68–3.35
Mg, mmol/l	$1.13^{b} \pm 0.10$	$1.58^{a} \pm 0.24$	$1.08^{b} \pm 0.08$	$0.90^{c} \pm 0.07$	0.011	0.86 ± 0.10[3]	0.7–1.15
Fe, µmol/l	$19.00^{a} \pm 1.14$	$14.77^{b} \pm 4.38$	$22.78^{a} \pm 3.19$	$20.96^{a} \pm 2.41$	0.007	21.78 ± 6.70[3]	13.1–25.1
Cu, µmol/l	$33.04^{a} \pm 4.67$	$29.39^{a} \pm 3.67$	$19.86^{b} \pm 5.11$	$17.18^{b} \pm 4.31$	0.021		
Zn, µmol/l	$13.02^{b} \pm 1.86$	$25.44^{a} \pm 9.34$	$11.23^{b} \pm 0.80$	$12.70^{b} \pm 3.10$	0.005		

AP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase; TG – triacylglycerols; TCH – total cholesterol; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; P – phosphorus; Ca – calcium; Mg – magnesium; Fe – iron; Cu – copper; Zn – zinc; %HDL – HDL to TCH ratio; [1] the overall effect of the season; [2] Pritchard et al. (2009); [3] Paden et al. (2014); [4] Winnicka (2021); [a-c] – mean values within a row for individual parameters with different superscripts are significantly different at $P \leq 0.05$

and $P = 0.049$, respectively) in the spring-summer period than in the autumn-winter period. AP activity was highest in blood samples collected during winter ($P = 0.046$). Plasma levels of TCH and TG were significantly higher ($P = 0.028$ and $P = 0.016$, respectively) in summer and winter. On the other hand, significantly ($P = 0.041$) lower blood HDL plasma levels were determined in the samples collected from mares in autumn, while LDL levels were lower in winter samples ($P = 0.043$). The highest ($P = 0.002$) concentration of P was recorded in winter, Ca in autumn ($P = 0.047$), and Mg and Zn in summer ($P = 0.011$ and P = 0.005, respectively). Fe levels were significantly higher in spring, autumn, and winter ($P = 0.007$), whereas Cu concentrations were elevated during the spring-summer period ($P = 0.021$).

Discussion

The values of RBC and WBC parameters (except Ht) determined in the blood of cold-blooded mares fell within the reference ranges (Miglio et al., 2019; Winnicka, 2021). A study on humans has shown seasonal variations in the activity of various genes, including those linked to immune responses, as reflected in blood parameters (Dopico et al., 2015). In this study, total leukocyte counts in mares remained stable across seasons, and seasonal differences were observed only in the leukogram. The percentage of granulocytes was significantly lower in spring compared to other seasons, whereas lymphocyte percentages showed the opposite trend. Similar findings were reported by Ono et al. (2021) in Noma horses, where both neutrophil and lymphocyte counts varied seasonally. When comparing winter and summer periods, the latter authors observed a significantly higher number of neutrophil granulocytes in summer and a higher lymphocyte count in winter. In addition, they found differences in total leukocyte count and eosinophil count, both of which were higher in summer. Dmoch et al. (2008) reported the highest WBC counts in

