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Examination of the haematological profile of pregnant Polish Holstein-Friesian black-and-white cattle in the early stage

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Abstract

Introduction: Cattle health and welfare are monitored *via* the analysis of the haematological profile, and it shows cattle's ability to adapt to changing environmental conditions, pregnancy and lactation; profile changes also indicate reproductive disorders. The literature lacks reports of the examination of the haematological profile in cows up to the 50th day of pregnancy (dop). Therefore, this research examined that in cows up to this pregnancy stage. **Material and Methods:** A total of 101 Polish Holstein-Friesian black-and-white cows were divided into groups. The control groups consisted of non-pregnant heifers (group C00) and non-pregnant cows (group C0), and the experimental groups were pregnant heifers (group T1 at dop ≤ 28 and group T2 at dop ≥ 29–dop < 45) and pregnant cows (group T3 at dop ≥ 29–dop ≤ 50). In addition, the T3 group was divided into cows pregnant for up 45 dop and cows between 45 and 50 dop. Blood samples were collected in March and April 2021 from each animal and analysed. A transrectal ultrasound examination was performed to detect and confirm pregnancy. **Results:** Statistically significant differences ($P \leq 0.01$) between the group of cows at dop < 45 dop and those at dop ≥ 45–dop ≤ 50 dop were noted in granulocyte percentage (GRA%), white and red blood cell counts (WBC/RBC), platelets (PLT), platelet distribution width (PDW), haematocrit (HCT) and lymphocyte percentage (LYM%). No statistically significant differences were found in the mean corpuscular haemoglobin, monocytes (MON), monocyte percentage (MON%), mean platelet volume (MPV), thrombocrit or red blood cell distribution width (RDW). Similar statistically significant differences ($P \leq 0.01$) emerged between the groups of heifers in PLT, GRA, RBC, lymphocytes, LYM% and HCT, and no significant differences were found between MPV, MON, MON% or RDW. **Conclusion:** Examining the haematological profile in high-yielding cattle is vital in maintaining herd profitability and high reproduction, which depend on the quick diagnosis of disorders facilitated by haematology. This study analysed the haematology profile of dairy cattle at dop ≤ 50 for the first time, indicating changes in lymphocyte levels, which suggests that the animals experienced direct stress during the study.

Keywords: Polish Holstein-Friesian cow, haematological profile, blood examination, pregnant cows, reproduction.

Introduction

The main goal of raising dairy herds is to maintain the high level of milk production that can be achieved by a high level of reproduction (9, 10). Reproduction is an essential factor in the profitability of dairy cattle breeding because it affects the continuity of milk production and the length of calving intervals (15). To achieve sustainable animal production, the reproductive potential must be increased without compromising animal welfare. The most frequently used resource to diagnose and evaluate the health of dairy cattle is the examination of the haematological profile (9, 18). This examination facilitates the detection of productive and reproductive disorders occurring in the cows, as well as the identification of factors that change biomarkers, such as disease, pregnancy or stress (1, 14, 21, 26). The haematological profile of cows varies mainly with the climate, season, age, body condition, and nutrition received (9, 21). It is well known that changes in the profile of cattle blood are also observed during pregnancy and lactation (22, 25). This is due to the adaptation of cows to pregnancy and the increased demand for nutrients and energy, which is associated with an increase in the number of oxygen-transporting erythrocytes and the content of haemoglobin and haematocrit (22, 30, 31). Haematological profile examination is crucial in monitoring animals' health and identifying factors that cause changes in biomarkers, such as disease, stress and pregnancy (18, 22). In ruminants, blood parameters are related to the physiological state, the herd management methods, and the season of the year (18). A lower red blood cell content may indicate anaemia, while a decreased haematocrit value may indicate dehydration. Differences in leukocyte content may be due to infection (30). Limited changes and fluctuations in other blood parameters are observed in cows that have adapted to climatic conditions (18). The authors of the many publications on cattle haematology have not focussed on examining the profile of cows until the 50th day of pregnancy (dop). The aim of the research was to determine the haematological profile of black-and-white Polish Holstein-Friesian dairy cows at intervals up to the 50th dop.

Material and Methods

Ethical committee approval. The experiment was performed as part of routine clinical activities monitoring the health of the females in the herd. Nevertheless, the procedures were notified to and approved by the animal experimentation ethics committee overseeing the research of the Department of Environment Hygiene and Animal Welfare, Wrocław University of Environmental and Life Sciences (BDd/P/7/216).

Cattle and herd management. The research was carried out on 101 black-and-white Polish Holstein-Friesian cows in the second lactation. The animals were divided into groups according to the length of pregnancy (Fig. 1).

The control groups consisted of non-pregnant heifers (group C00, n = 7) and non-pregnant cows (group C0, n = 8), and the experimental groups consisted of pregnant heifers (group T1 at dop ≤ 28, n = 13; and group T2 at dop ≥ 29–dop < 45, n = 11) and pregnant cows (group T3 n = 62; at dop ≥ 29–dop, n = 35; ≤ 50, n = 27) (Fig. 1).

The animals involved in the experiment were in good condition and healthy. They were selected from animals up to their third lactation at random in order to provide a cross-section of animals in the selection. If animals with elevated blood parameters were included in the experimental group, they likely had a disease which was only subclinical, because animals with clinical symptoms would not be scheduled for mating. The animals were at the peak of lactation. The heifers' ages were comparable, while the cows' differences in age were slightly greater. Cows were between 0.9 and 6.2 years old. The youngest group consisted of non-pregnant heifers from the C00 group, the ages of which ranged from 0.9 to 1.6 and averaged 1.17 years. The oldest animals were in the T3 group and were 2.1–6.2 and on average 4.2 years old.

