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CHANGES IN THE HAEMATOLOGICAL PROFILE OF ROMANIAN BLACK AND SPOTTED DAIRY CALVES

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Abstract

The aim of this study was to evaluate the haematological profile of Romanian Black and Spotted dairy calves during the first three months of life. Forty dairy calves, clinically healthy, from the Experimental Farm of the Research and Development Institute for Bovine Balotesti were screened for hemoleukogram (CBC) evaluation using the Abacus Junior Vet 5 haematology analyser. Comparisons between age groups (t_1: 3-7 days; t_2: 30 days; t_3: 60 days; t_4: 90 days, n = 10 heads/age group) were performed using One-Way ANOVA with post hoc Tukey test. Significant differences in the erythrogram components for platelets (PLT) and mean platelets volume (MPV) were observed (PLT: t_1 vs t_2: $p = 0.0001$; t_1 vs t_3: $p = 0.0014$; t_1 vs t_4: $p = 0.0000$; MPV: t_1 vs t_3: $p = 0.0148$). Statistical differences for leukocytes (WBC) and monocytes (MO) were also observed (WBC: t_1 vs t_2: $p = 0.0397$; t_1 vs t_3: $p = 0.0214$; MO: t_1 vs t_3: $p = 0.0284$). The main blood parameters studied were significantly different during the first three months of life of the calves, expressing a haematological adaptation pattern of the un-weaned dairy calves.

Key words*: age, cattle, dairy calves, haematological adaptation, hemoleukogram.*

INTRODUCTION

The hemoleukogram (CBC) was found to offer valuable information in the diagnosis, surveillance, and estimating of a prognosis regarding the progression of a disease in an individual (Jones & Alison, 2007; Roland, 2014). The study of haematological profile of newborn dairy calves during the adaptation period to the environmental challenges and immunological immaturity is important (Novo et al., 2015), given that this crucial neonatal period is characterized by high morbidity and mortality (Mee, 2008). The values of different blood variables in calves and other young animals are changing with age (Mohri et al., 2010; Moosavian et al., 2010; Brscic et al., 2015). Additionally, the geographical position, management factors, breed of the animal and laboratory factors can affect the haematological reference intervals (Mohri et al., 2007; George et al., 2010). Changes can be observed in the haematological profile of calves due to the transition from the intrauterine environment to the external environment (Knowles et al., 2000; Benesi et al., 2012a). The reference values of different blood variables are well established for adult cattle, however, for calves, there is a scarcity on data available. This study evaluated the haematological profile of healthy newborn calves from birth up to 3 months of age.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU* regarding handling and protection of animals used for scientific purposes.

Forty dairy calves (Romanian Black and Spotted, n=10 heads/age group), from the Experimental Farm of the Research and Development Institute for Bovine Balotesti, were screened for hemoleukogram (CBC) during the first three months of life. The blood samples were taken at the following time: t_1: 3-7 days, t_2: 30 days, t_3: 60 days, and t_4: 90 days. The calves were housed in individual hutches and received a daily ration of 6 liters of milk, divided in two meals per day. Alfalfa hay, concentrate feed, and water were offered *ad libitum*. Blood samples were collected aseptically from the jugular vein (1-2 ml) of each animal, in vacutainer tubes with anticoagulant using disodium ethylene diamine tetra acetic acid (EDTA). Haematological parameters (red blood cells count, haemoglobin concentration, hematocrit percentage, red blood

cells distribution width, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet percentage, mean platelets volume, platelets distribution width, total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage) were determined using the automated hematology analyzer Abacus Junior Vet 5 (Diatron, Hungary). The analyses were carried out in the Animal Physiology and Biochemistry Laboratory of the institute. Results were expressed as a mean (± standard deviation). Comparisons between age groups were performed using One-Way ANOVA with post hoc Tukey test. Significance was declared when $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSIONS

The mean and standard deviation of the erythrogram components are shown in Table 1. No significant effect between the studied age groups ($p > 0.05$) was found for RBC ($F_{(3; 39)} = 0.88$; $p = 0.4602$), HGB ($F_{(3; 39)} = 1.45$; $p = 0.2440$), HTC ($F_{(3; 39)} = 1.40$; $p = 0.2560$), and RDW ($F_{(3; 39)} = 0.57$; $p = 0.6369$). The obtained value for red blood cell count (RBC) was $9.36 \pm 1.24\ 10^6/\mu l$ in the first 7 days of life (t_1) and decrease to $8.95 \pm 1.0710^6/\mu l$ at the 90 days of life (t_4). The HGB concentration ranged from 10.58 ± 1.72 g/dl at 7 days of life (t_1) to 9.48 ± 0.79 g/dl at 60 days of life (t_3), and 10.12 ± 0.95 g/dl at 90 days of life (t_4). The obtained HCT percentage ranged from $30.80 \pm 5.04\%$ at 7 days of life (t_1) to $27.24 \pm 2.49\%$ at 90 days of life (t_4). After the first week, RDW increased till $26.31 \pm 3.48\%$ at 60 days of life (t_3) and decreased further to $24.67 \pm 3.78\%$ at 90 days of life (t_4). The current results are not in accordance with those reported by Baccili et al. (2018) in Holstein calves. Moreover, no changes in erythrogram components in calves from birth up to 30 days of life were observed by Benesi et al., 2012a. Mohri et al. (2007) reported that the HGB, MCH, and MCHC decrease during the first month of life and then start to increase up to the age of 3 months.

