

ANALYSIS OF CHANGES IN SELECTED HEMATOLOGICAL PARAMETERS IN BLOOD FROM PRIMIPAROUS DAIRY COWS DURING LAST TRIMESTER OF PREGNANCY AND FIRST TWO MONTHS OF LACTATION

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ABSTRACT

Dynamic changes in an organism of heifers at the end of pregnancy, and in primiparous cows in early lactation, may imply changes in morphological parameters. Their evaluation may contribute to a better understanding of changes, occurring in an organism of pregnant and lactating young cows. Hence, the aim of the study was to investigate, analyze and evaluate the changes in hematological parameters of peripheral blood (RBC, MCV, HGB, MCH, MCHC, WBC, PLT and HCT) in pregnant heifers/primiparous cows, of Polish Holstein-Friesian of Black-and-White variety, during the last trimester of pregnancy and in the first two months of lactation, with particular emphasis on transition period. Blood parameters were analyzed with the use of analyzer: scil Vet abc Plus+ (HORIBA Medical), on the following dates: 90, 30, 14 and 7 days before parturition and 7, 14, 30 and 60 days after parturition. Results of the study indicate that the most abrupt changes were observed for hematocrit, erythrocyte count, leukocytes, thrombocytes and hemoglobin concentration in blood. Parameters of red blood cells, at the end of pregnancy and first two months of lactation, did not exhibit any statistically significant changes. Noteworthy, is the convergence of trends in hematocrit and hemoglobin during analyzed period. High and stable hematocrit at the end of pregnancy was due to the stable erythrocyte count in this period. Decrease in RBCs after calving, and lower values during first two months of lactation, caused postpartum decrease in hematocrit and its stabilization at a lower level. These observations denote indirectly changes in blood volume, during analyzed period, what is supported by: tendencies observed for hemoglobin concentration, and stable MCV and MCHC. Obtained results suggest high efficiency of homeostatic mechanisms in pregnant and lactating heifers/primiparous cows. Our results may be useful for comparative studies both in physiology and health disturbances research in cows during the pre and post-partum period.

Key words: heifers, primiparous dairy cows, pregnancy, lactation, blood, hematological indices

INTRODUCTION

At the end of pregnancy and during first months after parturition, dynamic adaptive changes in intensity of metabolic processes occur in a female body. These changes are connected with fetal growth rate, mammo-genesis, onset of colostrum and milk production, with reproductive system regeneration and preparation for new fertilization and pregnancy [Darul and Kruczyńska 2005, Brucka-Jastrzębska and Kawczuga 2007, Mordak 2009].

Analysis of blood deliver valuable information about dynamic, metabolic changes in an organism [Szefner-Mirzolemska 2009]. Hematological analysis are often applied in veterinary practice, in order to assess potential risk of disease occurrence or as a tool for early diagnosis of health disturbances. The results, which differ significantly from reference values, indicate disorders of homeostasis and suggest organ dysfunction or infection. Nevertheless, when interpreting the results of studies, we should also take into account the physiological state of

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an organism (e.g. age, sex, stage of pregnancy or lactation) and conditions of living, especially animal nutrition [Kupczyński and Chudoba-Drozdowska 2002, Brucka-Jastrzębska et al. 2007, Mordak 2008, Quiroz-Rocha et al. 2009].

Dynamic changes in the body of heifers at the end of pregnancy, and in primiparous cows in initial period of lactation, may cause changes in hematological parameters. Their evaluation may contribute to a better understanding of changes occurring in the body of pregnant and lactating young cows.

Hence, the aim of the study was to investigate, analyze and evaluate changes in hematological parameters, in peripheral blood of cows of Polish Holstein-Friesian of Black-and-White variety, during the last trimester of first pregnancy and in the first two months of lactation, with special emphasis on the transition period.

MATERIAL AND METHODS

The studies were carried out on 8 pregnant heifers/primiparous cows, of Polish Holstein-Friesian of Black-and-White variety, aligned genetically (95–100% HF; half-sisters), during the last trimester of pregnancy and in the first two months of lactation. Animals were kept on a large farm, in a free-stall housing system. Animals were fed according to INRA feeding norms [INRA 2009], in conformity with the TMR system. The main components of fodder were silages (grass meadow, corn, alfalfa) and meal (rapeseed, barley), supplemented with mineral – vitamin additives. Cows had free access to water. All animals were clinically healthy, without any periparturient health disturbances (the herd were under constant veterinary supervision).