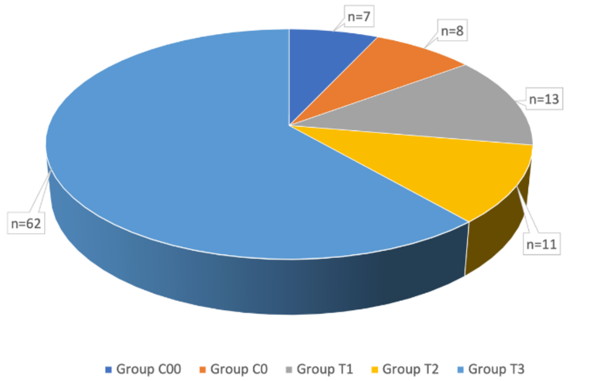

Fig. 1. The division of animals into groups according to the length of pregnancy
Group C00 – control group of non-pregnant heifers; Group C0 – control group of non-pregnant cows; Group T1 – experimental heifers at day of pregnancy (dop) ≤ 28; Group T2 – experimental heifers at dop ≥ 29–dop < 45; Group T3 – experimental cows at dop ≥ 29–dop ≤ 50

The experiment was conducted on a dairy cattle farm in a town in the Wielkopolskie Voivodeship in Poland. The cows were housed in free-stall barns and had free access to fresh water and open ventilation. The farm used the Total Mixed Ration feeding system.

Ultrasound examination of bovine pregnancy. During the research, an ultrasound examination was performed to detect and confirm pregnancy in cattle. For this purpose, transrectal ultrasonography (TRUS) was used. The examination was performed using a real-time B-mode ultrasound device (Dramiński USG iScan 2 MULTI) equipped with a 6–8 MHz linear endo-rectal transducer.

Blood collection and analysis. Blood samples for research were collected in March and April 2021 from all 101 cows. Temperature conditions were close to the thermal comfort range of cows. After the morning milking and before feeding, 5 mL of blood was collected from the tail vein into a tube sprayed with ethylenediaminetetraacetic acid (EDTA) (Profilab, Warsaw, Poland). Immediately after collecting, the blood samples were gently mixed. They were kept in an electric travel cooler (22) until they arrived at the Laboratory of the Faculty of Biology and Animal Husbandry of Wroclaw University of Environmental and Life Sciences. In the laboratory, a haematological analysis of blood samples was performed using a Mythic 18 Vet analyser (Orphée, Plan-les-Ouates, Switzerland). This device measures the white blood cell count (WBC) and red blood cell count (RBC), and analyses lymphocytes (LYM), monocytes (MON), granulocytes (GRA) and platelets (PLT). The analyser also determines the haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), red blood cells distribution width (RDW), mean platelet volume (MPV) and platelet distribution width (PDW), and thrombocrit (PCT).

Statistical analysis. The obtained results were analysed statistically. First, the mean ($\overline{x}$) and standard deviation (SD) were calculated. Then, the significance of differences between the means was estimated using the Students *t*-test and Fisher's least significant difference test. Finally, correlation values were estimated using Pearson's correlation for pairs of analysed variables using the CORR procedure in SAS Enterprise Guide (SAS/STAT 9.4; SAS Institute, Cary, NC, USA).

Results

Statistically significant differences at the level of $P \leq 0.01$ in the percentage of granulocytes (GRA%) were observed between group 1 of pregnant cows up to the 45th dop and group 2 of pregnant cows between the 45th and 50th dop (Table 1). Group 1 (n = 35) and group 2 (n = 27) constituted by the research group T3 (n = 62). Statistically significant differences at the same level between those two groups were also found in the WBC and RBC, PLT, PDW, and percentage of lymphocytes (LYM%). In addition, statistically significant differences at the level of $P \leq 0.05$ were found between group 1 and the other two groups in the content of MCHC and MCV (Table 1). However, no significant differences were found between group 3 of non-pregnant heifers and group 1 nor between group 3 and group 2 in MCH, MON, MON%, MPV, PCT or RDW.

Table 1. Average values of haematological parameters of Polish black-and-white Holstein-Friesian cattle, comparing those of non-pregnant heifers to those of pregnant cows grouped by length of pregnancy

Parameter	Group 1 $\overline{x}$ ± SD	Group 2 $\overline{x}$ ± SD	Group 3 $\overline{x}$ ± SD
WBC (10^3/µL)	8.34 ± 2.03**	7.30 ± 2.38**	9.66 ± 4.46
GRA (10^3/µL)	4.26 ± 1.04	3.72 ± 1.31**	5.34 ± 3.86
GRA% (%)	51.76 ± 8.86**	51.70 ± 11.04**	50.86 ± 12.66
LYM (10^3/µL)	3.43 ± 1.38**	3.07 ± 1.50*	3.58 ± 1.08
LYM% (%)	40.63 ± 8.85**	40.91 ± 11.02**	41.34 ± 12.40
MON (10^3/µL)	0.64 ± 0.23	0.52 ± 0.15	0.75 ± 0.33
MON% (%)	7.61 ± 1.86	7.40 ± 1.42	7.80 ± 0.55
RDW (10^3/µL)	19.42 ± 1.06	19.88 ± 1.72	20.36 ± 1.75
RBC (10^6/µL)	7.63 ± 1.10**	7.44 ± 1.46**	8.40 ± 0.58
HGB (g/dL)	11.06 ± 1.58**	11.40 ± 2.32*	11.59 ± 0.54
MCH (pg)	14.51 ± 0.84	15.36 ± 1.12	13.85 ± 1.13
MCHC (g/dL)	34.34 ± 0.74*	34.60 ± 1.05	35.45 ± 0.93
MCV (µm^3)	42.28 ± 2.58 *	44.44 ± 3.40	39.13 ± 3.56
MPV (µm^3)	6.54 ± 0.81	6.50 ± 0.73	6.83 ± 0.66
PCT (%)	0.18 ± 0.11	0.19 ± 0.13	0.17 ± 0.08
PDW (%)	31.96 ± 13.08**	29.53 ± 13.71**	42.23 ± 11.63
PLT (10^3/µL)	290.17 ± 191.98**	300.00 ± 219.29**	248.88 ± 117.54
HCT (%)	32.15 ± 4.11**	31.95 ± 7.52**	32.73 ± 1.92