blood samples collected from horses in spring and additionally identified seasonal variations in the percentage of neutrophils and basophils in the leukogram. A study on the effect of various factors on the blood parameters of Shetland ponies has shown that the WBC count and monocyte percentage decreased significantly in winter (Shawaf et al., 2018). It is known that low ambient temperatures in winter can act as a stress factor by stimulating the hypothalamic-pituitary-adrenal (HPA) axis, leading to the suppression of the immune system (Hu et al., 2016). This was supported by findings in Polish Konik horses, where a significant decrease in leukocyte count was observed during winter (Krumrych and Wiśniewski, 1992). However, an opposite trend, i.e., an increased WBC count during winter months is also possible. Some authors have reported an enlargement of lymphoid organs in autumn and winter, suggesting an adaptive body response (Nelson and Demas, 1996). The intensity of the stress factor (temperature), the duration of exposure to this factor, and the nature of its changes, which may be sudden or successive, are likely key determinants in this context. In their experiment on cart horses, de Souza et al. (2018) found no seasonal differences in WBC parameters, except for eosinophil count, which peaked in winter. In contrast, Carthusian broodmares had the highest eosinophil counts in spring, coinciding with highest parasite exposure (Satué et al., 2014). In the present study, the RBC count in cold-blooded mares remained stable in all seasons, while significant seasonal variations were observed in HB concentration and Ht value. The lowest values of both of these blood parameters were recorded in spring. A meta-analysis on healthy individuals living in various climatic conditions demonstrated seasonal fluctuations not in RBC counts but in HB and Ht levels, which reached the highest values in winter (Kuzmenko et al., 2021). Exposure to low ambient temperatures can also stimulate erythropoiesis, as demonstrated in a previous experiment on mice (Maekawa et al., 2013). This mechanism enhances oxygen supply to tissues during periods of increased metabolic demand. In addition, cold temperatures activate the sympathetic nervous system, which in turn may lead to spleen contraction (given its sympathetic innervation), resulting in the release of stored blood cells into circulation (Stewart and McKenzie, 2002). On the other hand, extreme temperatures recorded in summer can lead to haemoconcentration due to sweating and water loss. As a result, fluctuations in RBC parameters may occur in both cold and hot months, with additional influence from atmospheric pressure. A study by Gill et al. (1982) on Arabian mares showed that high atmospheric pressure in the winter contributed to a decrease in RBC counts, while low pressure in September and March was correlated with an increase in their numbers in the blood. Ono et al. (2021) observed higher Ht, HB and RBC counts during winter in Noma horses, whereas Satué et al. (2014) reported peak RBC counts in summer and elevated Ht values in summer and autumn in Carthusian broodmares. The increase in Ht values recorded during this period was attributed not only to the activation of thermoregulatory mechanisms, but also to the higher intensity of horses' exertion due to their time spent on the pasture. Research on the seasonal effect on blood parameters in Polish Konik horses (Krumrych and Wiśniewski, 1992) revealed the highest RBC counts and HB levels in summer and autumn, with maximum Ht values recorded in summer and the lowest in winter.

The results of the present study demonstrate seasonal variations not only in the haematological parameters but also in many biochemical indices. However, it should be noted that the levels of all parameters studied were within ranges considered physiologically normal (Winnicka, 2021). Although blood glucose levels are tightly regulated by hormones and typically fall within a relatively narrow range, they can still undergo significant fluctuations (Martyniak and Tomasik, 2021). In the horses studied, maximum glucose concentration was recorded in summer and winter, and the lowest in spring. Dmoch et al. (2008) analysed blood samples from horses of different breeds and reported the highest glucose concentration in winter and lower in spring and summer, confirming seasonal dependence of this blood parameter. In contrast, a study by Ono et al. (2021) on Noma horses, and an experiment by Shawaf et al. (2018) involving Shetland ponies, did not confirm seasonal fluctuations in blood glucose levels; however, both studies were based on blood samples collected only during winter and summer. Data from a human population survey (Martyniak and Tomasik, 2021) indicated that higher glucose concentrations were recorded in autumn and lower in spring, which was consistent with our findings. A likely explanation of this dependency is the post-winter intensification of exercise and dietary modification at this time of year. The analysis of total plasma protein concentration in the horses also revealed seasonal variations, with the highest concentration determined in summer and the lowest in the autumn and winter months. These differences