The studies were performed on blood, drawn from external jugular vein (*vena jugularis externa*), each time at the same hour (at approximately 11.00), on the following dates: 90, 30, 14 and 7 days before parturition, and 7, 14, 30 and 60 days after parturition. Blood samples were collected using sterile needles, into sterile tubes coated with anticoagulant (EDTA K2), and were then centrifuged (3000 rpm; temp. 4°C) and transported to analytical laboratory of Department of Physiology at Pomeranian Medical University in Szczecin, where the analyses were performed. In collected material were determined: the total number of: white blood cells (WBC), platelets (PLT), red blood cells (RBC); mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HGB), hematocrit (HCT). The tests were performed with the use of analyzer: scil Vet abc Plus+ (HORIBA Medical). Obtained results were presented in tables, mean values and standard deviations were calculated. In order to confirm the significance of differences, between values before and after parturition, the results

were analyzed with the use of one-way ANOVA, with Duncan's multiple range test.

The study design was approved by Local Ethical Committee for Experiments on Animals in Szczecin (resolution no: 22/2009, dated 10.07.2009).

RESULTS AND DISCUSSION

Obtained results were presented in figures 1–8. The average number of leukocytes in the peripheral blood of cows ranged from 8.17 to $11.86 \cdot 10^3 \cdot \text{mm}^{-3}$ (Fig. 1). Statistically significant changes in the number of leukocytes were observed during the last two weeks before the delivery (an increase of $1.68 \cdot 10^3 \cdot \text{mm}^{-3}$, compared to the values observed 30 days prior calving) and during first two weeks of lactation (decrease of approximately $3 \cdot 10^3 \cdot \text{mm}^{-3}$). At the end of the first month of lactation, the number of leukocytes increased and stabilized at a level similar to that observed in the 7th and 8th month of pregnancy.

Similar tendencies, i.e. an increase in the number of white blood cells in cows blood, about two weeks before parturition, and a decrease in the first weeks after calving, were observed by Kehrlí et al. [1989]. Krakowski [2003], also reported an increase in the number of WBC in cows blood, immediately before parturition. Todorović and Davidović [2012], showed the highest number of leukocytes, in dairy cows of Holstein-Friesian breed, immediately before parturition (a high percentage of neutrophils and monocytes, in parallel with low number of lymphocytes), and a lowering rate of WBC after calving (the lowest number of leukocytes was observed on 45th day of lactation).

Meirom et al. [1999], Mir et al. [2008], Oliveira and Hansen [2009], Szefner-Mirzolemska [2009], highlight changes in the total number of leukocytes and changes in percentage of subpopulations of these cells during periparturient period. Serrano et al. [2011], showed that during intensive milk production in HF herd, decrease in the number of leukocytes in blood is observed. The authors explain it by influx of white blood cells from peripheral blood to the udder. In contrast, Gavan et al. [2010] demonstrated increased number of white blood cells in blood from primiparous, lactating cows, during first weeks after calving. Sattar and Mirza [2009], showed that pregnant heifers had higher number of leukocytes in blood, compared to parturient cows. However, it is worth noting, that increased number of white blood cells may indicate a pathological condition, such as infection or inflammation of e.g. mammary gland [Meglia et al. 2001].

Platelet count differed statistically significantly between the period of pregnancy and lactation (Fig. 2). In last trimester of pregnancy ranged from 347.92 to $389.25 \cdot 10^3 \cdot \text{mm}^{-3}$, and during lactation from 522.86 to $696.71 \cdot 10^3 \cdot \text{mm}^{-3}$. Noteworthy, is statistically sig-

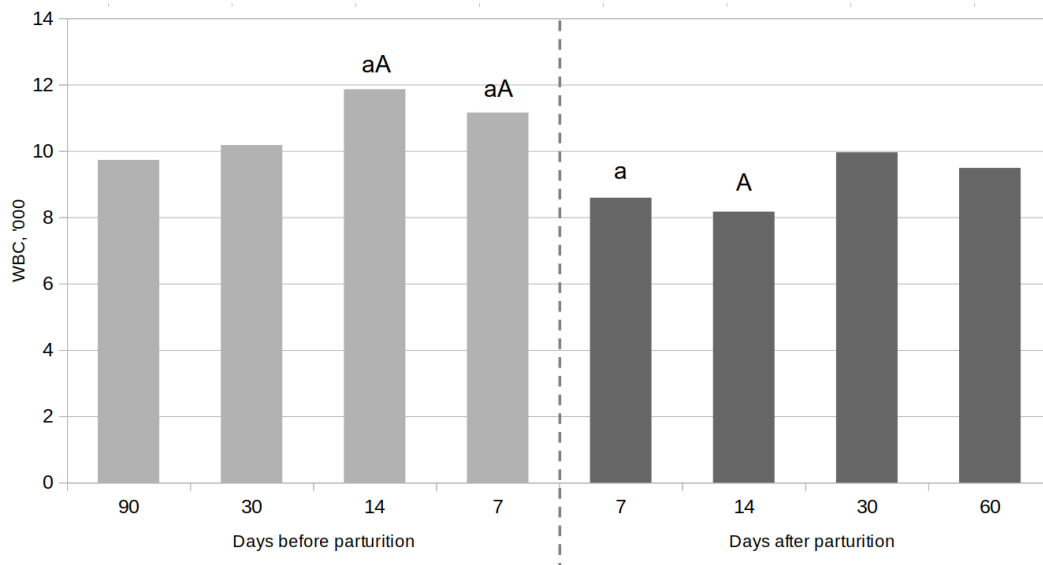


Fig. 1. The average number of leukocytes (WBC, $10^3 \cdot \text{mm}^{-3}$) in the peripheral blood of cows before and after parturition; significant differences at $P < 0.05$ (a) and $P < 0.01$ (A) are marked between values, before and after parturition