Group 1 – pregnant cows up to the 45th day of pregnancy (dop); Group 2 – pregnant cows between the 45th and 50th dop; Group 3 – non-pregnant heifers (group C00); $\overline{x}$ – mean; SD – standard deviation; ** – statistical differences significant at the level of $P \leq 0.01$; * – statistical differences significant at the level of $P \leq 0.05$; WBC – white blood cell count; GRA – granulocytes; GRA% – percentage of granulocytes; LYM – lymphocytes; LYM% –percentage of lymphocytes; MON – monocytes; MON% – percentage of monocytes; RDW – red blood cell distribution width; RBC – red blood cell count; HGB – the haemoglobin concentration; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume; MPV –mean platelet volume; PCT – thrombocrit; PDW – platelet distribution width; PLT – platelets; HCT – haematocrit

Deviations from the reference values according to Astuti *et al.* (3) (Table 3) were found in the values of GRA in the group of animals up to dop 45 (4.26 ± 1.04 (10^3/μL)) and the group of non-pregnant heifers (5.34 ± 3.86 (10^3/μL)) (Table 1). Moreover, all groups of animals had higher GRA% than the reference values (Tables 1 and 3). Abnormalities were also found in LYM%, MON%, PCT and PDW for the examined groups of animals.

Table 2. Comparison of average haematological parameters of Polish black-and-white Holsteina-Friesian cattle setting heifers against pregnant cows

Parameter	H x̄ ± SD	C x̄ ± SD
WBC (10^3/μL)	9.11^a ± 2.85	6.74^b ± 1.76
GRA (10^3/μL)	4.35 ± 2.17**	4.02 ± 1.30
GRA% (%)	46.49^A ± 9.57**	58.80^B ± 5.66*
LYM (10^3/μL)	4.09^A ± 1.27**	2.20^B ± 0.49
LYM% (%)	46.10^A ± 9.60**	33.45^B ± 4.82
MON (10^3/μL)	0.67 ± 0.23	0.51 ± 0.21
MON% (%)	7.41 ± 1.14	7.75 ± 1.98
RDW (10^3/μL)	19.96 ± 1.30	19.44 ± 1.66
RBC (10^6/μL)	8.46^a ± 0.94**	6.56^b ± 0.49*
HGB (g/dL)	12.21^A ± 1.67*	9.95^B ± 0.98*
MCH (pg)	14.44 ± 1.01	15.19 ± 1.16
MCHC (g/dL)	35.08 ± 0.91*	33.95 ± 0.57
MCV (μm^3)	41.20^A ± 3.09	44.72^B ± 3.06*
MPV (μm^3)	6.76 ± 0.77	6.27 ± 0.62
PCT (%)	0.14 ± 0.07	0.26 ± 0.13
PDW (%)	40.59^A ± 11.38*	20.25^B ± 4.03*
PLT (10^3/μL)	206.52^A ± 110.26**	402.41^B ± 224.99**
HCT (%)	34.18^A ± 6.05**	29.31^B ± 2.62

H – heifers (Groups C00 + T1 + T2); C – pregnant cows; x̄ – mean; SD – standard deviation; ** – statistical differences significant at the level of P ≤ 0.01; * – statistical differences significant at the level of P ≤ 0.05; a, b, c – values in columns with different lowercase letters differ significantly (P ≤ 0.05); A, B, C – values in columns with different uppercase letters differ highly significantly (P ≤ 0.01); WBC – white blood cell count; GRA – granulocytes; GRA% – percentage of granulocytes; LYM – lymphocytes; LYM% – percentage of lymphocytes; MON – monocytes; MON% – percentage of monocytes; RDW – red blood cell distribution width; RBC – red blood cell count; HGB – haemoglobin concentration; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume; MPV –mean platelet volume; PCT – thrombocrit; PDW – platelet distribution width; PLT – platelets; HCT – haematocrit

Table 3. Reference values for haematological parameters of dairy cattle

Parameter	Reference value
WBC	4.0–12.0 (10^3/μL)
GRA	0.6–4.0 (10^3/μL)
GRA%	15.0–50.0 (%)
LYM	2.5–7.5 (10^3/μL)
LYM%	45.0–75.0 (%)
MON	0.1–0.8 (10^3/μL)
MON%	2.0–7.0 (%)
RDW	16.0–24.0 (10^3/μL)
RBC	5.0–10.0 (10^6/μL)
HGB	8.0–15.0 (g/dL)
MCH	11.0–18.0 (pg)
MCHC	30.0–37.0 (g/dL)
MCV	37.0–60.0 (μm^3)
MPV	5.5–7.5 (μm^3)
PCT	0.250–0.650 (%)
PDW	10.0–20.0 (%)
PLT	150–730 (10^3/μL)
HCT	24.0–46.0 (%)

WBC – white blood cell count; GRA – granulocytes; GRA% – percentage of granulocytes; LYM – lymphocytes; LYM% – percentage of lymphocytes; MON – monocytes; MON% – percentage of monocytes; RDW – red blood cell distribution width; RBC – red blood cell count; HGB – haemoglobin concentration; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume; MPV – mean platelet volume; PCT – thrombocrit; PDW – platelet distribution width; PLT – platelets; HCT – haematocrit

These parameters were higher than the norms, with two exceptions – LYM% and PCT – the values of which in the analysed groups of cows were lower than the reference values (Tables 1 and 3). The highest deviations from the references values were found in the third group, *i.e.* non-pregnant heifers, regarding PDW content (42.23 ± 11.63 (%)). In contrast, HCT, HGB, LYM, MON, PLT, RBC, WBC, MCH, MCHC, MCV, PLT, and RDW were normal (Tables 1 and 3).

