

THE HAEMATOLOGICAL PROFILE OF NEWBORN CALVES OBTAINED BY CROSSBREEDING WITH MEAT SIRE BREEDS

Marinela ENCULESCU¹, Radu NEAMT², Daniela Maria VIDMICH¹, Ioana NICOLAE¹

¹Research and Development Institute for Bovine, 21, Bucuresti-Ploiesti, 077015, Balotesti, Romania

²Research and Development Station for Bovine, 32, Bodroglui, 310059, Arad, Romania

Corresponding author email: marinelaenculescu2006@yahoo.com

Abstract

Hemogram is a basic test used to establish the haematological status and diagnosis of various haematological conditions in animals. The purpose of this study was to establish the physiological status of crossbred calves compared to Romanian Black Spotted calves at birth by determining their haematological profile. Our researches were carried out in the Dairy cows' Experimental Farm of I.C.D.C.B. Balotesti, on a number of 45 newborn calves. They have been distributed in four experimental groups, each one of 9 calves (E₁: F₁ Charolaise x Romanian Black Spotted, E₂: F₁ Blanc Blue Belgique x Romanian Black Spotted, E₃: F₁ Aberdeen Angus x Romanian Black Spotted, E₄: F₁ Limousine x Romanian Black Spotted) and the control group of 9 calves (M: Romanian Black Spotted). The haematological parameters (red blood cells, haemoglobin, hematocrit, red blood cells distribution width, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelets volume, platelets distribution width, total white blood cells, lymphocytes, monocytes, neutrophil) were determined using the automated hematology analyzer Abacus Junior Vet 5. The results were expressed as a mean (\pm standard error), standard deviation (sd) and coefficient of variation (V). The analysis of variance (ANOVA) single-factor was applied to test the significance of differences. The obtained results showed no statistical significant differences between experimental groups ($F_{critical} < F_{0.05}$) for all the haematological parameters studied. However, for MCHC (mean corpuscular hemoglobin concentration), significant differences ($p \leq 0.05$) between the experimental groups was observed, as following: $F_{critical} = 2.93$; $F_{0.05} (8; 44) = 2.00$; $F_{critical} > F_{0.05}$. The recorded values obtained in this work could help to a better interpretation of clinical pathology data and diagnosis of neonatal diseases in calves.

Key words: calves, crossbreed, haematological profile, newborn.

INTRODUCTION

Hematology has become an area of great interest, considering the increased incidence of haematological diseases in farm species. Haematological examinations data are analyzed corelatively, for the diagnosis diseases of the blood and hematopoietic organs or subclinical conditions. (Gherariu et al., 1985). The haematological profiles provide reliable information on the health and functional status of the organism (Kumar and Pauchaura, 2000). Erythrocytes/red blood cells are the main mass of the blood cells (Stancioiu, 1999). In cattle, erythrocytes have an average diameter of 5-6 μ m (Roland et al., 2014) and a relatively long life span of 130-160 days (Brockus, 2011; John, 2010). The component by which erythrocytes exert their function as respiratory gas transporter is hemoglobin. In combination with hematocrit and hemoglobin concentration,

the number of erythrocytes is useful in detecting and monitoring of anemia and erythrocytosis/polycythemia. For a better assessment, the erythrocyte mass is correlated with hematocrit. The red blood cells numbers is influenced by the plasmatic volume changes such as physiological status and hydro-electric balance (Means, 2004). The red blood cells distribution width (RDW) indicates whether all the red cells are about the same, width, size and shape (Terzano et al., 2005). Platelets are involved in haemostasis and of tissue repair processes (Russell, 2010). Leucocytes count/white blood cells, play an essential role in immune defense and include different subpopulations (lymphocytes, monocytes, neutrophil, eosinophil and basophil). They are the results of the dynamic production of bone marrow, the release of the cells to the peripheral circulation and the storage in different organs (Yaquib et al., 2013).