Table 1. Mean values of RBC, HGB, HTC and RDW in healthy calves from birth up to 3 months of age

Period/Haematological indicators	RBC, $10^6/\mu l$	HGB, g/dl	HTC, %	RDW, %
	X ± sd	X ± sd	X ± sd	X ± sd
t_1	9.36 ± 1.24	10.58 ± 1.72	30.80 ± 5.04	24.66 ± 2.93
t_2	8.57 ± 0.95	9.59 ± 1.61	28.13 ± 5.36	24.65 ± 3.55
t_3	8.88 ± 1.11	9.48 ± 0.79	27.24 ± 2.49	26.31 ± 3.48
t_4	8.95 ± 1.07	10.12 ± 0.95	29.74 ± 3.48	24.67 ± 3.78
X	8.94 ± 1.09	9.94 ± 1.36	28.98 ± 4.32	25.07 ± 3.39

RBC = red blood cells count; HGB = haemoglobin concentration; HCT = hematocrit percentage; RDW = red blood cells distribution width; t_1: 3-7 days; t_2: 30 days; t_3: 60 days; t_4: 90 days.

The obtained average values for MCV, MCH, and, MCHC (Table 2) were not different statistically ($p > 0.05$) between the studied age groups (MCV: $F_{(3; 39)} = 1.21$; $p = 0.3195$; MCH: $F_{(3; 39)} = 0.71$; $p = 0.5494$; MCHC: $F_{(3; 39)} = 0.52$; $p = 0.6661$).

Table 2. Mean values of MCV, MCH, and MCHC in healthy calves from birth up to 3 months of age

Period/Haematological indicators	MCV, fl	MCH, pg	MCHC, g/dl
	X±sd	X±sd	X±sd
t_1	32.7 ± 2.79	11.29 ± 0.84	34.39 ± 1.32
t_2	31.9 ± 3.30	10.36 ± 0.90	34.24 ± 1.46
t_3	30.9 ± 2.64	10.77 ± 1.04	34.87 ± 1.54
t_4	33.5 ± 3.78	11.44 ± 1.41	34.16 ± 1.20
X	32.45 ± 3.18	11.16 ± 1.06	34.42 ± 1.36

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; t_1: 3-7 days; t_2: 30 days; t_3: 60 days; t_4: 90 days.

Variations for MCV and MCH were detected by a gradual decrease of their values at the first week of life up to 30 days of life (MCV:31.9 ± 3.30 fl; MCH:10.36 ± 0.90 pg). This may coincide with the replacement of foetal HGB with adult HGB. The decrease in HTC and MCV in the first month of life can be considered as transient microcytic anemia,

considering the reference ranges for adult cattle (Mohri et al., 2007). The MCHC showed similar mean values between 34.39 ± 1.32 g/dl (t_1) and 34.16 ± 1.20 g/dl (t_2). Novo et al. (2015) reported the following values: 36.4 ± 1.6 fl for MCV, 11.3 ± 0.8 pg for MCH, and 31.0 ± 2.0 g/dl for MCHC in calves at 30 days of life.

The means and standard deviations of the PLT, MPV and PDW are shown in Table 3. A significant differences between age groups for platelets (PLT: $F_{(3;\ 39)} = 11.23$; $p = 0.0000$), and mean platelets volume (MPV: $F_{(3;\ 39)} = 3.52$; $p = 0.024$) were observed (PLT: t_1 vs t_2: $p = 0.0001$; t_1 vs t_3: $p = 0.0014$; t_1 vs t_4: $p = 0.0000$; MPV: t_1 vs t_3: $p = 0.0148$). The obtained results in this study indicate that PLT count increase rapidly in the first week of life, after which the dynamics of PLT count differ. The number of platelets (PLT) in calves was 681.9 ± 204.47 10^3/µl in the first 7 days of life (t_1), decreased rapidly to 353.4 ± 149.31 10^3/µl at 30 days of life (t_2), then slowly increased at 408.5 ± 91.94 10^3/µl (t_3), and decrease slightly to 338.9 ± 137.62 10^3/µl at 90 days of age (t_4). No statistically significant changes were noted in the PDW percentage ($F_{(3;\ 39)} = 1.60$; $p = 0.2040$). In dairy calves mean values recorded for PDW were 33.69 ± 2.26% at t_1, decreased (30.67 ± 3.42%) at t_3, and increased (31.57 ± 4.16%) at t_4. Panousis et al. (2018) published mean values of 603.8 ± 294.6 10^9/l for PLT, and 7.77 ± 1.48 fl for MPV in calves.

