

RESEARCH ARTICLE

HEMATOLOGICAL PROFILE IN HOLSTEIN DAIRY COWS ACCORDING TO THE DIFFERENT PHYSIOLOGICAL STAGE

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Original submission:

29 December 2022

Revised submission:

22 February 2023

Accepted:

18 May 2023

How to cite this article:

Mekroud M, Khelifi-Ouhami NM, Titi A, Arzour-Lakehal N, Mekroud A, Ouchene N. 2023. Hematological profile in Holstein dairy cows according to the different physiological stage. *Veterinaria*, 72(2), 174-182.

ABSTRACT

This study aims to investigate the variations of hematological and ionic parameters of Holstein dairy cows according to the physiological stage, as an indicator of their health status. The survey was conducted between January and March, 2020 and carried out on 4 lots of 15 dairy cows each. The analysis of blood parameters included: red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), lymphocytes (LYM), monocytes (MONO), neutrophils (NEU), eosinophils (EOS), basophils (BASO) and ionic parameters such as potassium (K), chlorine (Cl), calcium (Ca), phosphorus (P) and sodium (Na).

The RBC level was lowest in the 2nd third of gestation ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) and in postpartum period ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$), in comparison to the 1st third ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) and 3rd third (dry period) of gestation ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$). The same observation was noted for HCT ($p < 0.0001$). MCV was highest in dry cows ($p < 0.01$). MCH and MCHC were highest in cows that were in the postpartum period ($p < 0.001$). For WBC, the count was higher in the 1st third of gestation compared to the other stages, ($11.3 \pm 2.2 \times 10^3/\mu\text{L}$) versus ($6.4 \pm 4.1 \times 10^3/\mu\text{L}$), ($5.2 \pm 1.3 \times 10^3/\mu\text{L}$), and ($8.0 \pm 4.9 \times 10^3/\mu\text{L}$) for the 2nd third, 3rd third of gestation, and postpartum, respectively ($p < 0.001$).

In postpartum cows, the ionic parameters had the lowest values of potassium (K), calcium (Ca) and phosphorus (P) compared to the other stages ($p < 0.001$).

This investigation can be used as a basis for information on the hematological and ionic profile of dairy cattle in order to determine the health status before the onset of clinical signs.

Keywords: Algeria, dairy cows, hematological parameters, ions, physiological stage

INTRODUCTION

Through the various phases of the production cycle, dairy cows are confronted with changes in diet, management and environment, as well as changes in physiological parameters. These changes are reflected by hematological analyses (Herdt, 2000). Therefore, the evaluation of the hematological profile at the herd level can help in the diagnosis and prognosis of diseases, and provide an overview of the feeding and management strategies adopted in dairy farms, allowing the rapid assessment of animal responses to the different phases of the lactation cycle (Bezerra et al., 2017; Herdt, 2000; Roland et al., 2014). It is also useful for the classification of certain anemias (Brockus, 2011).

The hematological parameters can be influenced by several factors including age, breed, sex, physiological phases of the animal, season and farming system (Brucka-Jastrzebska et al., 2007; Farooq et al., 2012; 2017; Shehab-El-Deen et al., 2010).

Throughout the different physiological phases, dairy cows are confronted with multiple subclinical disorders (like subclinical hypocalcemia, subclinical ketosis) that are often associated with severe changes in several blood parameters (Duffield, 2000; Goff, 2008; Mezzetti et al., 2019). Variations in the hematological parameters of dairy cows during different physiological phases have been investigated in several studies (Brscic et al., 2015; Cozzi et al., 2011; Moretti et al., 2017).

In Algeria, the cattle herd represents about 6% of the total livestock population (sheep, goats, cattle, camels, and horses) with a total of 1.9 million cattle of which 52% are dairy cows (MADR, 2018).

This study aims to investigate the variations of the hematological and ionic parameters of Holstein dairy cows, according to the physiological stage (first and second thirds of gestation, dry period and post-partum) as an indicator for physiological status assessment. The results obtained for the first time in Algeria could be used as reference data.