MATERIALS AND METHODS

The experimental procedures used in this study were 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-five calves, (crossbreed and Romanian Black Spotted, 9 calves/group), from the Research and Development Institute for Bovine Balotesti, were screened for haematological profile at birth. The analyses were carried out in the Animal Physiology and Biochemistry Laboratory of the institute. Blood samples were collected aseptically from the jugular vein (1-2 ml) of each animal, in vacutainer tubes with anticoagulant using potassium-ethylenediamine tetraacetic acid (EDTA/K3) with a concentration of 1.27 mg EDTA/K3 per ml of blood. 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,

platelets count, mean platelets volume, platelets distribution width, total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage) were determined using automated hematology analyzer Abacus Junior Vet 5. Results were expressed as a mean (\pm standard error), standard deviation (sd) and coefficient of variation (V). The analysis of variance (ANOVA) single-factor was applied to test the significance of differences.

RESULTS AND DISCUSSIONS

The recorded mean values for RBC and HGB (Table 1) were situated in the normal physiological limits, except with groups E_2 : F_1 BBB x BNR and M: BNR for red blood cell (the means values were above normal physiological limits), but without statistical significant differences ($F_{critical} < F_{0.05}$). The same mean values have been obtained by others authors in newborn calves (Botezatu et al., 2014; Anton et al., 2009).

Table 1. The result of RBC, HGB, HTC, RDW parameters in newborn calves

Groups/Breed ¹	Parameters ²	$\bar{X} \pm s_x$	sd	V%
E_1 : F_1 CH x BNR	RBC, mil/mm ³	8.95 \pm 0.83	2.65	29.61
E_2 : F_1 BBB x BNR		10.58 \pm 0.45	1.51	14.22
E_3 : F_1 AA x BNR		8.99 \pm 0.34	1.09	12.12
E_4 : F_1 LI x BNR		8.44 \pm 0.54	1.72	20.38
M: BNR		9.35 \pm 0.48	1.51	16.15
$F_{critical}=1.82$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.144).				
E_1 : F_1 CH x BNR	HGB, g/dl	10.34 \pm 0.34	1.11	10.74
E_2 : F_1 BBB x BNR		11.54 \pm 0.55	1.56	13.52
E_3 : F_1 AA x BNR		11.78 \pm 0.50	1.47	12.48
E_4 : F_1 LI x BNR		10.08 \pm 0.58	1.86	18.45
M: BNR		10.61 \pm 0.59	1.89	17.81
$F_{critical}=1.97$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.118).				
E_1 : F_1 CH x BNR	HTC, %	29.15 \pm 1.30	4.81	16.50
E_2 : F_1 BBB x BNR		33.85 \pm 1.52	4.15	12.26
E_3 : F_1 AA x BNR		32.32 \pm 1.43	4.56	14.11
E_4 : F_1 LI x BNR		29.48 \pm 1.25	3.98	13.50
M: BNR		30.82 \pm 1.58	5.02	16.29
$F_{critical}=1.71$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.166).				
E_1 : F_1 CH x BNR	RDW, %	23.36 \pm 0.50	1.58	6.76
E_2 : F_1 BBB x BNR		25.60 \pm 0.23	1.08	4.22
E_3 : F_1 AA x BNR		24.12 \pm 0.39	1.26	5.22
E_4 : F_1 LI x BNR		24.48 \pm 0.94	3.01	12.30
M: BNR		24.45 \pm 0.22	0.71	2.90
$F_{critical}=1.96$; $F_{0.05}(8; 44)=2.00$; $F_{critical} < F_{0.05}$; (p=0.119).				

¹ CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.
²RBC= erythrocytes/red blood cells count, HGB=hemoglobin concentration, HCT= hematocrit percentage, RDW=red blood cells distribution width.