may be due to the higher protein content of the forage, as fresh grass, which is the basis of the horses' diet in summer, may be richer in protein than hay (Bockisch et al., 2023). In addition, horses dehydrate more easily at high temperatures in summer. However, an experiment by Dmoch et al. (2008) did not confirm the effect of season on equine blood protein levels. Similar observations were made by Ono et al. (2021) in Noma horses and by Shawaf et al. (2018) in Shetland ponies; however, these authors analysed total protein levels only in summer and winter. The end product of protein metabolism in horses is urea, whose level depends on dietary protein intake, protein breakdown, and kidney filtration efficiency. Thus, urea level is a valuable diagnostic indicator for assessing kidney function. In the mares under study, higher plasma urea concentrations were recorded in winter than in summer, but the differences observed were statistically insignificant. When investigating the seasonal effect on blood parameters in Noma horses, Ono et al. (2021) found significant fluctuations in urea nitrogen, with markedly lower concentrations in summer compared to winter. Like urea, CREAT is an important indicator of kidney function. Plasma CREAT levels in the present study indicated proper kidney functions but showed significant seasonal variations. The lowest CREAT concentration was determined in samples collected in summer, while the highest was observed in autumn. Ono et al. (2021) also reported season-dependent changes in this equine blood parameter, but in contrast to the results of our study, they recorded a significantly higher serum creatinine levels in summer than in winter. Seasonal variations in this marker have also been observed in humans, with a maximum recorded in summer and a minimum in the winter months (Martyniak and Tomasik, 2021). The blood parameters showing seasonal variations also included TG and other lipid profile components, i.e., total cholesterol and its fractions. The lowest concentrations of TG, total CHOL, and HDL were observed in blood samples collected in autumn. In contrast, significantly higher concentrations of TG and CHOL were determined in blood samples from summer and winter. Dmoch et al. (2008) reported maximum TG concentrations in the blood of horses in spring and summer and minimum concentrations in winter, which differed slightly from our observations. The total CHOL concentrations determined by these authors also differed from those observed in the mares in our study, as its lowest value was recorded in summer, and significantly higher in spring and winter. The analysis of the activity of selected enzymes (AST, ALT, LDH and AP) in the blood of mares in individual seasons showed a consistent pattern, namely their higher activity (except for AP) in spring and summer. This may be attributed to increased physical activity of horses in this period due to grazing in the pasture. During increased muscle effort, peroxidative processes may lead to compromised myocyte membrane integrity and the release of muscle enzymes, such as CK, AST and LDH, into the bloodstream (Pal et al., 2018). Seasonal variations in enzymatic activity in equine blood were also confirmed by Vranković et al. (2015), who showed that AST and CK activity in the plasma of Holstein stallions was significantly higher in summer compared to winter, while ALT activity was significantly lower in this period compared to the other seasons. Dmoch et al. (2008) studied horses of different breeds and observed significantly higher ALT and LDH activity in spring compared to summer and winter. On the other hand, Hasković and Suljević (2011) investigated seasonal changes in enzyme activities (AST, ALT, CK, LDH, AP, GGT) in Bosnian mountain horses in spring and autumn, and observed significant increases in autumn. Another research into the seasonal variability of blood parameters in Noma horses (Ono et al., 2021) demonstrated no effect of season on AST but reported higher LDH and AP activities in summer compared to winter, which stayed in contrast to CK activity. Vranković et al. (2015) also noted a significant increase in AP activity during summer, which differed from our findings, where AP activity reached its maximum in winter. A similar pattern was observed in a study on human population, which linked seasonal fluctuations in AP activity to variations in vitamin D metabolite concentrations in the blood (Devgun et al., 1981). According to the latter authors, changes in AP activity were likely related to the activity of the bone isoenzyme involved in bone mineralization.

In addition to biochemical blood markers, the effect of seasonality was also observed in the concentrations of all minerals tested, including macronutrients and trace elements. The analysis of P concentration in the blood of mares demonstrated its highest levels in winter and significantly lower levels in summer. Krumrych et al. (1995) noted relatively small fluctuations in P concentration in the blood of Polish Konik horses across seasons; however, its minimal level was obtained in autumn. The P concentration determined in mares during summer, when their diet consisted primarily of green