Table 2 shows a comparison of the haematological parameters of heifers and pregnant cows, in which statistically significant differences were noted. Differences at the level of P ≤ 0.01 pertained to PLT, GRA%, and GRA. Less significant but statistically valid differences at the level P ≤ 0.05 between heifers and cows were found in HGB and PDW. Heifers had a significantly higher haemoglobin content (12.21 ±

1.67 (g/dL)) than cows (9.95 ± 0.98 (g/dL)), for which the reference value is 8.0–15.0 (g/dL) (Table 3) (3). Both heifers and pregnant cows were characterised by a higher content of GRA than the reference values, from which heifers showed the greatest deviation at 4.35 ± 2.17 ($10^3/\mu L$) against the norm of 0.6–4.0 ($10^3/\mu L$) (Tables 2 and 3). Moreover, heifers had a significantly higher ($P \leq 0.01$) content of LYM (4.09 ± 1.27 × $10^3/\mu L$) than cows (2.20 ± 0.49 × $10^3/\mu L$) and had a higher LYM% (46.10 ± 9.60 for heifers *vs*. 33.45 ± 4.82 for cows) (Table 2). The cows' LYM% value was significantly lower than the 45.0–75.0 reference value (Tables 2 and 3). There were also statistically significant differences at the level of $P \leq 0.05$ between heifers and cows in the contents of MCHC, MCV and PDW (Table 2). Heifers were characterised by a mean PDW content (40.59 ± 11.38%) twice as high as the reference value, which for this parameter is 10.0–20.0%. In contrast, the mean value of PCT obtained for heifers was lower than the reference value (0.250–0.650%) and amounted to 0.14 ± 0.07% (Tables 2 and 3). Moreover, in both groups, the reference percentage of monocytes was slightly exceeded, being 7.41 ± 1.14 for heifers and 7.75 ± 1.98 for cows. There were no significant differences between the groups in RBC, WBC, MON, MON%, MPV, PCT or MCH (Table 2).

The Pearson's correlation values considering all dairy cattle haematological parameter data are presented in Table 4.

Highly significant correlations ($P \leq 0.01$) were noted between WBC and: RBC, HGB, HCT LYM, MON, and PDW (Table 4). Correlations of regular significance ($P \leq 0.05$) were detected between WBC and MON%, between RBC and GRA, and between MON% and GRA%. Thrombocrit correlated only with PLT and PDW at the levels of $P \leq 0.01$ and $P \leq 0.05$, respectively. The mean platelet volume correlated with PDW (Table 4). The results also indicated that RBC correlated with HGB, MCHC, MCV, HCT and LYM (Table 4).

Pregnancy was confirmed in the examined cows by transrectal ultrasound and Figs 2–8 reproduce images of the sounding.

The figures show transrectal ultrasound projections of pregnancies from days 29 to 39.

Table 4. Pearson's correlation values for haematological parameters of cows' blood

	WBC	RBC	HGB	HCT	PLT	LYM	MON	GRA	LYM%
WBC	1	0.64145**	0.58184**	0.55768**	0.12123ns	0.71413**	0.83838**	0.87833ns	0.03639ns
RBC		1	0.94734**	0.90814**	0.12114ns	0.68151**	0.44398**	0.35080**	0.44345**
HGB			1	0.95365**	0.03649ns	0.66429**	0.32751*	0.26443*	0.48052**
HCT				1	0.06239ns	0.58727**	0.28291*	0.28316*	0.37719*
PLT					1	0.04063ns	0.09542ns	0.13425ns	0.15948ns
LYM						1	0.60442**	0.29617*	0.64392**
MON							1	0.69739**	0.00841ns
GRA								1	0.48615**
LYM%									1

	MON%	GRA%	MCV	MCH	MCHC	RDW	MPV	PCT	PDW
WBC	0.23363*	0.07747ns	-	-	-	0.36385*	0.02595ns	0.12563ns	0.41233**
RBC	0.30012*	0.37933*	0.45821**	0.20889*	0.64494**	0.25965*	0.01210ns	0.12181ns	0.56941**
HGB	0.41980**	0.39406**	0.19525*	0.08335ns	0.73003**	0.25594*	0.01719ns	0.03862ns	0.45478**
HCT	0.44251**	0.28904*	0.17111ns	0.05845ns	0.59783**	0.23689*	0.07609ns	0.06153ns	0.36950*
PLT	0.04885ns	0.14702ns	0.17235ns	0.17466ns	0.07183ns	0.03623ns	0.15619ns	0.99610**	0.30944*
LYM	0.15027ns	0.60204**	0.27350*	0.09556ns	0.45777**	0.28579*	0.06002ns	0.03566ns	0.40430**
MON	0.27067*	0.04035ns	0.38371*	0.26092*	0.28212*	0.36855*	0.09669ns	0.09607ns	0.31521*
GRA	0.27286*	0.52385**	0.24411*	0.12630ns	0.30521*	0.29278*	0.01533ns	0.13912ns	0.21823*
LYM%	0.03879ns	0.98379**	0.13328ns	0.03809ns	0.23664*	0.07820ns	0.11351ns	0.15558ns	0.30458*

	MON%	GRA%	MCV	MCH	MCHC	RDW	MPV	PCT	PDW
MON%	1	0.21733*	0.30243*	0.43501**	0.46124**	0.06582ns	0.10003ns	0.03840ns	0.07662ns
GRA%		1	0.18446ns	0.11527ns	0.14839ns	0.08820ns	0.12886ns	0.14511ns	0.28382*
MCV			1	0.93347**	0.02221ns	0.23940*	0.05386ns	0.16434ns	0.45209**
MCH				1	0.37543*	0.16699ns	0.03119ns	0.16664ns	0.28139*
MCHC					1	0.16761ns	0.05376ns	0.07050ns	0.36066*
RDW						1	0.05475ns	0.03287ns	0.20184*
MPV							1	0.09250ns	0.36006*
PCT								1	0.28095*
PDW									1