MATERIAL AND METHODS

Study area

The study was conducted between January and March, 2020 on a dairy cattle farm covering an area of 1500 hectares in the wilaya of Mila, northeast Algeria (36°20'47" N 6°20'19" E). This region has an altitude of 486 m, known for its warm Mediterranean climate with dry summers. The average annual precipitation in this region is 501.7 mm.

Animals

The study was conducted on Holstein cows aged between 2 and 5 years. The milk production varies from 30 to 40 liters/cow/day. The dry period is at the end of the 7th month of gestation. Cows are artificially inseminated.

For the purpose of the study, the cows were divided into lots in a homogeneous way with regard to health status, age groups and season of study in order to keep only one factor of variation, which is the physiological stage. Clinically sick cows were excluded from our study.

The breeding system of the farm is intensive. The animals receive their feed (hay, barley, concentrate, silage and straw) *in situ*. The watering is *ad libitum*. Milking is done in the milking room twice a day (morning and evening).

Blood samples

Four lots of 15 cows each were selected in the: 1st third period of gestation (1T), 2nd third period of gestation (2T), 3rd third period of gestation or dry period (3T) and postpartum (PP). Each animal was sampled only once.

Blood samples were taken from the coccygeal vein. Animals should be calm to avoid stress-related changes of the hemogram (Jones and Allison, 2007). The samples were transported under isothermal conditions respecting the cold chain, and the time frame for analysis (within 24 hours) was respected to ensure accurate and reliable results (Adewoyin, 2014; Warren et al., 2013).

Blood samples were collected in the morning and divided into two tubes: one has EDTA for blood count formula and another is heparinized for ion analysis. Blood smears for white blood cells were made at the farm and dried, then placed in the boxes for later staining.

Laboratory analysis

The following hematological analyses were performed: red blood cell count (RBC, $\times 10^6/\mu\text{L}$), hemoglobin concentration (HGB, g/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin content (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), platelets (PLT, $\times 10^3/\mu\text{L}$), and white blood cell count (WBC, $\times 10^3/\mu\text{L}$).

All these parameters were measured using a hematology automat (MINDRAY BC3000 Plus, Ref SKU-BC-3000Plus, China) and (BIOTA, Ref VABIO 360, Turkey).

Relative WBC values (neutrophils (NEU), eosinophils (EOS), basophils (BAS), lymphocytes (LYM), and monocytes (MON)) were determined on the basis of microscopic observation of blood smears stained by May-Grunwald/ Giemsa staining technique (RAL 555 kit, RAL diagnostic, Martillac, France).

For ions, the dosage of sodium (Na), potassium (K), chlorine (Cl), calcium (Ca) and phosphorus (P) was determined by:

- The EasyLyte electrolyte analyzer (EasyLyte plus ANALYZER, USA) which is an automated system that was used to make measurements of Na, K and Cl ions.
- Thecolorimetry for Ca and P ions.

For each physiological stage, average values of each blood parameter were calculated.

Data analysis

The statistical program used was R i386 3.0.2 for Windows Front-end GUI. The ANOVA test supplemented by the Chi-square test and multiple range tests (multiple ranges) were used for statistical analysis. The threshold value of different tests was $p < 0.05$.

RESULTS

The data concerning changes in hematological and ionic parameters according to the physiological stage in dairy cows are presented in Tables 1, 2 and 3.

The results showed that the changes were not significant for HGB and PLT (Table 1). However, the changes were significant for RBC, HCT, MCV, MCH and MCHC.

RBC were lower in 2T ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) and PP ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$) compared to 1T ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) and 3T ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$) (Table 1).