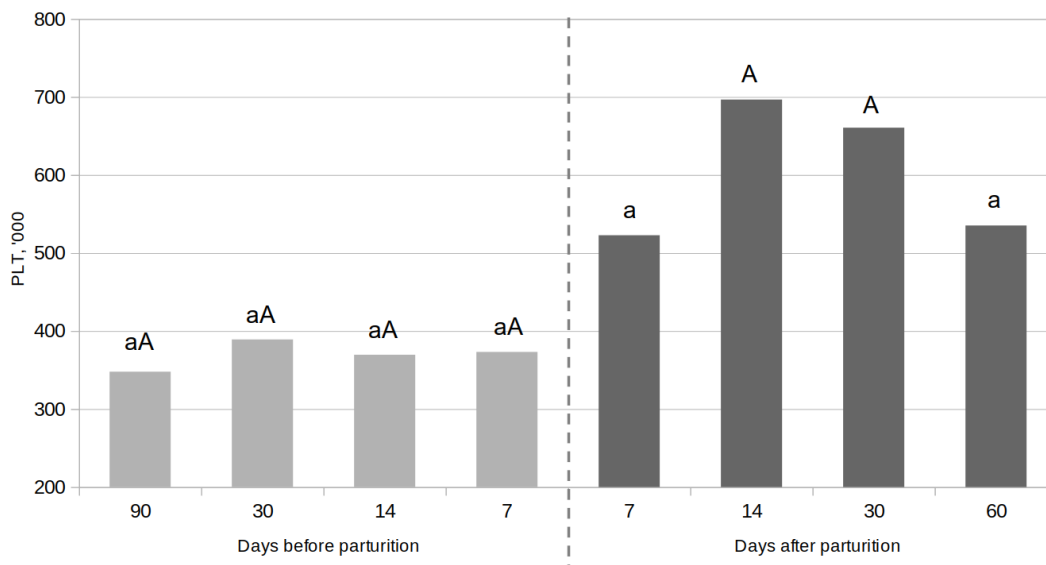


Fig. 2. Platelet count (PLT, $10^3 \cdot \text{mm}^{-3}$) in the peripheral blood of cows before and after parturition; significant differences at $P < 0.05$ (a) and $P < 0.01$ (A) are marked between values, before and after parturition

nificant ($P < 0.01$), nearly double increase in the number of thrombocytes, during first two weeks after calving, compared to values observed one week before the delivery. From 2nd week of lactation, platelet count decreased, but on 60th day of lactation was statistically significantly higher ($P < 0.01$), than that observed during pregnancy.

Stability of platelet count in blood of pregnant cows was demonstrated in studies of Brucka-Jastrzębska et al. [2007] and Szefer-Mirzolemska [2009]. Thibodeaux et al. [1990], showed that thrombocytopenia observed in humans and mice, does not occur at early stages of preg-

nancy in cattle. In addition, the authors observed no differences in blood platelet count, between pregnant and non-pregnant heifers.

Number of red blood cells in blood of tested cows ranged from 6.94 to $8.21 \cdot 10^6 \cdot \text{mm}^{-3}$ (Fig. 3). In the third trimester of pregnancy, RBC count was statistically significantly higher, than during first two months of lactation. It is worth to emphasize, that decrease in number of erythrocytes in blood, was statistically significant ($P < 0.05$) in the first week after parturition, and that this parameter stabilizes until the end of second month of