**– $P \leq 0.01$; *– $P \leq 0.05$; ns – $P > 0.05$; WBC – white blood cell count; RBC – red blood cell count; HGB – haemoglobin concentration; HCT – haematocrit; PLT – platelets; LYM – lymphocytes; MON – monocytes; GRA – granulocytes; LYM% – percentage of lymphocytes; MON% – percentage of monocytes; GRA% – percentage of granulocytes; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; RDW – red blood cell distribution width; MPV – mean platelet volume; PCT – thrombocrit; PDW – platelet distribution width

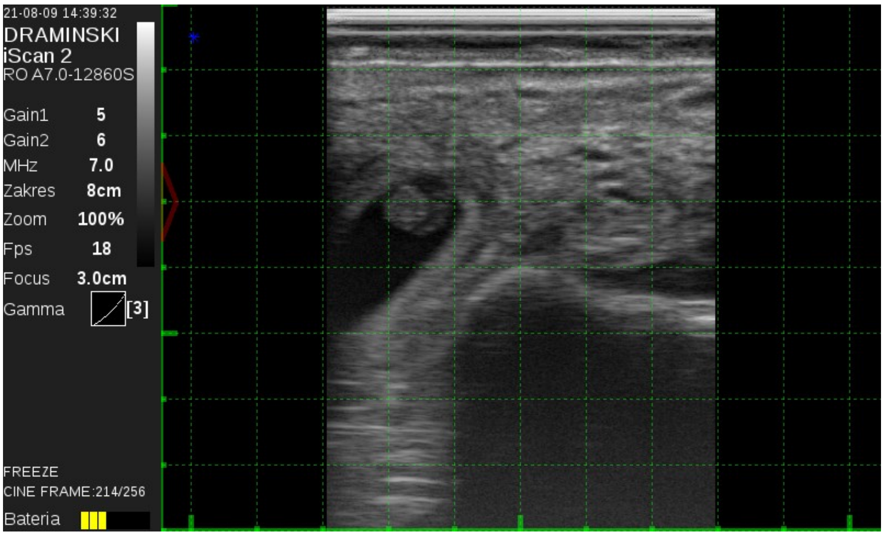

Fig. 2. Transrectal ultrasound image showing late embryo development at around the 29th day of pregnancy

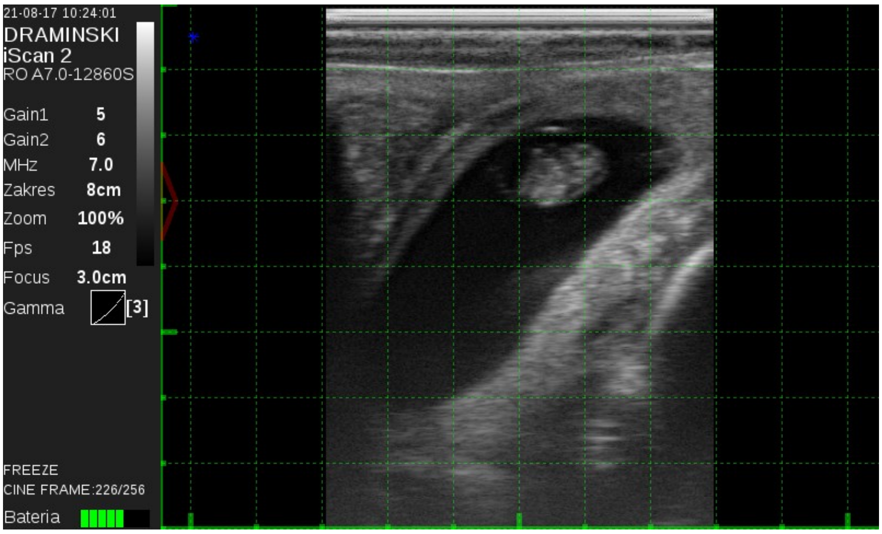

Fig. 3. Transrectal ultrasound image showing late embryo development at around the 30th day of pregnancy

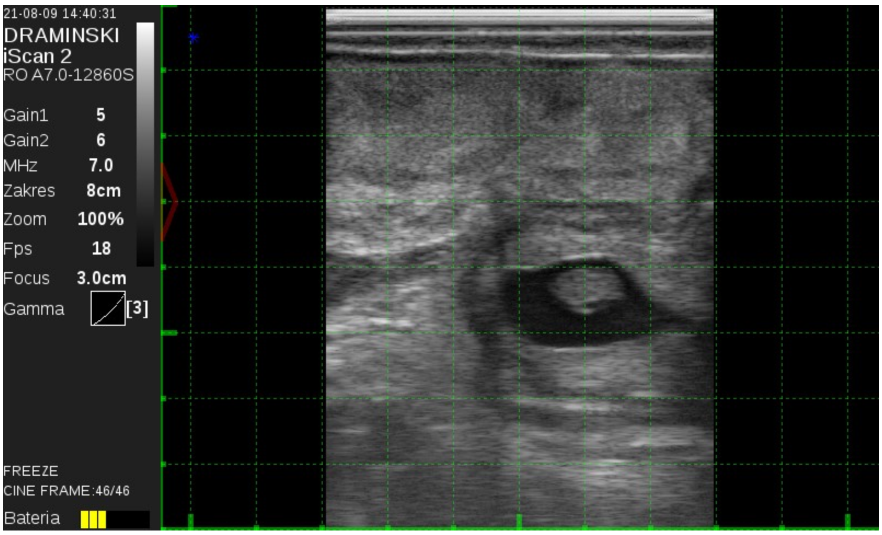

Fig. 4. Transrectal ultrasound image showing late embryo development at around the 31st day of pregnancy