Table 1 Red blood cell parameters and erythrocyte indices according to cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P value
RBC ($\times 10^6/\mu\text{L}$)	6.6 ^a (6 – 7.2)	6.0 ^b (5.2 – 6.8)	6.7 ^a (6.2 – 7.2)	5.6 ^c (4.8 – 6.4)	0.0001
HGB (g/dL)	10.6 ^a (10 – 11.2)	10.2 ^a (9.3 – 11.1)	10.6 ^a (9.9 – 11.3)	10.2 ^a (10.1 – 10.3)	-
HCT (%)	30.6 ^b (28.6 – 32.6)	28.1 ^b (23.4 – 32.8)	33.6 ^a (31.4 – 35.8)	26.8 ^c (22.4 – 31.2)	0.0001
MCV (μm^3)	46.4 ^b (43.7–49.1)	47.0 ^b (43.4 – 50.6)	49.9 ^a (47.5 – 52.3)	47.7 ^b (45.5 – 49.9)	0.01
MCH (pg)	16.0 ^c (14.7 – 17.3)	17.2 ^b (16.1 – 18.3)	15.8 ^c (14.6 – 17)	18.2 ^a (17.5 – 18.9)	0.0001
MCHC (g/dL)	34.5 ^a (33.7 – 35.3)	36.8 ^a (32.6 – 41)	31.7 ^b (30.3 – 33.1)	37.1 ^a (31.5 – 42.7)	0.001

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P value
PLT ($\times 10^3/\mu\text{L}$)	332.7 ^a (250 – 415.4)	319.6 ^a (191.4 – 447.8)	288.4 ^a (207.5 – 369.3)	347.9 ^a (225.1 – 470.7)	-

1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Values that have different letters in the same line are significantly different ($p < 0.05$)

HCT was lower in PP ($26.8 \pm 4.4\%$), compared to 1T, 2T and 3T, ($30.6 \pm 2.0\%$) ($28.1 \pm 4.7\%$), ($33.6 \pm 2.2\%$), respectively ($p < 0.0001$) (Table 1).

Dry cows had the highest MCV values ($49.9 \pm 2.4 \mu\text{m}^3$) ($p < 0.01$). MCH ($18.2 \pm 0.7 \text{ pg}$) and MCHC ($37.1 \pm 5.6 \text{ g/dL}$) were highest in PP ($p < 0.001$) (Table 1).

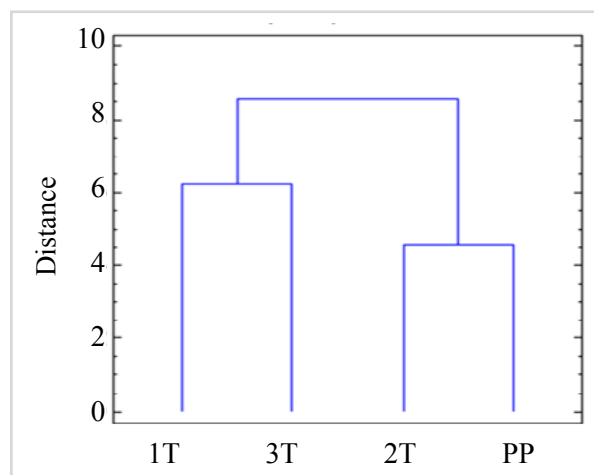


Figure 1 Red blood cell dendrogram and erythrocyte indices according to cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

The dendrogram presented in Figure 1 shows that the changes of the hematological parameters of red

blood cells, according to the physiological stage, are distributed into 2 groups of which the first includes 1T and 3T and the second includes 2T and PP (Figure 1).

Hematological analysis of white blood cells presented in Table 2 showed no significant variation for EOS and BASO. The highest values of WBC, LYM, MONO and NEU were observed in 1T ($p < 0.001$).

The neutrophil/lymphocyte ratio (NEU/LYM) was equal to 1.04, 1.15, 0.91, 0.96 at 1T, 2T, 3T and PP respectively.

The dendrogram shows that the changes of WBC hematological parameters according to the physiological stage were divided into three groups (Figure 2). The first group is represented by 1T, which is significantly different from the other two groups. The second is composed by 2T and 3T, which are very close to the third group, which is PP (Figure 2).

Regarding the ionic elements presented in Table 3, there was no significant difference for Na. For K, Ca, and P, cows showed the lowest values in PP ($3.8 \pm 0.3 \text{ mmol/L}$), ($86.3 \pm 7.5 \text{ mmol/L}$), and ($48.2 \pm 10.7 \text{ mmol/L}$), respectively ($P < 0.001$). Cl was highest in 2T and 3T ($P < 0.01$) (Table 3).