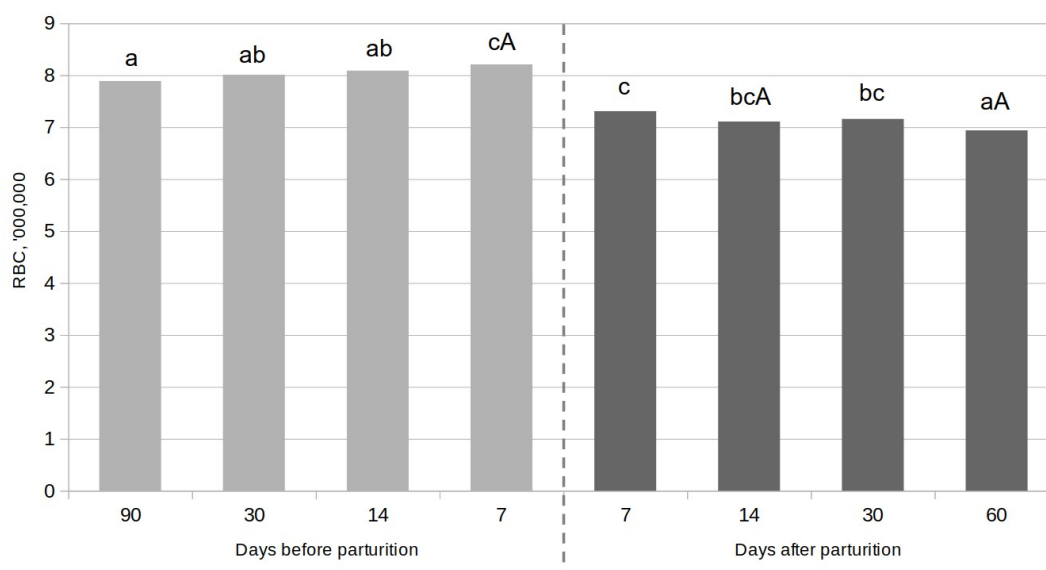


Fig. 3. The average number of red blood cells (RBC, $10^6 \cdot \text{mm}^{-3}$) in the peripheral blood of cows before and after parturition.; significant differences at $P < 0.05$ (a, b, c) and $P < 0.01$ (A) are marked between values, before and after parturition

lactation. Worth mentioning, is the fact that the content of erythrocytes in blood of examined cows, both, during pregnancy and lactation, was very high and exceeded the upper limit of reference values.

Klinkon and Zadnik [1999], observed an increase in the number of erythrocytes in blood of cows, during last 10 days before the delivery, and a decrease on 1st, 2nd, 3rd and 4th day of lactation. In contrast, Brucka-Jastrzębska and Kawczuga [2007], Sattar and Mirza [2009] and Sudha Rani and Prabhakar Rao [2021] reported that in late pregnancy in cows, a decrease in a number of RBCs is observed.

Increased erythrocyte count in blood of pregnant cows (compared to non-pregnant) was reported by Darul and Kruczyńska [2005]. Also, Krakowski [2003], found increased erythrocyte number in cows, in the second trimester of pregnancy. Brucka-Jastrzębska et al. [2007], suggest that during pregnancy, especially at early stages, increased erythropoiesis is observed. On the contrary, Shibu et al. [2011], showed that number of erythrocytes in blood of pregnant heifers (over the entire period of gestation), was statistically lower than in non-pregnant heifers. Different results were obtained by Johnson et al. [1990]. The authors did not observe statistically significant differences in RBC number between pregnant and non-pregnant animals.

Lower number of erythrocytes in blood of cows after calving was observed by Hejlasz et al. [1987], and Gāvan et al. [2010]. Also, Nazifi et al. [2008], reported a decrease in RBCs in cows blood during lactation (erythrocyte number was higher on 30th day of lactation, compared to 60th day of lactation). Lower number of red

blood cells in blood of cows, in the first month of lactation, was also noted by Shibu et al. [2011].

These diversified results, presented by different authors, indicate that during pregnancy, increased erythropoiesis is observed, in order to cover fetus and mother's increased demand for oxygen [Joshi et al. 2018, de Oliveira et al. 2019, Merdana et al. 2020, Sudha Rani and Prabhakar Rao 2021]. However, it is widely known, that especially during third trimester of pregnancy, there is an increase in volume of total body water, and increase in volume of blood. This results in a reduction of RBC count, while increasing the absolute number of these cells. The results indicate, that in analyzed heifers, both, during pregnancy and first months of lactation, blood oxygenation were sufficient.

Mean corpuscular volume (MCV) in heifers during third trimester of pregnancy, remained at a similar level and fluctuated in the range of 47.43 to $49.00 \mu \cdot \text{m}^{-3}$ (Fig. 4). After calving, statistically significant decrease of MCV was observed. On 30th day it reached $44.57 \mu \cdot \text{m}^{-3}$ ($P < 0.05$), and $43.57 \mu \cdot \text{m}^{-3}$ on 60th day ($P < 0.1$).

However, no statistically significant changes were observed in mean corpuscular hemoglobin concentration (MCHC), both during third trimester of pregnancy and two months of lactation (Fig. 5). Average value of MCHC during entire analyzed period was $33.14 \text{ g} \cdot \text{dL}^{-3}$ (32.76 – 33.71).

Also, mean corpuscular hemoglobin (MCH) showed slight changes and the average value of this parameter during pregnancy and lactation was 15.48 pg (14.53 – 16.11). Noteworthy, is gradual decrease in hemoglobin concentration in erythrocytes with time in lactation (Fig. 6).