Table 3. Mean values of PLT, MPV and PDW in healthy calves from birth up to 3 months of age

Period/Haematological indicators	PLT, 10^3/µl	MPV, fl	PDW, %
	X ± sd	X ± sd	X ± sd
t_1	681.9 ± 204.47	5.86 ± 0.44	33.69 ± 2.26
t_2	353.4 ± 149.31	5.67 ± 0.31	32.59 ± 2.83
t_3	408.5 ± 91.94	5.29 ± 0.36	30.67 ± 3.42
t_4	338.9 ± 137.62	5.59 ± 0.47	31.57 ± 4.16
X	445.67 ± 202.199	5.60 ± 0.44	32.13 ± 3.32

PLT = platelet count; MPV = mean platelets volume; PDW = platelets distribution width; t_1: 3-7 days; t_2: 30 days; t_3: 60 days; t_4: 90 days.

The means and standard deviations of the leukogram components are shown in Table 4. Statistical differences for the total white blood cells count (WBC: $F_{(3;\ 39)} = 3.81$; $p = 0.0180$) and monocytes (MO: $F_{(3;\ 39)} = 3.37$; $p = 0.0287$) were observed (WBC: t_1 vs t_2: $p = 0.0397$; t_1 vs t_3: $P = 0.0214$; MO: t_1 vs t_3: $p = 0.0284$). A gradual decrease of the total number of white blood cells with the increase of age was observed (t_1: 11.75 ± 2.55 10^3/µl; t_2: 9.09 ± 1.94 10^3/µl; t_3: 9.82 ± 2.28 10^3/µl; t_4: 8.85 ± 1.61 10^3/µl). Lymphocytes ($F_{(3;\ 39)} = 1.64$; $p = 0.1955$) showed value of 53.01 ± 16.64 % in the first 7 days of life (t_1), followed by an increase, with maximum values (64.86 ± 5.67%) observed at 60 days of life (t_3), and a decrease (57.25 ± 19.09%) at 90 days of life (t_4). Monocytes grow in the first 7 days of life (t_1: 8.97 ± 10.51%), followed by a gradual decrease (t_2: 2.88 ± 3.03 %; t_3: 1.5 ± 0.68%) then they are no longer influenced by age. Values between 35.15 ± 7.94% and 39.6 ± 16.55% for neutrophils during the first 90 days after birth (t_4) were recorded ($F_{(3;\ 39)} = 2.20$; $p = 0.1043$).

Table 4. Mean values of WBC, LY, MO and NE in healthy calves from birth up to 3 months of age

Period/Haematological indicators	WBC, 10^3/µl	LY, %	MO, %	NE, %
	X ± sd	X ± sd	X ± sd	X ± sd
t_1	11.75 ± 2.55	53.01 ± 16.64	8.97 ± 10.51	35.15 ± 7.94
t_2	9.09 ± 1.94	51.61 ± 13.69	2.88 ± 3.03	45.52 ± 12.20
t_3	9.82 ± 2.28	64.86 ± 5.67	1.5 ± 0.68	33.66 ± 5.36
t_4	8.85 ± 1.61	57.25 ± 19.09	3.15 ± 3.11	39.6 ± 16.55
X	9.87 ± 2.34	56.68 ± 15.03	4.12 ± 6.19	38.48 ± 11.85

WBC = total white blood cells count; LY = lymphocytes percentage; MO = monocytes percentage; NE = neutrophil percentage; t_1: 3-7 days; t_2: 30 days; t_3: 60 days; t_4: 90 days.

Leukogram variations in calves within the first week of life could be caused by the stress following the postpartum adaptation, caused by the elevation in circulating levels of endogenous glucocorticoids in the blood of the calves and their effects on different blood leukocytes (Benesi et al., 2012a). In this case, the leukocyte formula can acquire a neutrophilic profile. In the current study, the leukocyte formula had a lymphocyte profile. The total means values of the hemoleukogram (CBC) components in the dairy calves at 0-3 months of life are shown in Figure 1. The recorded values in this study were not in accordance with values reported by Parvu et al. (2003) in un-weaned dairy calves.