Table 2 Values of the WBC according to the cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P values
WBC ($\times 10^3/\mu\text{L}$)	11.3 ^a (9.1 – 13.5)	6.4 ^{bc} (2.3 – 10.5)	5.2 ^c (3.9 – 6.5)	8.0 ^b (3.1 – 12.9)	0.001
LYM ($\times 10^3/\mu\text{L}$)	5.10 ^a (4.08 – 6.12)	2.69 ^{bc} (0.96 – 4.42)	2.33 ^c (1.59 – 3.07)	3.80 ^b (1.67 – 5.93)	0.0001
MON ($\times 10^3/\mu\text{L}$)	0.51 ^a (0.14 – 0.88)	0.20 ^c (0.04 – 0.36)	0.32 ^{bc} (0.02 – 0.62)	0.45 ^b (0.20 – 0.70)	0.05
NEU ($\times 10^3/\mu\text{L}$)	5.28 ^a (3.79 – 6.77)	3.10 ^{bc} (0.87 – 5.33)	2.11 ^c (1.44 – 2.78)	3.63 ^b (0.81 – 6.45)	0.001

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P values
EOS ($\times 10^3/\mu\text{L}$)	0.42 ^a (0.02 – 0.82)	0.34 ^{bc} (0.00 – 0.78)	0.40 ^b (0.14 – 0.66)	0.11 ^c (0.00 – 0.25)	-
BAS ($\times 10^3/\mu\text{L}$)	0.0	0.0	0.0	0.0	-

1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Values that have different letters in the same line are significantly different ($p < 0.05$)

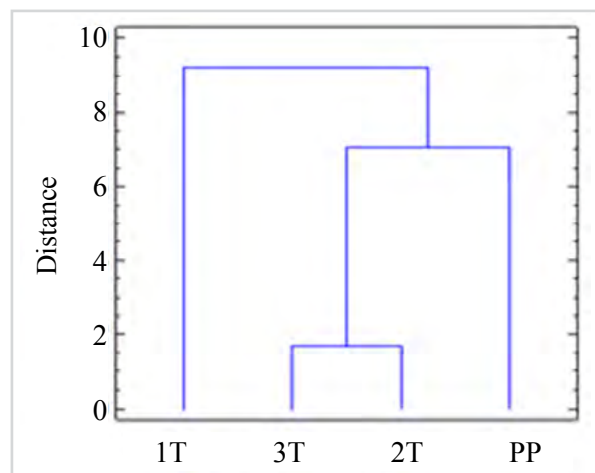


Figure 2 Dendrogram of the variation of WBC according to the cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

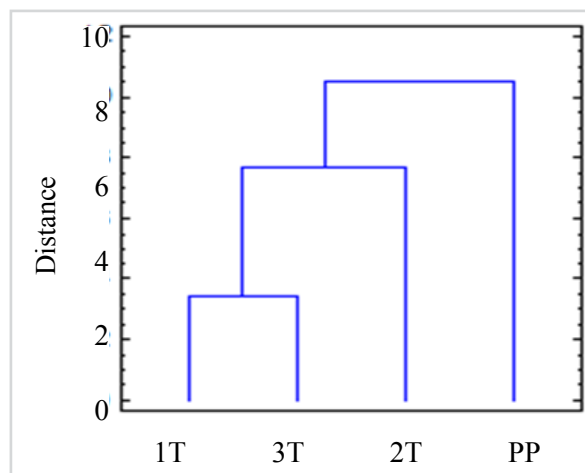


Figure 3 Dendrogram of the ions according to the cows' physiological stage. 1T: 1st third of gestation, 2T: 2nd third of gestation, 3T: dry, PP: postpartum

Table 3 Variations in ion concentrations according to the cows' physiological stage

