

REFERENCE VALUES AND FREQUENCY DISTRIBUTION OF HEMATOLOGICAL PARAMETERS IN COWS DURING LACTATION AND PREGNANCY*

BRANISLAVA BELIĆ, MARKO R. CINCOVIĆ,
LJUBICA KRČMAR, BOJANA VIDOVIĆ¹

SUMMARY: The hematological parameters were investigated in 40 Holstein-Friesian. The cows were in the second and third lactation. The blood samples were taken in four productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). The findings of hematological values in Holstein-Friesian cows corresponded with reference values for a given type of animal. The periparturient period is characterized by a reduced number of red blood cells, decreased concentrations of hemoglobin, reducing the total number of leukocytes with the growth of neutrophils. The characteristic findings of the entire population of cows, regardless of the period of lactation is to draw the number of neutrophils and lymphocytes to the right and turn left into hemoglobin. This supports the exposure to stress in daily production. All parameters are arranged in the normal (Gaussian) distribution.

Key words: *hematology, dairy cows, the reference values, frequency distribution of parameter.*

INTRODUCTION

Hemogram testing in animals is an indispensable diagnostic procedure. For the evaluation of hematologic findings, it is important to know the reference values for blood elements. Reference values depend on many elements, such as the period of lactation, breed, sex, age, etc. (Mirzadeh et al., 2010). A large comparative analysis (George et al., 2010) showed that the most recent literature defines the limits of normal values in the widest variations were found in healthy cows. The study was used to compare

Original scientific paper / *Originalni naučni rad*

¹Branislava Belić, PhD MED, Associate professor; Marko R. Cincović, DVM, Teaching Assistant; Ljubica Krčmar, DVM, postgraduate student; Bojana Vidović, DVM, postgraduate student.

Corresponding author: Marko R. Cincović, cin_vet@yahoo.com, Faculty of Agriculture, Department of veterinary medicine, D.Obradovića 8, 21000 Novi Sad. University of Novi Sad.

*The paper is part of the research work on the project "Analiza i identifikacija stresogenih faktora kod mlečnih krava u cilju unapređenja održivosti stočarske proizvodnje na teritoriji AP Vojvodine" (srb.), financed by Provincial secretariat for science and technological development, The Autonomous province of Vojvodina.

hematological results obtained from the literature of the fifties of last century and contemporary sources. It was found that the concentration of hemoglobin and the number of neutrophils, eosinophils and lymphocytes and neutrophils ratio is statistically significantly different between these two sets of data.

The aim of this study was to investigate the effect of lactation on haematological values in dairy cows, and to form a frequency distribution for each value, especially compared to the average reference values from recent world literature, as well as Schalms veterinary hematology (Weiss and Wardrop Ed, 2010) and Large Animal Internal Medicine (Morris, 2009-Smith B. Ed).

MATERIAL AND METHOD

Biochemical parameters were examined in 40 Holstein-Friesian cows. The cows were in the second and third lactation and were grown under the same conditions of diet (standard recipe meals based on corn silage, TMR) and care (free rearing system on deep litter). The blood samples were taken in four main productive periods as follows: in the dry period (2-3 weeks before delivery), the postpartum period (0-2 weeks postpartum), in the middle of lactation (15-17 weeks postpartum) and late lactation (2-3 weeks before the start of drying). The blood was collected and quickly processed in the semi-automated hematology analyzer Hemavet 950th directly to a number: RBC ($\times 10^{12}/L$), WBC ($\times 10^9/L$), neutrophils, basophils, eosinophils, lymphocytes, monocytes and platelets ($\times 10^9/L$). The concentration of hemoglobin was determined by mentioned analyzer (g / l). The computation is determined by the erythrocyte indices: MCV (fl), MCH (pg) and MCHC (%). The following formula was used:

$$MCV = (\text{hematocrit} / \text{red blood cells of millions in mL}) \times 10$$

$$MCH = (\text{Hb (g / dl) of millions of red blood cells in mL}) \times 10$$

$$MCHC = (\text{Hb (g / dl) / hematocrit}) \times 100.$$

RESULTS

The most pronounced haematological changes were seen in cows in the period after calving. Immediately after calving, the hematological picture is characterized by: the fall in erythrocyte count, hemoglobin level and decrease of the calculated parameters, decreasing the number of leukocytes, but the number of neutrophils increases (Table 1).