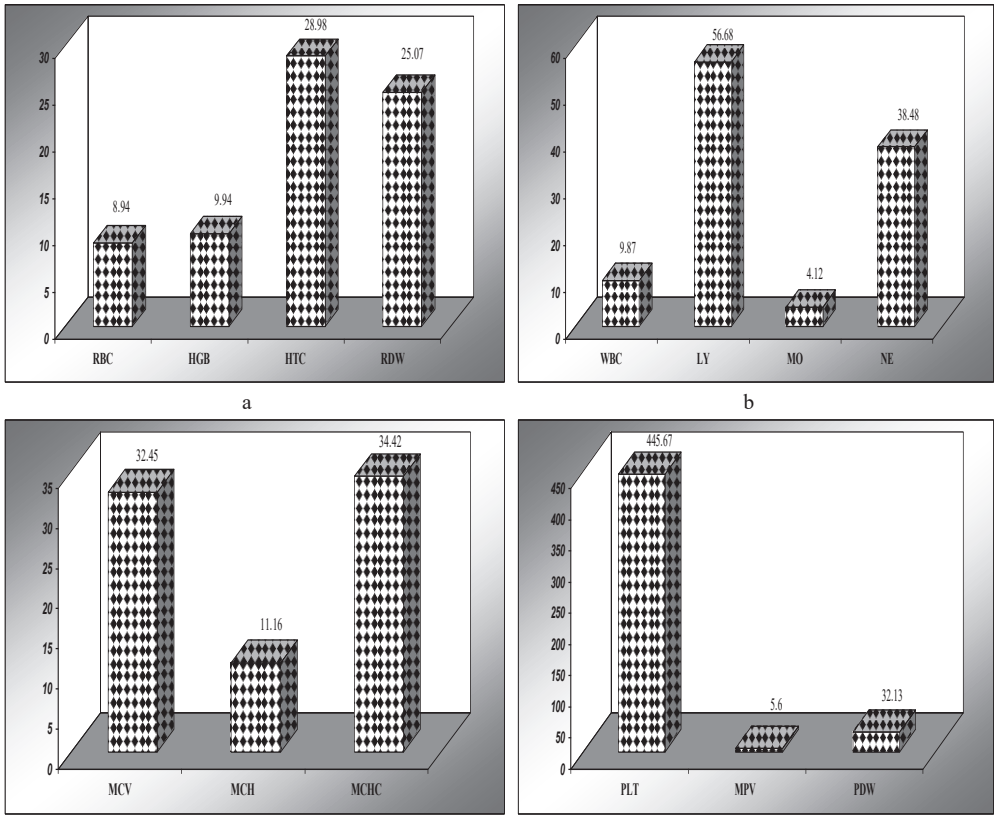

Figure 1 a, b, c, d. Mean values of hemoleukogram (CBC) in healthy dairy calves at 0-3 months of life

Botezatu et al. (2014) reported the following values: 9.1 10^6/mm^3 for RBC, 12.5 g/dl for HGB, 34.2% for HCT, 42 μ^3 for MCV, 12.8 pg for MCH, 35.5 g/dl for MCHC, 454 10^3/mm^3 for PLT, 8.1 μ^3 for MPV, 9.1 10^3/mm^3 for WBC, 65% for LY, 0.65% for MO, and 34% for NE, in Holstein-Friesian calves between 0 to 3 months. In comparison with adult cows' reference values reported by Parvu et al. (2003), calves had lower mean of HGB, HTC, MCV, MCH, higher mean of RBC, WBC, NE, and similar value for LY and MO. Klinkon and Jezek (2012), Jonson and Alison (2007), Brun–Hansen (2006) reported that, in young calves, RBC counts and HTC percentage might be higher, and, MCV and MCHC might be lower than in adults cows. The total white blood cells count (WBC) in blood of calves is higher comparative with adult animals and is more variable as values of other haematological variables. Different types of leucocytes have different life spans so their number can change rapidly and blood serves only as transport medium from the place of origin to the place of inflammation (Roland, 2014; Kraft, 1999a). In young animals, the number of leukocytes,

including neutrophils is higher than in adults. With age, this situation changes, the number of neutrophils decreasing in favour of lymphocytes (Abramowicz et al., 2019).

CONCLUSIONS

The obtained results, showed significant variations in the components of the CBC of calves during the first 3 months of life. These haematological variations could be a consequence of the stress related to environmental adaptation challenges that calves are facing.

Current results, collected with those of other authors, could represent a first step in setting up reference values for the haematological profiles in clinically healthy un-weaned dairy calves.

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