Parameters	1T (CI, 95%)	2T (CI, 95%)	3T (CI, 95%)	PP (CI, 95%)	P values
Na (mmol/L)	139.9 ^a (138.1 – 141.7)	140.6 ^a (138.9 – 142.3)	139.0 ^a (137.1 – 140.9)	139.8 ^a (138 – 141.6)	-
K (mmol/L)	4.2 ^a (4.0 – 4.4)	4.2 ^a (3.8 – 4.6)	4.3 ^a (4.1 – 4.5)	3.8 ^b (3.5 – 4.1)	0.001
Cl (mmol/L)	98.7 ^c (97.2 – 100.2)	100.5 ^{ab} (98.4 – 102.6)	102.1 ^a (100.5 – 103.7)	100.3 ^{bc} (96.5 – 104.1)	0.01
Ca (mg/L)	97.9 ^a (95.6 – 100.2)	99.0 ^a (94.7 – 103.3)	96.5 ^a (93.4 – 99.6)	86.3 ^b (78.8 – 93.8)	0.001
P (mg/L)	62.4 ^b (54.2 – 70.6)	70.2 ^a (62.9 – 77.5)	68.7 ^b (60.6 – 76.8)	48.2 ^c (37.5 – 58.9)	0.001

It appears that the mineral content varies in three groups (Figure 3). The first group is represented by 1T and 2T, whose values are quite close. The second group, composed of dry cows, differs significantly from the previous group and from the group that follows, composed of cows from the PP (Figure 3).

DISCUSSION AND CONCLUSION

Performing a hematological profile of clinically normal cattle must take into account many factors such as age, physiological status of the animal, season and climate (Brucka-Jastrzebska et al., 2007; Dzavo et al., 2020; Farooq et al., 2017).

The results of the investigated blood parameters reported in the present survey were within the range of reference values for healthy dairy cattle (Bellier and Cordonnier, 2010; Krimer, 2011).

In this study, RBC, HGB and HCT contents were high in the beginning of gestation (1T). This may be associated with increased erythrocyte release from the spleen due to the stress of gestation (Kumar and Pachauri, 2000).

The lowest RBC and HCT values were reported in cows in PP. This finding could be due to anemia caused by calving (Brockus, 2011) and increased production of pro-inflammatory cytokines that could cause a reduction in erythropoiesis (Chikazawa and Dunning, 2016).

The findings of this investigation are consistent with others (Herman et al., 2018; Paiano et al., 2020).

For MCV, dry cows have the highest values, while lactating cows (PP) have the lowest values. This is consistent with the results of Sattar and Mirza (2009). This observation suggests a change in plasma volume induced by the cessation of milk production (van den Brink et al., 2023).

MCH and MCHC contents in parturient cows were the highest. The same observations were found by Sattar and Mirza (2009).

The total number of platelets is influenced by the quantity of consumption, production, sequestration,

and loss (Russell, 2010). The change in platelet count during the four stages investigated in this study was not significant. The same finding was made by Mirzadeh et al. (2010).

The WBC count is higher in early pregnancy cows (1T) compared to the other groups. Our results are consistent with Abdelatif and Alameen (2012) and Ouahrani and Bordjah (2016).

The increase in the number of WBC in PP compared to 3T observed in this survey is due to the increase in antepartum cortisol (Preisler et al., 2000). In addition, cows must satisfy the needs of their fetus at the end of gestation, which increases the energy demand reaching the peak around one month before parturition, to synthesize colostrum and milk (Esposito et al., 2014).

In the present study, NEU and LYM levels were higher in early lactation (PP) than in the third trimester of gestation (3T), and EOS, BAS and MON were not affected by the periparturient period. This is consistent with the results of Ate et al. (2009) and Sattar and Mirza (2009).

Regarding ions, K, Ca and P, recorded the lowest levels in postpartum cows (PP). This is explained by the occurrence of hypocalcemia coupled with hypophosphatemia during calving due to the high demand by the mammary gland, a few days before calving. In the absence of any health problems, this hypocalcemia is physiological (Goff, 2014; Goff, 2008; Horst et al., 2005). This finding is in accordance with Cerutti et al. (2018), Mukača and Katica (2018) and Hadzimusic and Krnic (2012).

Ca and P concentration is regulated by parathormone, which stimulates Ca reabsorption from the urine and bone and stimulates P absorption from the intestine (Cavestany et al., 2005; Goff et al., 2014; Peterson et al., 2005). The sudden high mobilization of circulating Ca and P by the mammary gland at the onset of lactation, however, leads to a decrease in their blood concentration (Goff and Liesegang, 2014).