Table 1. Average values of haematological parameters of blood under the stage of lactation
Tabela 1. Prosečne vrednosti hematoloških parametara krvi shodno stadijumu laktacije

Parameters <i>Parametri</i>	Precalving <i>Zasušene</i>	Fresh cows <i>Početak laktacije</i>	Mid lactation <i>Sredina laktacije</i>	End lactat. <i>Kraj laktacije</i>	Ref.value <i>Ref.vredn. literatura</i>
Erythrocytes $\times 10^{12}/L$	6.2	4.82*	7.04	8.11	5-10
Hemoglobin (g/l)	100.44	85.06*	90.5	103.3	80-150
MCV (fl)	50.5	48.21*	52.3	54.8	40-60
MCH (pg)	14.4	12.89*	13.22	14.11	11-17
MCHC (%)	35.1	34.4	35.2	35.4	30-36

Leukocytes x 10 ⁹ /L	12.55	12.03	13.5	13.02	5-13
Neutrophils x 10 ⁹ /L	4.45	5.19**	4.23	4.3	1.7-6
Basophils x 10 ⁹ /L	/	/	/	/	Rare/Retki
Eosinophils x 10 ⁹ /L	0.67	0.58**	0.77	0.89	0.1-1.1
Monocytes x 10 ⁹ /L	0.71	0.65	0.67	0.74	0.1-0.7
Lymphocytes x 10 ⁹ /L	5.12	4.83*	5.38	6.99	1.8-8.1
Platelet x 10 ⁹ /L	410	370	390	405	100-800

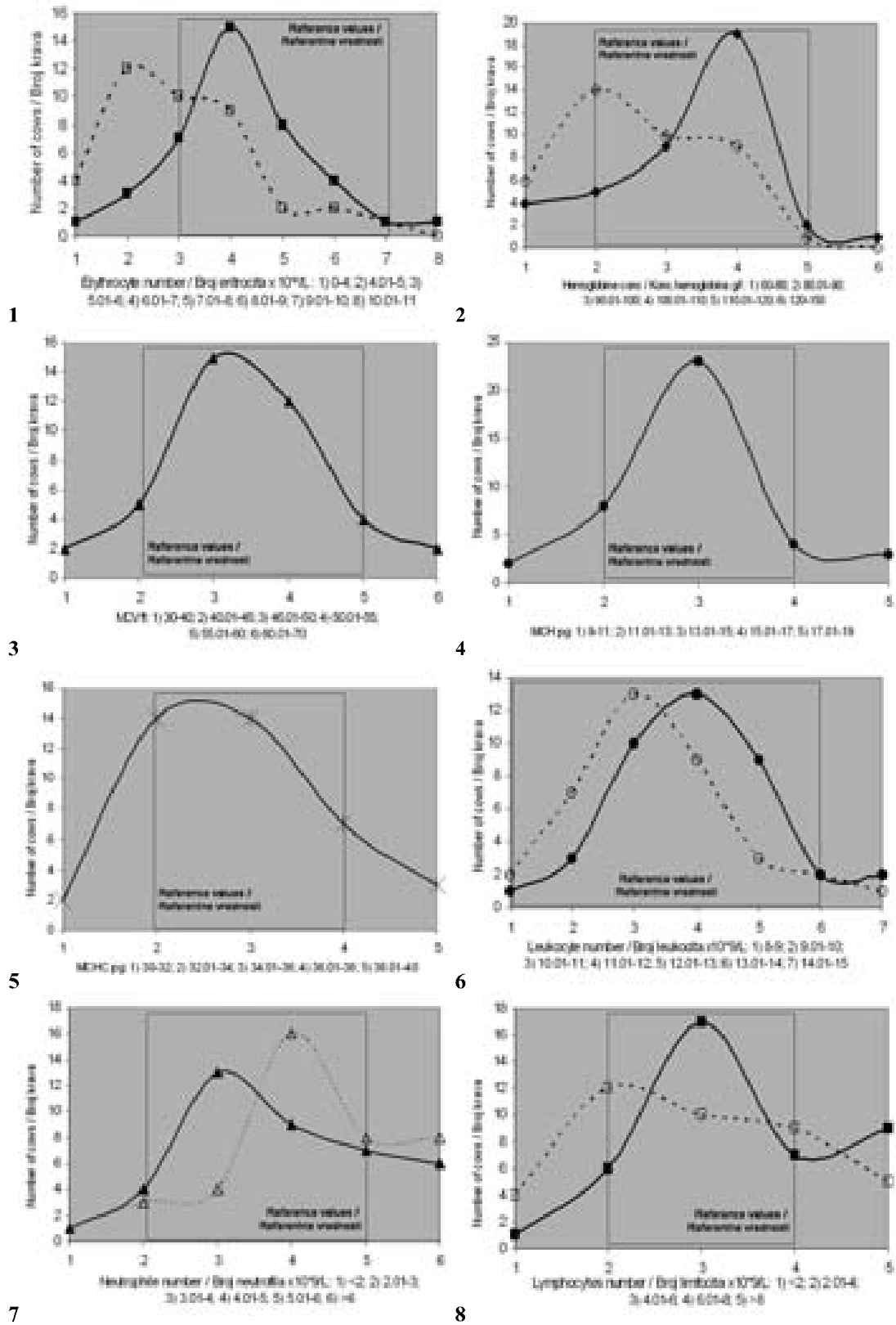
*p<0.05, ** p<0.01.