forage, was reduced compared to the reference values. This suggests that the forage may not have covered the P requirements during this period. It is well-established that mineral content in plants, including P, varies depending on soil conditions, which are, in turn, influenced by weather factors such as temperature or precipitation. Ca concentrations determined in the blood of mares showed that, similarly to P, the lowest levels of this element were found in summer, slightly below the reference values (Pritchard et al., 2009; Winnicka, 2021), while the maximum levels were recorded in autumn rather than winter. Similar results for Ca were reported by Vranković et al. (2015) in Holstein stallions. In contrast, Grela et al. (2003), who analysed blood samples from English Thoroughbred foals, observed higher levels of not only Ca but also other minerals, including Mg and P, during the spring-summer period. Krumrych et al. (1995), in their study on Polish Konik horses, found the highest Ca levels in summer and the lowest in autumn and winter. Dmoch et al. (2008) reported yet another pattern, noting the lowest Ca concentration in spring, and significantly higher levels in summer and winter. Changes observed in blood Ca levels over the year may be influenced by diet, but also by endogenous vitamin D synthesis, which in our climate zone is most intense during the period of highest solar radiation, i.e., June and July. The highest concentration of vitamin D in the blood is usually observed in autumn (Tuchendler and Bolanowski, 2010). Magnesium concentrations in the blood of mares examined in the present study reached the highest levels in summer and the lowest in winter. The results of other authors indicate that maximum levels of Mg in equine blood occur in summer (Dmoch et al., 2008) or during the spring-summer period (Grela et al., 2003; Vranković et al., 2015). Examining blood samples of Shetland ponies, Gromadzka-Ostrowska et al. (1985) also demonstrated slightly higher Mg levels in spring but generally concluded that seasons had no significant effect on this mineral. This observation aligned with data from human studies, which recorded slight seasonal fluctuations in blood Mg levels throughout the year (Martyniak and Tomasik, 2021). The blood samples of mares under study were analysed for trace elements, i.e. Cu, Fe, and Zn. They exhibit similar physicochemical properties, which may result in significant interactions between them, affecting their bioavailability. A deficiency in these elements can lead to decreased erythropoiesis (e.g., reduced RBC counts and HB content) (Knottenbelt and Pascoe, 2003); however this phenomenon was not observed in the present study. Trace elements are also cofactors of many important enzymes, thus their determination seems highly advisable. Identifying key periods of their deficiency during the year could help guide dietary modifications to prevent imbalances. In the present study, the lowest iron concentration in the mares' blood was recorded in summer, while the highest in autumn. This contrasts with findings by Vranković et al. (2015), who observed a completely different pattern in Holstein stallions. A study by Gromadzka-Ostrowska et al. (1985) showed that plasma Fe and Cu concentrations in Shetland mares were lower in winter and autumn, whereas reached their peak values in spring. Similarly, Krumrych et al. (1995) assayed the highest iron concentration in blood samples collected in spring, although they recorded relatively low seasonal fluctuations. Dmoch et al. (2008) found no statistically significant differences in serum Fe concentration in horses but noted its lowest level in spring. In the present study, the analysis of Cu concentrations in the blood plasma of mares demonstrated its maximum levels in the spring and summer periods, with significantly lower concentrations recorded in autumn and winter. Biricik et al. (2005) in Standardbred mares found the highest serum Cu levels in summer and the lowest in spring. Conversely, Krumrych et al. (1995) found no seasonal variations in Zn and Cu concentrations in Polish Konik horses. The present study identified the maximum blood Zn levels in summer, while they were relatively similar and significantly lower during the remaining seasons. In contrast, a study by Gromadzka-Ostrowska et al. (1985) on mares of Shetland ponies reported elevated blood Zn levels in January. According to the latter authors, seasonal variations in blood levels of this element could be influenced by changes in the secretion of melatonin, a hormone produced by the pineal gland.

Conclusions

The results of the current study reveal seasonal variations in most of the assessed haematological and biochemical blood parameters in cold-blooded mares. This confirmed the influence of changing weather conditions, seasonal dietary adjustments, and varying activity levels on equine blood composition, as observed by other researchers. The season has proven to be a significant factor that should be taken into account when interpreting blood analysis results for cold-blooded horses.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Biricik H., Ocal N., Gucus A.I., Ediz B., Uzman M., 2005. Seasonal changes of some mineral status in mares. J. Equine Vet. Sci. 25, 346–348, https://doi.org/10.1016/j.jevs.2005.07.002

Bockisch F., Taubert J., Coenen M., Vervuert I., 2023. Protein evaluation of feedstuffs for horses. Animals 13(16), 2624, https://doi.org/10.3390/ani13162624