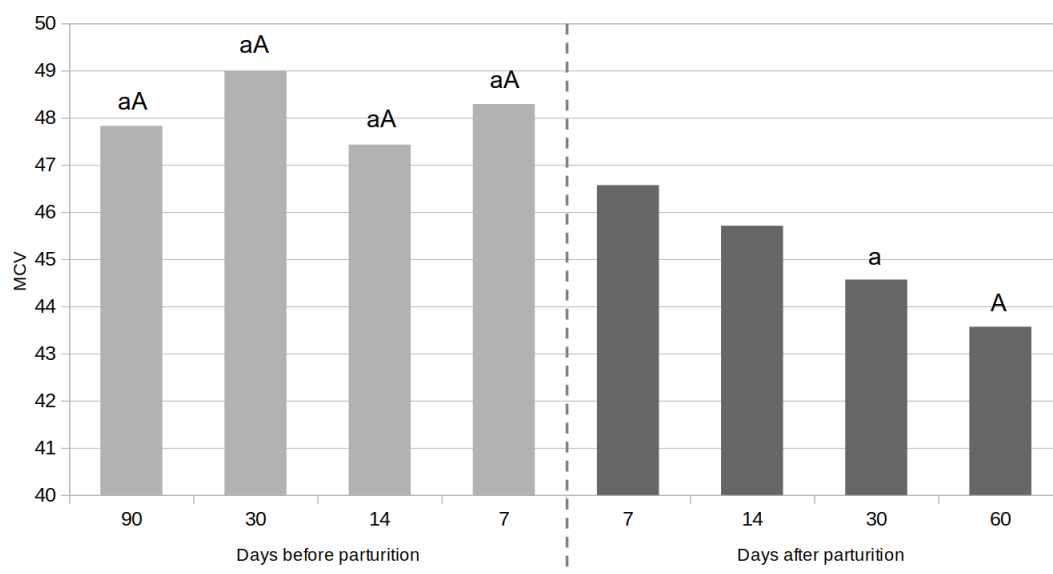


Fig. 4. Mean corpuscular volume (MCV, $\mu \cdot \text{mm}^{-3}$)

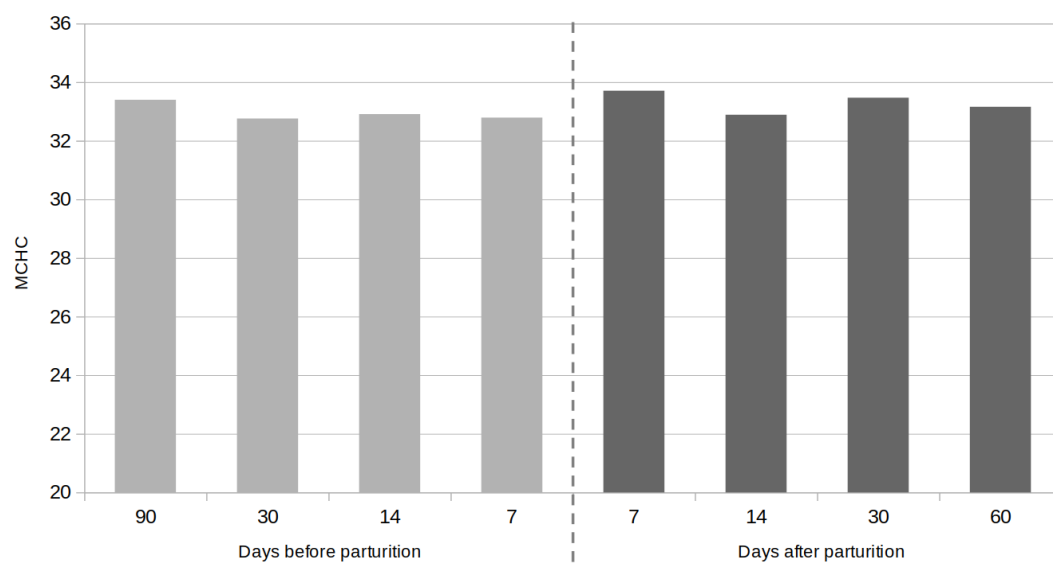


Fig. 5. Mean corpuscular hemoglobin concentration (MCHC, $\text{g} \cdot \text{dL}^{-3}$)

Different tendency in parameters of red blood cells, than noted in our experiment, was reported by [Shibu et al. \[2011\]](#). The authors observed an increase in MCV in the last month of pregnancy, and its elevated level in early lactation. The authors also showed decrease in MCHC in subsequent months of pregnancy, with the lowest value observed immediately before parturition. Moreover, they observed higher MCV and MCH in pregnant heifers, compared to non-pregnant cows.

[Mir et al. \[2008\]](#), reported lower MCV in blood of cows in the middle of pregnancy, compared to early and late pregnancy. The authors showed that MCH was higher

during first and second trimester of pregnancy, compared to third trimester. This stage of pregnancy was also characterized by an increase in MCHC.

[Sattar and Mirza \[2009\]](#), noted higher parameters of red blood cells in cows around parturition, lower in pregnant cows in lactation. Also, [Gāvan et al. \[2010\]](#), showed that MCV, MCH and MCHC in cows, in early lactation, were lower and stabilized at a lower level of reference values. The authors suggest that the reason may be decreased concentration of iron in blood.