The average percentage for HTC was $33.85 \pm 1.52\%$ for group E₂: F₁ BBB x BNR and $32.32 \pm 1.43\%$ for group E₃: F₁ AA x BNR comparative with groups E₁: F₁ CH x BNR, E₄: F₁ LI x BNR and M: BNR where registered values below reference values indicated by the literature. Decrease of the HTC indicated anemia and impaired ability to carry oxygen from red blood cells. The RDW and MCV are useful for classifying the types of anemia. The increase of RDW is the first sign early in iron deficiency or other trace mineral deficiencies associated with macrocytic or microcytic anemia-the MCV is normal or lower and RDW is higher (Brockus, 2011; Brun Hansen, 2006; Glader, 2004; Kincaid, 1999). Together with MCHC and MCH (Table 2), MCV can permit

the early detection of processes that will cause anemia. In newborn calves, the recorded means values for RDW were higher (from $23.36 \pm 0.50\%$ to $25.60 \pm 0.23\%$) and obtained means values for MCV were below normal physiological limits (from 33.00 ± 0.57 fl to 35.44 ± 0.86 fl). The coefficient of variation calculated for RBC, HGB, HTC, MCH was lower than 20.38%, expressing a homogeneous population. However, for RBC (group E₁: F₁ CH x BNR) the coefficient of variation was 29.61%. For RDW, the values were below than critical threshold of 10% for groups E₁: F₁ CH x BNR, E₂: F₁ BBB x BNR, E₃: F₁ AA x BNR and M: BNR, indicated a very homogeneous population.

Table 2. The result of MCV, MCH, MCHC parameters in newborn calves

Groups/Breed ¹	Parameters ²	$\bar{X} \pm s_x$	sd	V%
E ₁ : F ₁ CH x BNR	MCV, fl	34.33±1.06	3.39	9.87
E ₂ : F ₁ BBB x BNR		32.33±1.01	3.43	10.61
E ₃ : F ₁ AA x BNR		35.44±0.86	2.74	7.73
E ₄ : F ₁ LI x BNR		34.66±0.89	2.29	6.61
M: BNR		33.00±0.57	1.65	5.00
<i>F_{critical}=1.86; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.137).</i>				
E ₁ : F ₁ CH x BNR	MCH, pg	12.57±0.46	1.54	11.25
E ₂ : F ₁ BBB x BNR		11.41±0.43	1.39	12.18
E ₃ : F ₁ AA x BNR		12.54±0.50	0.90	7.18
E ₄ : F ₁ LI x BNR		11.98±0.44	1.41	11.77
M: BNR		11.56±0.22	0.59	5.10
<i>F_{critical}=1.78; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.160).</i>				
E ₁ : F ₁ CH x BNR	MCHC, g/dl	38.64±0.58	1.86	4.81
E ₂ : F ₁ BBB x BNR		36.81±0.37	1.17	3.18
E ₃ : F ₁ AA x BNR		37.12±1.08	3.44	9.27
E ₄ : F ₁ LI x BNR		35.44±0.83	2.86	8.07
M: BNR		35.17±0.26	2.22	6.31
<i>F_{critical}=2.96; F_{0.05}(8; 44)=2.00; F_{critical} > F_{0.05}; (p=0.031).</i>				

¹CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.

²MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration.

In case of MCHC (Figure 1), significant differences ($p \leq 0.05$) between the experimental groups were observed, as follows:

$F_{critical} = 2.93; F_{0.05}(8; 44) = 2.00; F_{critical} > F_{0.05}$

Also, a very homogeneous population was obtained for MCHC (values between 3.18%-9.27%) for all experimental groups studied.