However, the Na level was not significantly different between the different stages investigated. This observation is consistent with that of Mukača

and Katica (2018).

An increase in plasma electrolyte concentration in the postpartum period has been reported in cattle affected by renal dysfunction or related to stress conditions caused by adrenocorticotropin hormone release and inflammatory conditions occurring around calving (Harshfield et al., 2009). In our study, all cows were healthy and were raised under optimal conditions of which the lowest ion values were observed in PP.

Significantly strong variation was noted for the majority of hematological parameters according to the physiological stage (RBC, HCT, MCV, MCH, WBC, and number of LYM, MON and NEU) and ions (K, Cl, Ca and P). The most marked variations were noted around parturition (peripartum period) by a decrease in red line and ion values, while white line cells show an increase.

Consequently, this study may be useful in providing baseline information on the hematological profile

of dairy cattle for the assessment of physiological status. This survey requires additional investigation concerning the differences in hematological parameters according to age and sex.

ACKNOWLEDGEMENTS

Farmers are acknowledged for their cooperation during sample collection.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design – MM, ON; Supervision – TA, ALN, MA, ON; Analysis and/or Interpretation – KTNA, ON; Literature Search – MM, ON; Writing Manuscript – MM, KTNA, TA, ALN, MA, ON; Critical Review – KTNA, ON.

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HEMATOLOŠKI PROFIL HOLSTEIN MLIJEČNIH KRAVA PREMA RAZLIČITIM FIZIOLOŠKIM STADIJIMA

SAŽETAK

Cilj ovog istraživanja jeste ispitati varijacije hematoloških i jonskih parametara kod holstein mliječnih krava u različitim fiziološkim stadijima kao indikatora zdravstvenog stanja. Istraživanje je provedeno između januara i marta, 2020. godine na 4 grupe od po 15 mliječnih krava. Analiza krvnih parametara je uključivala: eritrocite (RBC), hematokrit (HCT), hemoglobin (HGB), srednji korpuskularni volumen (MCV), srednji korpuskularni sadržaj hemoglobina (MCH), srednju korpuskularnu koncentraciju hemoglobina (MCHC), trombocite (PLT), leukocite (WBC), limfocite (LYM), monocite (MONO), neutrofile (NEU), eozinofile (EOS), bazofile (BASO) i jonske parametre kao što su kalijum (K), hlor (Cl), kalcijum (Ca), fosfor (P) i natrijum (Na).

RBC su bili najniži u 2. trećini gestacije ($6.0 \pm 0.8 \times 10^6/\mu\text{L}$) i u postpartalnom periodu ($5.6 \pm 0.8 \times 10^6/\mu\text{L}$) u odnosu na 1. trećinu ($6.6 \pm 0.6 \times 10^6/\mu\text{L}$) i 3. trećinu (suhi period) gestacije ($6.7 \pm 0.5 \times 10^6/\mu\text{L}$) ($p < 0.0001$). Isto je uočeno za HCT ($p < 0.0001$). MCV je bio najviši kod suhih krava ($p < 0.01$). MCH i MCHC su bili najviši kod krava u postpartalnom periodu ($p < 0.001$). WBC su bili najviši u 1. trećini gestacije u usporedbi s ostalim stadijima ($11.3 \pm 2.2 \times 10^3/\mu\text{L}$) prema ($6.4 \pm 4.1 \times 10^3/\mu\text{L}$), ($5.2 \pm 1.3 \times 10^3/\mu\text{L}$), i ($8.0 \pm 4.9 \times 10^3/\mu\text{L}$) u 2. trećini, 3. trećini gestacije i postpartalno ($p < 0.001$).

Kod postpartalnih krava od jonskih parametara su najniži bili kalijum (K), kalcijum (Ca) i fosfor (P) u usporedbi sa drugim stadijima ($p < 0.001$).

Ovo istraživanje se može koristiti kao osnovna informacija o hematološkom i jonskom profilu mliječnih krava kako bi se odredilo njihovo zdravstveno stanje prije pojave kliničkih znakova.

Ključne riječi: Alžir, fiziološki stadij, hematološki parametri, joni, mliječne krave