Concentration of hemoglobin (HGB) in blood of examined cows ranged from 10.06 to 12.93 $\text{g} \cdot \text{dL}^{-3}$ (Fig. 7).

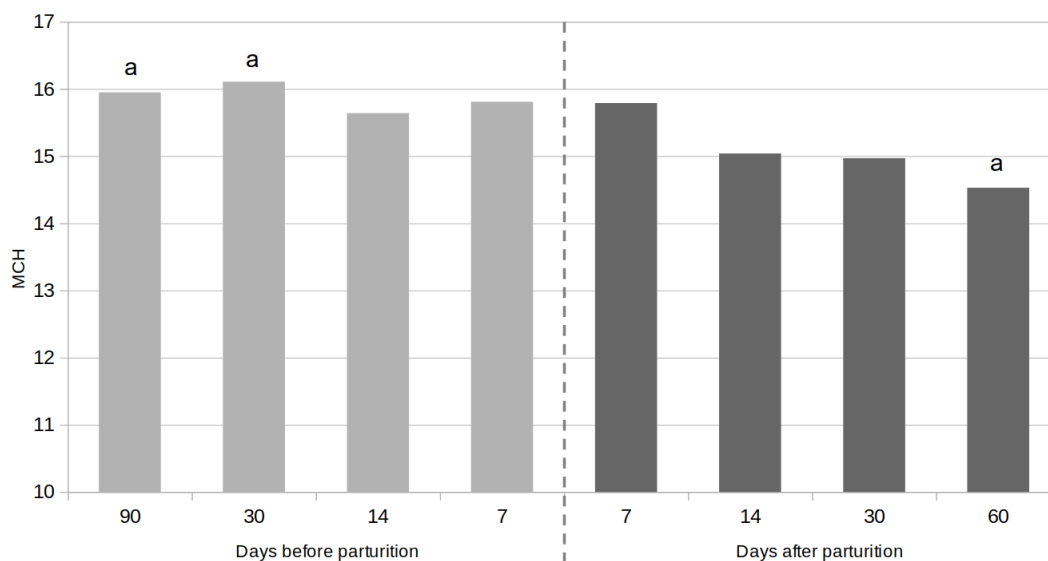


Fig. 6. Mean corpuscular hemoglobin (MCH, pg); significant differences at $P < 0.05$ (a) are marked between values, before and after parturition

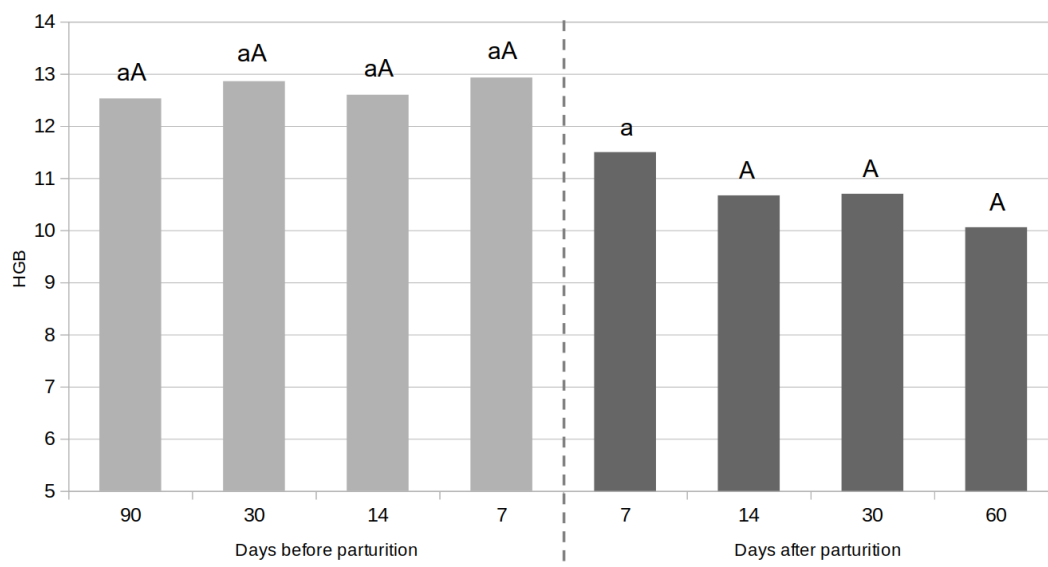


Fig. 7. Concentration of hemoglobin (HGB, $\text{g} \cdot \text{dL}^{-3}$) in the blood of cows before and after parturition; significant differences at $P < 0.05$ (a) and $P < 0.01$ (A) are marked between values, before and after parturition

It is worth to emphasize, that hemoglobin concentration in blood, in the last trimester of pregnancy, was stable (without statistically significant differences), and higher than in postpartum period. Mean HGB concentration in last trimester of pregnancy was $73 \text{ g} \cdot \text{dL}^{-3}$ (12.53–12.93), and during first two months of lactation was $10.73 \text{ g} \cdot \text{dL}^{-3}$ (10.06–11.50). Noteworthy, is a statistically significant ($P < 0.01$) decrease in hemoglobin concentration in blood of cows, during first two weeks after calving, and stabilization of this parameter in subsequent weeks of lactation.