Burlikowska K., Bogusławska-Tryk M., Szymeczko R., Piotrowska A., 2015. Hematological and biochemical blood parameters in horses used for sport and recreation. J. Cent. Eur. Agric. 16, 370–382, https://doi.org/10.5513/JCEA01/16.4.1634

Czech A., Kiesz M., Kiesz A., Próchniak T., Różański P., Klimiuk K., 2019. Influence of type of use, age and gender on haematological and biochemical blood parameters of Małopolski horses. Ann. Anim. Sci. 19, 85–96, https://doi.org/10.2478/aoas-2018-0031

de Souza A.F., Signor J., Schade J., Saito M.E., Muller T.R., Fonteque J.H., 2018. Seasonal variation in haematological parameters in crossbred horses used for urban traction from Lages, SC, Brazil. Arch. Vet. Sci. 23, 56–62, https://doi.org/10.5380/avs.v23i3.51021

Devgun M.S., Paterson C.R., Martin B.T., 1981. Seasonal changes in the activity of serum alkaline phosphatase. Enzyme 26, 301–305, https://doi.org/10.1159/000459198

Directive 2010/63/EU, 2010. https://environment.ec.europa.eu/topics/chemicals/animals-science_en (access 12.06.2024.)

Dmoch M., Polonis A., Saba L., 2008. Influence of seasons on the haematological and biochemical parameters of blood in horses. (in Polish). Med. Wet. 64, 930–933

Dopico X.C., Evangelou M., Ferreira R.C. et al., 2015. Widespread seasonal gene expression reveals annual differences in human immunity and physiology. Nat. Commun. 12, 7000, https://doi.org/10.1038/ncomms8000

Gill J., 1982. Two years studies of seasonal changes of haematological and proteinic indices in Arab horses. Med. Wet. 38, 309–312 (in Polish)

Grela E.R., Pastuszak J., Czech A., Kempa J., 2003. The influence of sex and feeding season on the hematological and biochemical parameters of Thoroughbred blood foals. (in Polish). Ann. Univ. Mariae Curie-Sklodowska Sect. EE 21, 199–205

Gromadzka-Ostrowska J., Zalewska B., Jakubów K., Goźliński H., 1985. Three-year study on trace mineral concentration in the blood plasma of Shetland pony mares. Comp. Biochem. Physiol. A 82, 651–660, https://doi.org/10.1016/0300-9629(85)90447-5

Hasković E., Suljević D., 2011. Seasonal changes in the activity of some enzymes in the serum of Bosnian mountain horse (*Equus caballus* L.). Veterinaria 60, 23–32

Hu G., Yang S., Hu W., Wen Z., He D., Zeng L., Xiang Q., Wu X., Zhou W., Zhu Q., 2016. Effect of cold stress on immunity in rats. Exp. Ther. Med. 11, 33-42, https://doi.org/10.3892/etm.2015.2854

Knottenbelt D.C., Pascoe R.R., 2003. Diseases and disorders of the horse, 3rd Ed. Harcourt Publishers, Spain, pp 180–202

Krumrych W., Wiśniewski E., 1992. Variability of hematological parameters of Polish horses in different seasons. (in Polish). Med. Weter. 48, 373–374

Krumrych W., Wiśniewski E., Danek J., Dąbrowska J., 1995. Seasonal variations of mineral components, glucose and proteins in the blood of Polish ponies. (in Polish). Med. Weter. 51, 168–169

Kuzmenko N.V., Tsyrlin V.A., Pliss M.G., 2021. Seasonal dynamics of red blood parameters in healthy people in regions with different types of climate: a meta-analysis. Izv.-Atmos. Ocean. Phys. 57, 1271–1292, https://doi.org/10.1134/S0001433821100078

Maekawa S., Iemura H., Kato T., 2013. Enhanced erythropoiesis in mice exposed to low environmental temperature. J. Exp. Biol. 216, 901–908, https://doi.org/10.1242/jeb.076760

Martyniak A., Tomasik P., 2021. Biological variability of the most common biochemical parameters. Diagn. Lab. 57, 109–118 (in Polish) https://doi.org/10.5604/01.3001.0015.5994