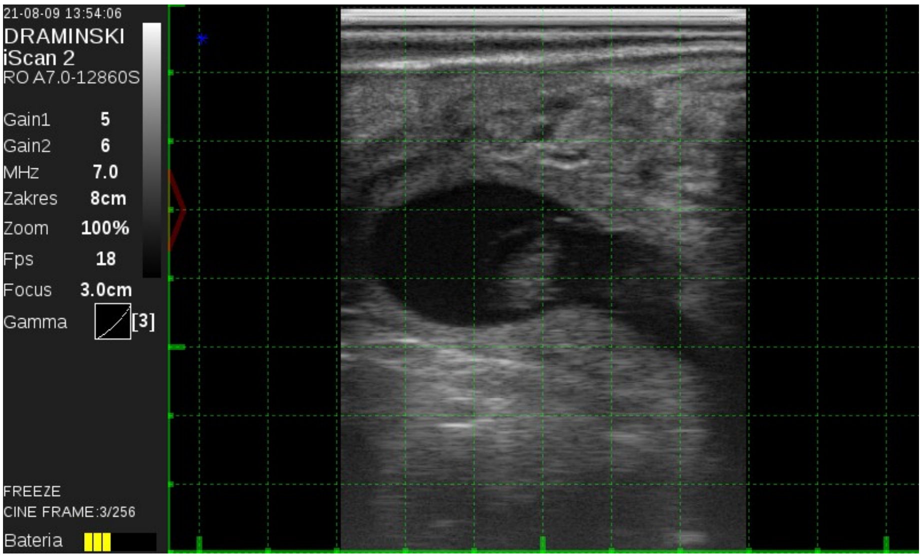

Fig. 5. Transrectal ultrasound image showing late embryo development at around the 32nd day of pregnancy

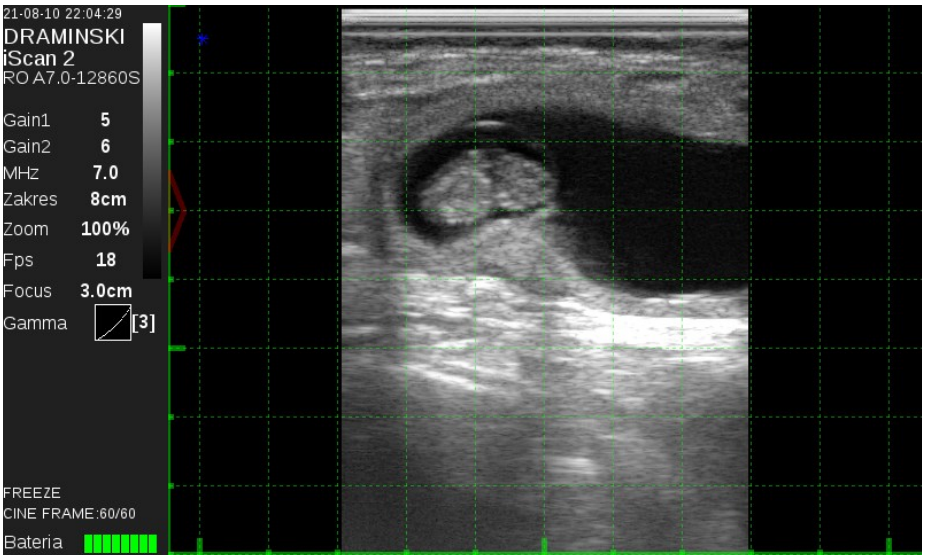

Fig. 6. Transrectal ultrasound image showing late embryo development at around the 35th day of pregnancy

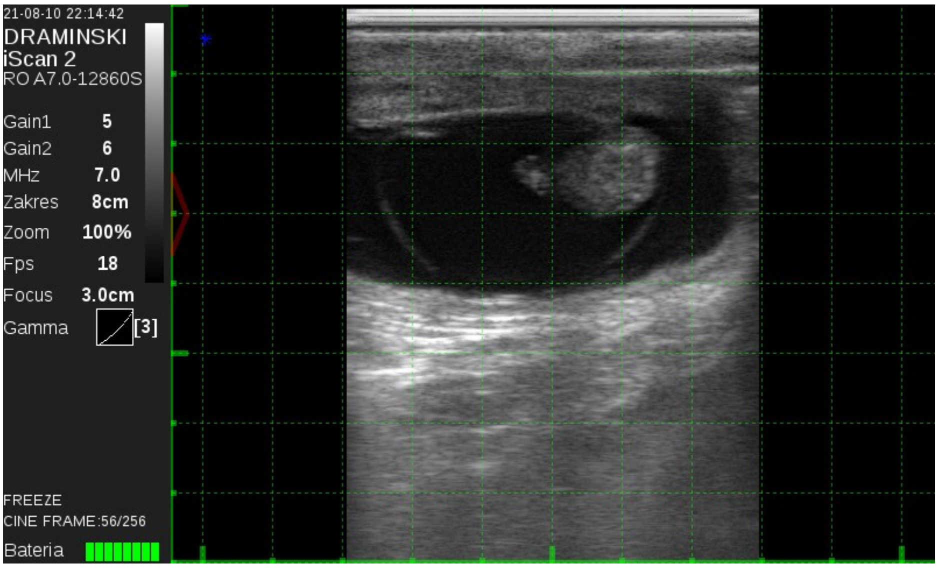

Fig. 7. Transrectal ultrasound image showing late embryo development at around the 37th day of pregnancy