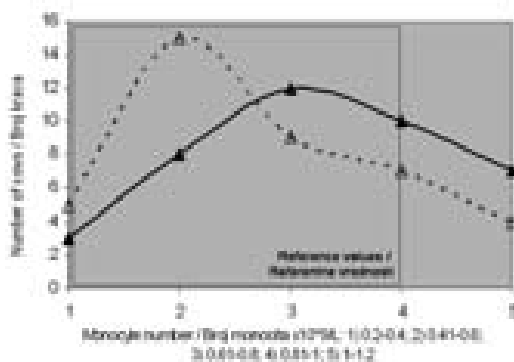
Table 2. The average number of cows whose hematological parameter values outside the reference (+ turn to the right, - turn left)

Tabela 2. Prosečan broj krava čije su vrednosti hematološkog metaboličkog parametra van referentnih (+ skretanje u desno, - skretanje u levo)

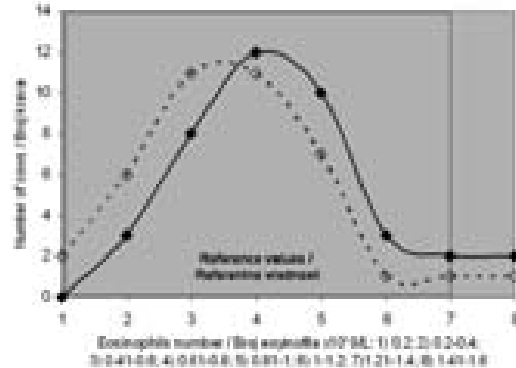
Parameters <i>Parametri</i>	Pecalving <i>Zasušene</i>		Fresh cows <i>Početak laktacije</i>		Mid lactation <i>Sredina laktacije</i>		End lactat. <i>Kraj laktacije</i>	
	+	-	+	-	+	-	+	-
Non referent values <i>Vrednost izvan referentnih</i>								
Erithrocytes x 10 ¹² /L	0	0	0	16	0	3	1	1
Hemoglobin (g/l)	0	0	0	6	0	0	1	0
MCV (fl)	0	1	0	1	1	0	1	0
MCH (pg)	0	0	0	1	1	0	2	0
MCHC (%)	0	1	0	1	2	0	1	0
Leukocytes x 10 ⁹ /L	0	0	1	0	0	0	2	0
Neutrophils x 10 ⁹ /L	3	0	8	1	2	0	1	1
Basophils x 10 ⁹ /L	/	/	/	/	/	/	/	/
Eosinophils x 10 ⁹ /L	0	0	1	0	2	0	0	0
Monocytes x 10 ⁹ /L	0	0	4	0	5	0	2	0
Lymphocytes x 10 ⁹ /L	1	1	5	4	4	0	4	0
Platelet x 10 ⁹ /L	0	0	0	0	0	0	0	0

The value of these parameters after calving was significantly different compared to other periods of lactation (p<0.05, p<0.01). In addition to the original values of parameters to be examined and the number of cows which were investigated parameters are outside normal ranges. These results are presented in Table 2 and show that most cows have values outside the reference period around calving. In order to accurately determine the haematological values important to examine the frequency distribution of parameters in cows. These results are presented in Graph. 1 to 11 It may be noted that most parameters showing normal distribution with a slight turn to the left in the periparturient period.