The trend of changes in hematocrit was similar to changes observed for hemoglobin (Fig. 8). High HCT was noted especially during last month of pregnancy (average 38.66%). Worth noting, is statistically significant ($P < 0.01$) hematocrit decrease, during first two weeks after calving, and its stabilization in subsequent weeks of lactation at a lower level (average 31.59%), compared to last trimester of pregnancy.

Increasing concentration of hemoglobin in blood of cows during last weeks before the delivery was observed by Steinhardt et al. [1994], with the highest value imme-

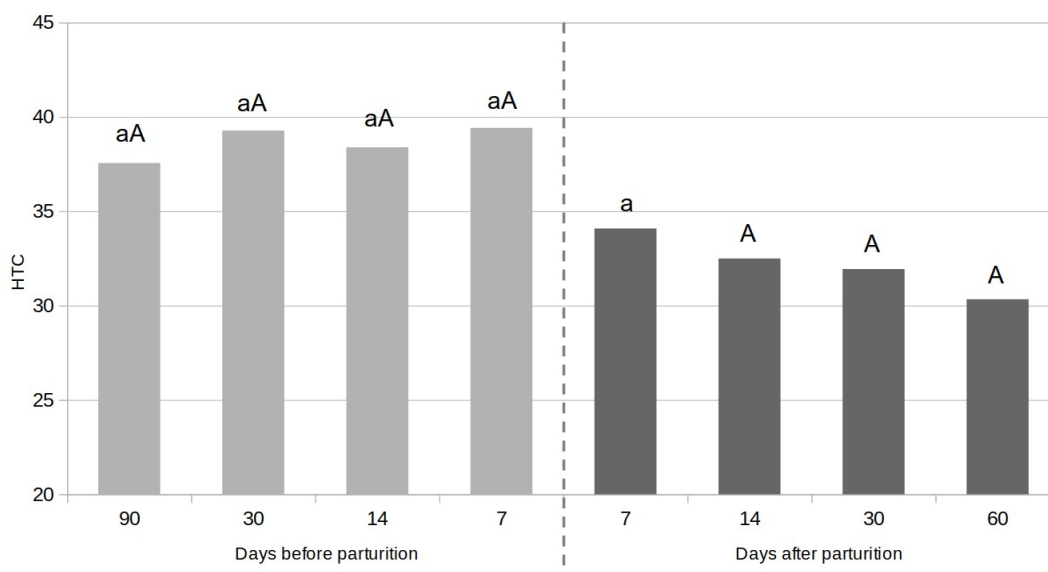


Fig. 8. Hematocrit (HTC, %) in the blood of cows before and after parturition; significant differences at $P < 0.05$ (a) and $P < 0.01$ (A) are marked between values, before and after parturition

diately before calving. Also Nazifi et al. [2008], reported higher concentration of hemoglobin in blood of pregnant cows, compared to values observed in first month of lactation. The authors did not observe statistically significant differences in hematocrit values, between values observed during first and second month of lactation. Higher concentration of hemoglobin and higher hematocrit in pregnant cows was also observed by Kumar and Pachauri [2000] and Brucka-Jastrzębska et al. [2007].

Mir et al. [2008], reported the highest hemoglobin concentration in blood of cows, during the second trimester of pregnancy, and subsequently its gradual decrease. Also, Shibu et al. [2011], showed decrease in hemoglobin concentration in pregnant cows (from the second trimester of pregnancy). The lowest value was observed in ninth month of pregnancy. Further decrease was observed during first month of lactation. Hematocrit in pregnant cows was also statistically significantly lower, than in non-pregnant cows, and decreased during first month of lactation. Rowlands et al. [1975] and Esievo and Moore [1979], observed a decrease in hemoglobin concentration in late pregnancy. Brucka-Jastrzębska and Kawczuga [2007] claim that lowering concentration of hemoglobin in cows blood, over the course of pregnancy, might be a result of an increase, observed for total blood volume.

Hejłasz et al. [1987] and Gävan et al. [2010], showed a decrease in hematocrit and hemoglobin values in cows after calving. Also Studziński et al. [2003], noted higher values of these parameters, 8 weeks before parturition, compared to values observed 12 weeks after delivery. Low concentration of hemoglobin in lactating cows, and the lowest values of hematocrit, observed at the end of

pregnancy, were reported by Sattar and Mirza [2009]. Also, Rowlands et al. [1975], Esievo and Moore [1979] and Mirzadeh et al. [2010], noted a decrease in hematocrit and hemoglobin concentration in early lactation. On the contrary, Gävan et al. [2010], showed an increase in hematocrit and hemoglobin concentration in early lactation. In contrast, Johnson et al. [1990], did not observe any statistically significant changes of these parameters between pregnant and non-pregnant cows. Similarly, Górski and Saba [2012], did not note any statistically significant changes in hemoglobin concentration in blood of cows during last two weeks of pregnancy and during first month of lactation.