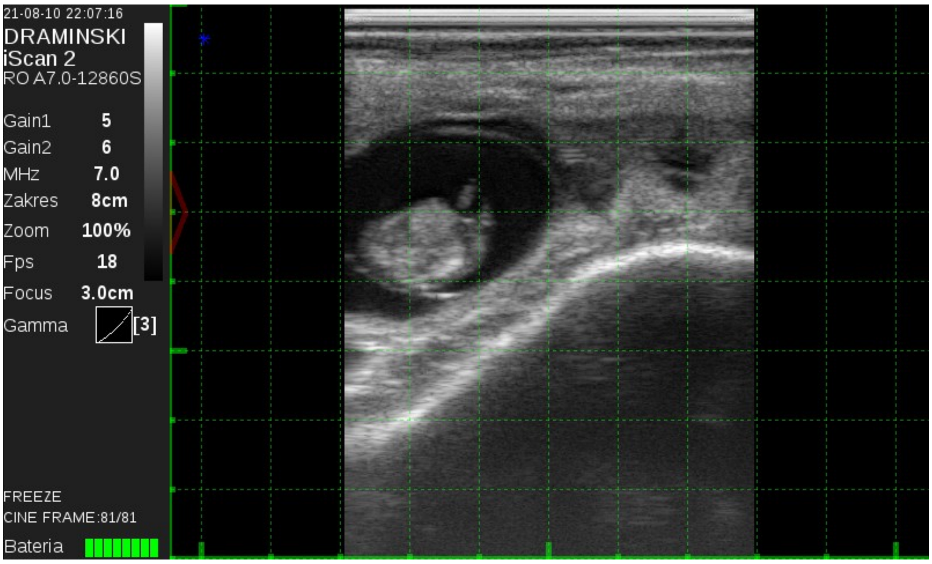

Fig. 8. Transrectal ultrasound image showing late embryo development at around the 39th day of pregnancy

Discussion

The aim of the research was to determine the haematological profile of black-and-white Polish Holstein-Friesian dairy cows at times up to the 50th day of pregnancy. In the current literature, the haematological profile of cows up to this stage of pregnancy has not been studied. The conducted research showed differences in the haematological indices depending on the physiological condition of cows. Significant differences were found mainly in the content of lymphocytes, granulocytes and platelets between heifers and pregnant cows. Similar differences were noted in the group of cows in pregnancy at or earlier than the 45th day and the group in pregnancy at between the 45th and 50th days of pregnancy compared to the group of non-pregnant cows. Examination of haematological parameters is the basis for monitoring the health and welfare of animals and is of great practical importance in the selection of animals and the assessment of their health condition, adaptation and resistance to stress, and their production and reproductive potential (4, 7, 8, 14). Blood, a connective tissue responsible for transporting nutrients, minerals, vitamins, enzymes and hormones, is involved in maintaining the physiological state, and its composition reflects the physiological state (7). The values of the typically investigated parameters are influenced by various factors, including age, nutrition, quality of conditions in which an animal is kept, lactation and pregnancy, which commonly lead to changes in these values (22, 26, 29, 30). According to Saeed *et al.* (29), heat stress also affects blood parameters in animals. The optimum ambient temperature for cows is 5 to 25°C. Above this range, it is more difficult for cows to thermoregulate (6). During heat stress, animals consume less feed to maintain optimal body temperature (37), and stress of this kind can have a negative effect on the success of fertilisation and embryo viability (2, 34).

During pregnancy, cattle blood exhibits an increase in erythrocyte content and parameters related to the red blood cells, which results from the mother's adaptation to the foetus' growth and start of lactation (27, 30). The red blood cell content may depend on the type and quality of the feed (28). Moreover, the uterus' requirements increase for blood and oxygen to feed the foetus and for gas exchange through the placenta. Because of the oxygen transport function of red blood cells, the demand for them during pregnancy increases (19). The increase in erythrocytes is due to the presence of erythropoietin secreted from the liver and kidneys. Erythropoiesis is affected by progesterone and placental somatropin and these are implicated in the increase in the number of erythrocytes (22). The increase in blood erythrocyte content occurs until late pregnancy, while their decrease is recorded on the day of calving (29). Pregnant cows have a higher red blood cell content than cows 55–60 days after calving, which results from the foetal oxygen demand (29). The conducted research did not show any values of red blood cells higher than the reference values for dairy cows (3, 23).

The increase in haemoglobin content during pregnancy is likewise due to the need to increase blood supply and transport oxygen to the developing foetus (22). Low content of haemoglobin in the blood of cows may be caused by inadequate nutrition which does not meet the nutritional requirements of the animals to which it is provided, as well as by the farm being at greater height above sea level than usual for *Bos taurus* (30). According to Setiawati *et al.* (30), the stress experienced by animals increases the haemoglobin content. This is due to the increased oxygen demand of tissue imposed by the metabolic processes occurring in times of stress (30). The increase is also noticeable in the peri-calving period when the cow's body prepares for parturition, during which the tissue's energy and oxygen requirements are higher. The decrease in blood haemoglobin content is also associated with progressive lactation (29).