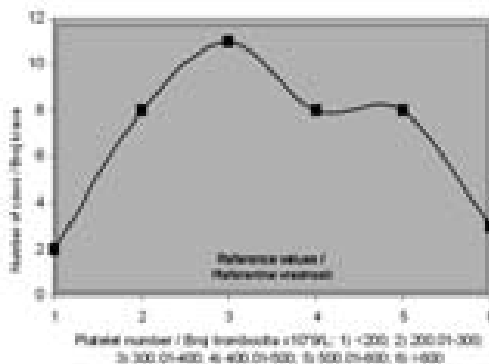




9



10



11

Graph 1-11. Frequency distribution of average values of the parameters examined in 40 cows (solid line), with a distinctive distribution of trends in the periparturient period for the parameters whose values are significantly different from the general average (dashed line), a rectangle shows the reference values obtained by analysis of the literature.

Grafici 1-11. Distribucija frekvencije prosečnih vrednosti parametara kod 40 ispitivanih krava (puna linija), sa naglašenom distribucijom kretanja u peripartalnom periodu za parametre čije se vrednosti signifikantno razlikuju za ovaj period (isprekidana linija), pravougaonik pokazuje referentne vrednosti dobijene anlizom literature.

DISCUSSION

The average values of the studied haematological parameters in accordance with the recommended reference values from world-class literature (Table 1).

The frequency distribution of these parameters (Graph. 1-11) enters the normal (Gaussian) distribution, as confirmed by statistical analysis of previous studies (George et al., 2010). Regardless of the lactation period a number of cows (average 2) will have values higher or lower than the reference (Table 2), but this does not affect significantly the parameters fatnesses and symmetry of distribution (parameters α and β , unrepresented results). Erythrocyte count and hemoglobin concentration significantly turns to the left in the periparturient period (Graph.1 and 2). The number of neutrophils and white blood cell count turns slightly to the right in the whole cow population (Graph. 7 and 8), while the diversion of neutrophils and prominent periparturient period. The value of monocytes decreases and turns to the left in the periparturient period (Graph. 9).

Previous studies have shown that the concentration of hemoglobin and red blood cells depend on the number of lactation in dairy cows (Belic et al., 2010). It was shown that during the period around calving erythrocyte count and hemoglobin concentration in cows decreased. This situation can be associated with milk production, because it was found that heifers have a significantly higher concentration of hemoglobin in relation to dairy cows (Satar and Mirza, 2009). Also, the concentration of hemoglobin and red blood cell count may be associated with stress in dairy cows, when their value declines (Belic et al., 2010). Gavan et al (2010) and Mirzadeh et al (2010) found significantly lower hemoglobin concentration and erythrocyte counts in the periparturient period, which agrees with our results. Reduced concentration of hemoglobin and erythrocyte counts during lactation was illustrated in the much older results (Rowlands et al., 1977).

The influence of lactation on the movement of white blood cells is minimal, except in the periparturient period (Detilleux et al, 1995). Another important indicator is the ratio of neutrophil to lymphocyte (N: L ratio). The neutrophil to lymphocyte ratio greater than 1 is considered that the cows were loaded inflammation or other stress (Latimer et al, 2003), which is present in the periparturient period in cows in our study (table 1). However, an important indicator of inflammation is the burden of certain elements of white grapes, and we have already mentioned that the number of neutrophils and lymphocytes slightly turns to the right. If the periparturient period is dominated by neutrophilia with monocytopenia cows more likely can to develop signs of metritis, while eosinopenia can lead to periparturient mastitis (Belic et al., 2010).

Establishing the reference values and factors that affect these values is important for clinical veterinary practitioners.

CONCLUSION

Hematologic values in Holstein-Friesian cows in our territory correspond with reference values for cattle. Some discrepancies exist in the periparturient period, at the beginning of lactation. All parameters are arranged according to the normal (Gaussian) distribution.