Massányi M., Halo M. Jr., Massányi P., Mlyneková E., Greń A., Formicki G., Halo M., 2022. Changes in haematological and biochemical parameters in blood serum of horses during exposition to workload stress. Heliyon 13, e12241, https://doi.org/10.1016/j.heliyon.2022.e12241

Miglio A., Morelli C., Maresca C., Felici A., Di Giambattista A., Antognoni M.T., 2019. Hematologic reference intervals for the Italian Heavy Draft horse. Comp. Clin. Pathol. 28, 833–840, https://doi.org/10.1007/s00580-019-02896-0

Mroczkowski S., Frieske A., Bohaczyk M., 2010. Polish draft horses. Prz. Hod. 12, 20–22 (in Polish)

NRC (National Research Council), 2009. Nutrient Requirements of Horses. National Academic Press, Washington, DC

Nelson R.J., Demas G.E., 1996. Seasonal changes in immune function. Quarterly Rev. Biol. 71, 511–548, https://doi.org/10.1086/419555

Ono T., Inoue Y., Hisaeda K., Yamada Y., Hata A., Miyama T.S., Shibano K., Kitagawa H., Ohzawa E., Iwata E., 2021. Effect of seasons and sex on the physical, hematological and blood biochemical parameters of Noma horses. J. Equine Sci. 32, 21–25, https://doi.org/10.1294/jes.32.21

Pađen L., Gomerčić T., Uras M., Arbanasić H., Galov A., 2014. Hematological and serum biochemical reference values for the Posavina and Croatian Coldblood Horse Breeds. Acta Veter. 64, 200–212, https://doi.org/10.2478/acve-2014-0019

Pal S., Chaki B., Chattopadhyay S., Bandyopadhyay A., 2018. High-intensity exercise induced oxidative stress and skeletal muscle damage in post pubertal boys and girls: A comparative study. J. Strength Cond. Res. 32, 1045–1052, https://doi.org/10.1519/JSC.0000000000002167

Pritchard J.C., Burn C.C., Barr A.R.S., Whay H.R., 2009. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Res. Vet. Sci., 87, 389–395, https://doi.org/10.1016/j.rvsc.2009.05.003

Satué K., Gardon J.C., Muñoz A., 2014. Season of the year should be considered in the interpretation of hematology in Carthusian broodmares. J. Hematol. Res. 1, 63–68, https://doi.org/10.12974/2312-5411.2014.01.02.4

Schaffartzik A., Hamza E., Janda J., Crameri R., Marti E., Rhyner C., 2012. Insect bite hypersensitivity in horses: what do we know? Vet. Immunol. Immunopathol. 147, 113–126, https://doi.org/10.1016/j.vetimm.2012.03.017

Shawaf T., Hussen J., Al-Zoubi M., Hamaash H., Al-Busadah K., 2018. Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia. Int. J. Vet. Sci. Med. 6, 61–64, https://doi.org/10.1016/j.ijvsm.2018.03.007

Stewart I.B., McKenzie D.C., 2002. The human spleen during physiological stress. Sports Med. 32, 361–369, https://doi.org/10.2165/00007256-200232060-00002

Tuchendler D., Bolanowski M., 2010. Seasonal variations in serum vitamin D concentrations in human. Endokrynol. Otył. Zab. Przem. Mat. 6, 36–41 (in Polish)

Udeh N.E., Orji E., Madubuike A.J., Ojiako C., Okite N., Felix G.C., 2021. Effect of season, age and sex on haematology and serum biochemistry of sport horses. J. Sustain. Vet. Allied Sci. 1, 140–144, https://doi.org/10.54328/covm/josvas.2021.032

Vranković L., Aladrović J., Beer-Ljubić B., Zdelar-Tuk M., Stojević Z., 2015. Seasonal changes in enzyme activities and mineral concentration in Holstein stallion blood plasma. Vet. Arh. 85, 235–246

Weiss D.J., Wardrop K.J. (Editors), 2010. Shalm's Veterinary Hematology. 6th edition. Wiley-Blackwell. Ames. Iowa (USA)

Winnicka A., 2021. Reference values for basic laboratory tests. Warsaw University of Life Sciences, Warsaw (in Polish)