Changes in the content of white blood cells may result from the migration of lymphocytes to the uterine membrane to protect against the penetration of pathogenic microorganisms and the development of infections. Moreover, it has been reported that such migration of blood morphotic elements allows for tolerance of the foetus by the mother (33). In determining pregnancy and foetoplacental development, steroid hormones, interferon-tau (IFNT), cytokines from endometrial tissues, and immune cells perform the primary functions (24). Fluctuations and changes in mechanisms cause pregnancy loss at up to 30 days of gestation in dairy cattle. The secretion of interferon-tau between days 15 and 18 of gestation in ruminants is vital for maintaining the corpus luteum, progesterone production, and supporting embryo growth, therefore disturbances in IFNT secretion can lead to miscarriage. In addition, when pregnancy is established, the content of monocytes and dendritic cells within the endometrial stroma increases as early as around the 14^{th} day of pregnancy (24). The increase in monocyte content occurs in early pregnancy and may be associated with a response to increased expression of monocyte chemoattractant (CCL2 and CCL8) and migration to the uterine endometrium in early pregnancy (36). In the endometrium, monocytes differentiate into dendritic cells that present the antigen to T-lymphocytes (24). This activation is involved in the formation of the placenta in cattle. In ruminants, modulation of the immune system is noted in the uterus and throughout the body during the peri-implantation period (32). The contribution of T-lymphocytes during embryo implantation has been described, and the total leukocyte content has been investigated between days 33 and 34 of gestation in cattle. An increase in the content of lymphocytes during pregnancy was found and was associated with the T-helper anti-inflammatory response (36). Granulocytes are presumed to be possible mediators of embryonic signals and are related to the level of expression of their genes as biomarkers for early pregnancy (20). Increased granulocyte content may be induced by increasing levels of progesterone (13, 24). A decrease in the leukocyte content in the blood (leukopenia) is observed in animals that experience a reduction in production or supply of corticosteroids (30). In turn, the increase in the number of leukocytes and neutrophils is caused by inflammatory reactions in the early stages of pregnancy that occur during the implantation of the embryo in the uterus (1, 22). In addition, an increase in leukocyte content can also be caused by summer parasitic infections and chronic viral infections (12, 18, 30). Also, these changes may be caused by disturbances in well-being, *i.e.* stress and pain, which favour more leukocytes in the bloodstream (22, 30). White blood cells (lymphocytes, monocytes, neutrophils, eosinophils and basophils) may be higher in summer because high temperatures stimulate the release of epinephrine and corticosteroids which increase the number of white blood cells (18). The authors' research showed statistically significant differences at the level of $P \leq 0.01$ in the leukocyte content between pregnant cows up to the 45^{th} day and those between the 45^{th} and 50^{th} days (Table 1) and differences at the level of $P \leq 0.05$ in heifers and in cows at different stages of pregnancy (Table 2). However, there were no higher values of WBC than the reference values for blood parameters of dairy cows (3, 23).

The MPV, PDW and PCT parameters are indicators of PLT; they indicate changes in the form and activation of platelets, and of them PDW is the most specific marker (11). The increase in parameters such as MCV, MCHC, MCH and RDW is especially noted in the later stages of pregnancy, during increased erythropoiesis (11). According to Saeed *et al.* (29), the analysis of MPV changes may be a potential marker for diagnosing heat stress in cattle. Heat-stressed cows have a reduced number of platelets and an elevated body temperature. Also, another potential marker of this type of stress may be RDW when there are few erythrocytes of different sizes. Moreover, during the summer period, higher MCHC values indicate animal tolerance for the environment (3, 37). The values of parameters such as MCV and MCH do not show significant differences when adaptation of animals to changing climatic conditions was stress free (29). In turn, higher MCHC in cattle is recorded mainly in the summer period (29). The HCT value drops in summer when the temperature of the surroundings and the body of the animal rises. This is when the blood vessels widen, leading to haemodilution (18, 29). In winter, a decrease in HCT is associated with milk production and recovery after delivery (18).

In the research, a larger content of platelets in groups 1 and 2 was noted over the content in the control groups, where the differences had statistical significance. Moreover, a higher PLT content was also observed in cows, which had a platelet content nearly twice as high as that in heifers. Nevertheless, the values obtained in the study were within the range of normal blood parameters for dairy cattle (23). A problem in high-yielding cows is preeclampsia or gestosis, which result from the failure of a mother's organism to meet the needs of a developing pregnancy (11, 17). Preeclampsia is a serious pregnancy complication that can harm the cow and foetus (16). It can present as HEELP syndrome (haemolysis, elevated liver enzymes and low platelet count) and, in more severe cases, as eclampsia (17). It can result in an acceleration of the heart rate as well as adverse obstetric effects such as foetoplacental insufficiency, but also in changes in the haematological profile (17). Preeclampsia is considered a multi-factorial disorder. Additionally, it has been reported that preeclampsia is associated with an increase in platelet function (17). Changes in PLT occur during pregnancy, which can lead to a hypercoagulable state and play a key role in the pathogenesis of preeclampsia (16). Platelets may contribute to the formation of microclots in the placenta. Therefore, analysis of thrombocyte levels may contribute to the early prediction of preeclampsia (17). An increased number of platelets in the blood may indicate a haemorrhage or a bleeding tendency, suspicion of which is provoked by visual symptoms such as ecchymosis, haematuria, epistaxis, and bloody diarrhoea (19). Decreased PLT values may be associated with an increased temperature in the summer season, which is associated with heat stress in animals (18). Thrombocytopaenia results from infection, mycotoxin poisoning, cancer, or viral diseases such as bovine viral diarrhoea (19).

In the research, statistical differences were observed between groups of animals up to the 45th and between the 45th and 50th days of pregnancy in parameters such as white and red blood cells and platelets. Differences in the content of red and white blood cells may result from the mother's body adapting to the developing foetus. Conversely, in high-yielding dairy cattle, elevated platelet counts may indicate preeclampsia or gestosis. The haematological parameters were within the veterinary reference standard ranges for the species. Changes in the content of white blood cells, including lymphocytes, are noted at the early pregnancy stage, during embryo implantation into the uterine endometrium. These changes can also indicate that cows are under stress, and the animals can be expected to have increased leukocyte values. In turn, fluctuations and changes in the cells of the immune system and hormones can lead to pregnancy loss by the 30th day. The haematological profile examination is usually performed for diagnostic purposes and to monitor the health of animals. It is an effective method to assess the body's homeostasis as well as to detect possible disorders or diseases and signal implementation of measures to prevent their development. However, more research is needed to detect potential pathologies of early pregnancy in cattle.

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