REFERENCE

- BELIĆ, B., CINCOVIĆ, M.R., STOJANOVIĆ, D., KOVAČEVIĆ, Z., MEDIĆ, S., SIMIĆ, V.: Hematology parameters and physical response to heat stress in dairy cows. *Contemporary agriculture*, 5(1-2)161-166, 2010.
- BELIĆ, B., CINCOVIĆ, M.R., STOJANOVIĆ, D., POTKONJAK, A., KOVAČEVIĆ, Z.: Erythrocytes parameters of cows in different periods of lactation. 21th Symposium of veterinarians of Serbia, Book of Abstracts, p. 56-57, Zlatibor, 2010.
- BELIĆ, B., CINCOVIĆ, M.R., STEVANČEVIĆ, M., BOŽIĆ, A., STOJANOVIĆ, D., KOVAČEVIĆ, Z.: Peripartalni hematološki nalaz kod mlečnih krava u cilju predviđanja sklonosti ka nastanku metritisa i mastitisa. *Zbornik XIX inovacije znanja u stočarstvu*, str.54, Zemun, 2010.
- DETILLEUX, J.C., KEHRLI, M.E., STABEL, J.R., FREEMAN, A.E., KELLEY, D.H.: Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Vet. Immunol. Immunopathol*, 44:251–267, 1995.

GEORGE, J.W., SNIPES, J., LANE, V.M.: Comparison of bovine hematology reference intervals from 1957 to 2006. *Vet. Clin. Pathol.*, 39(2)138–148, 2010.

LATIMER, K.S., PRASSE, K.W.: Leukocytes. In: Latimer KS, Mahaffey EA, Prasse KW, eds. *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology*. 4th ed. Ames, IA: Iowa State Press; p.46–79, 2003.

MIRZADEH, K.H, TABATABAI, S., BOJARPOUR, M., MAMOEL, M.: Comparative study of hematological parameters according strain, age, sex, physiological status and season in Iranian cattle. *Journal of Animal and Veterinary Advances*, 9(16):2123-2127, 2010.

MORRIS, D.D.: Alterations in the Erythron/Leukogram in Large Animal Internal Medicine 4th edition (Smith B., ed), Mosby inc. pp. 400-410, 2009

ROWLANDS, G.J., LITTLE, W., KITCHENHAM, B.A.: Relationship between blood composition and fertility in dairy cows-a field study, *Dairy Res.*, 44:1-7, 1977.

SATTAR, A., MIRZA R.H.: Haematological parameters in exotic cows during gestation and lactation under subtropical conditions. *Pakistan Vet. J.*, 29(3)129-132, 2009.

WEISS, D.J. (ed), WARDROP, K.J. (ed) (2010). *Schalms veterinary hematology*. 6th edition, Wiley-Blackwell.

REFERENTNE VREDNOSTI I DISTRIBUCIJA FREKVENCije HEMATOLOŠKIH PARAMETARA KOD KRAVA TOKOM LAKTACIJE I GRAVIDITETA

BRANISLAVA BELIĆ, MARKO R. CINCOVIĆ,
LJUBICA KRČMAR, BOJANA VIDOVIĆ

Izvod

Ispitivani su hematološki parametri kod 40 krava Holštajn-Frizijske rase. Krave su bile u drugoj i trećoj laktaciji. Krv je uzorkovana u okviru četiri osnovna produktivna perioda i to: u zasušenju (2-3 nedelje pred porođaj), u postpartalnom periodu (0-2 nedelje postpartum), u sredini laktacije (15-17 nedelja postpartum) i u kasnoj laktaciji (2-3 nedelje pred početak zasušenja). Nalaz hematoloških vrednosti kod Holštajn-frizijskih na našoj teritoriji krava odgovara referentnim vrednostima za datu vrstu. Karakteristika nalaza jeste skretanje broja neutrofila i limfocita u desno i skretanje koncentracije hemoglobina u levo. Ovo govori u prilog izloženosti stresu u svakodnevnoj proizvodnji. Peripartalni period odlikuje se smanjenim brojem eritrocita, sniženom koncentracijom hemoglobina, smanjenjem ukupnog broja leukocita uz porast broja neutrofila. Svi parametri su raspoređeni u okviru normalne (Gausove) distribucije.

Ključne reči: hematološki parametri, mlečne krave, referentne vrednosti, distribucija frekvencije parametara.

Received / *Primljen*: 15.04.2011.

Accepted / *Prihvaćen*: 18.05.2011.