CONCLUSION

Results of the study indicate, that the most abrupt changes were observed for hematocrit, erythrocyte count, leukocytes, thrombocytes and hemoglobin concentration in blood. Parameters of red blood cells, at the end of pregnancy, and first two months of lactation, did not exhibit any statistically significant changes. Noteworthy, is the convergence of trends in hematocrit and hemoglobin during analyzed period. High and stable hematocrit at the end of pregnancy, was due to the stable erythrocyte count in this period. Decrease in RBCs after calving, and lower values during first two months of lactation, caused postpartum decrease in hematocrit and its stabilization at a lower level. Changes in total number of WBC and thrombocytes, although very important from the physiological point of view, did not affect significantly hematocrit values. The above observations denote indirectly changes in blood volume during analyzed period. These

changes are also confirmed by similar tendencies observed for hematocrit and hemoglobin concentration – stable and higher before calving, and lower during first weeks of lactation. Additional confirmation is relatively stable MCV and MCHC. The above suggest the high efficiency of homeostatic mechanisms in pregnant and lactating heifers/primiparous cows. Our results may be useful for comparative studies both in physiology and health disturbances research in cows during the pre and postpartum period.

ACKNOWLEDGEMENTS

This work was supported by a research grant from the Ministry of Science and Higher Education, Poland (Project No. N311 112538).

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ANALIZA ZMIAN WYBRANYCH WSKAŹNIKÓW HEMATOLOGICZNYCH KRWI KRÓW MLECZNYCH W OSTATNIM TRYMESTRZE PIERWSZEJ CIĄŻY I PIERWSZYCH DWÓCH MIESIĄCACH LAKTACJI

STRESZCZENIE

Dynamiczne zmiany czynnościowe zachodzące w organizmie jałówek w końcowym okresie pierwszej ciąży i pierwiastek w początkowym okresie laktacji mogą implikować zmiany wartości wskaźników morfologicznych krwi. Ich ocena może przyczynić się do lepszego poznania zmian zachodzących w organizmie ciężarnych i laktujących młodych krów. Stąd celem niniejszej pracy było zbadanie, analiza i ocena zmian parametrów hematologicznych krwi obwodowej (RBC, MCV, HGB, MCH, MCHC, WBC, PLT oraz HCT), u jałówek rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej, w ostatnim trymestrze pierwszej ciąży i pierwszych dwóch miesiącach laktacji, ze szczególnym uwzględnieniem okresu przejściowego. Analizowano parametry krwi pobranej 90, 30, 14 i 7 dni przed porodem oraz w 7, 14, 30 i 60 dniu laktacji, wykorzystując analizator hematologiczny *scil Vet abc Plus+* (HORIBA Medical). Wyniki przeprowadzonych badań wskazują, że najbardziej istotne zmiany dotyczyły wskaźnika hematokrytowego, ogólnej ilości erytrocytów, leukocytów i trombocytów oraz stężenia hemoglobiny w osoczu krwi. Wskaźniki czerwonekrwinkowe, w końcowym okresie ciąży i pierwszych dwóch miesiącach laktacji, nie zmieniały się istotnie. Wykazano dużą zbieżność tendencji zmian wskaźnika hematokrytowego, koncentracji erytrocytów i stężenia hemoglobiny. Wysoka i stabilna wartość HCT w końcowym okresie ciąży, wynikała ze stabilnej koncentracji erytrocytów w tym czasie, a obniżenie ich koncentracji po porodzie i niższa zawartość we krwi, w pierwszych dwóch miesiącach laktacji, implikowały poporodowe obniżenie wartości HCT i jego stabilizację na niższym, niż w okresie ciąży poziomie. Powyższe obserwacje wskazują pośrednio na niewielkie zmiany objętości krwi w badanym okresie, o czym świadczą: tendencje zmian stężenia hemoglobiny w osoczu krwi oraz stabilność średniej objętości krwinki i średniego stężenia hemoglobiny w erytrocytach. Uzyskane wyniki wskazują na wysoką sprawność mechanizmów homeostatycznych organizmu ciężarnych i laktujących pierwiastek i mogą być przydatne w badaniach porównawczych, zarówno w badaniach fizjologii, jak i zaburzeń zdrowotnych u krów w okresie przed i poporodowym

Słowa kluczowe: jałówki, pierwiastki, okres okołoporodowy, pierwsza ciąża, laktacja, krew, wskaźniki hematologiczne