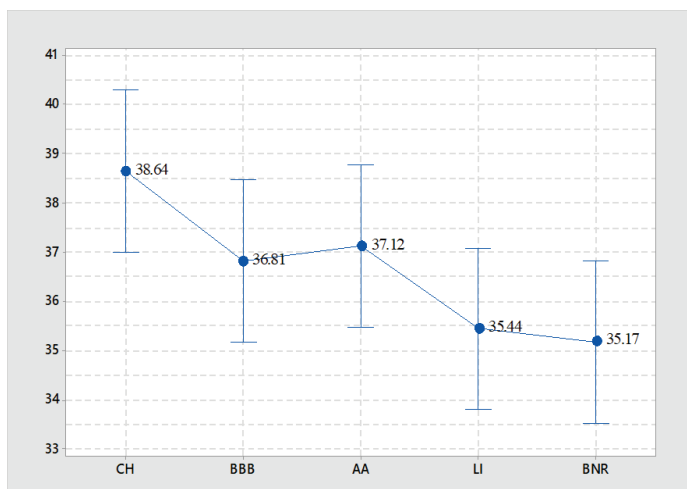


Figure 1. Average values of mean corpuscular hemoglobin concentration (MCHC) in newborn calves

In calves, platelets counts may be the same or above adult reference intervals (Brun Hansen, 2006). The obtained results for PLT, MPV and PDW were not statistically significant ($p>0.05$) between experimental groups (Table 3). An increased platelets count might be associated with an increased risk for thrombosis (Boudreaux, 2011), in our study the recorded means values for PLT were situated in normal physiological limits.

The obtained means values for MPV and PDW were below references values indicated by the literature. The MPV together with PDW can be used to differentiate conditions associated with lower platelets production and with increased platelets destruction.

The PLT and VTM are often low in conditions associated with alteration of platelets production (Perkins, 2004).

Table 3. The result of PCT, MPV, PDW parameters in newborn calves

Groups/Breed ¹	Parameters ²	$\bar{X} \pm s_x$	sd	V%
E ₁ : F ₁ CH x BNR	PLT, thousands/mm ³	505.40±85.58	272.30	53.88
E ₂ : F ₁ BBB x BNR		632.00±61.21	194.80	30.82
E ₃ : F ₁ AA x BNR		542.80±53.30	169.60	31.25
E ₄ : F ₁ LI x BNR		679.60±74.96	231.11	34.01
M: BNR		729.00±35.41	123.70	16.97
$F_{critical}=1.86; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.137).$				
E ₁ : F ₁ CH x BNR	MPV, fl	5.88±0.15	0.59	10.03
E ₂ : F ₁ BBB x BNR		5.57±0.03	0.10	1.80
E ₃ : F ₁ AA x BNR		5.64±0.11	0.37	6.56
E ₄ : F ₁ LI x BNR		5.98±0.06	0.22	3.68
M: BNR		5.90±0.14	0.46	7.80
$F_{critical}=1.84; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.140).$				
E ₁ : F ₁ CH x BNR	PDW, %	33.80±0.69	1.81	5.36
E ₂ : F ₁ BBB x BNR		32.76±0.23	1.36	4.15
E ₃ : F ₁ AA x BNR		33.04±0.15	0.50	1.51
E ₄ : F ₁ LI x BNR		32.68±0.20	0.65	1.99
M: BNR		33.80±0.40	1.30	3.85
$F_{critical}=1.78; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.151).$				

¹CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.

²PLT=platelets/thrombocytes count, MPV=mean platelet volume, PDWc=platelet distribution width.

There is limited data in the literature regarding the clinical interpretation of MPV and PDW. For PLT, the coefficient of variation was situated between 30.82-53.88% for groups E₁: F₁ CH x BNR, E₂: F₁ BBB x BNR, E₃: F₁ AA x BNR and E₄: F₁ LI x BNR, showed a heterogeneous populations. On the contrary, for PDW, the coefficient of variation was below 5.36%, in this case we had a very homogeneous populations (for E₂: F₁ BBB x BNR the values was 4.15%, for E₃: F₁ AA x BNR the values was 1.51% and for

E₄: F₁ LI x BNR the values was 1.99%). The obtained means values for WBC, LY and MO (Table 4) were situated above normal physiological limits, without statistical significant differences ($F_{critical} < F_{0.05}$). The high number of white blood cells may be the sign of a bacterial infection or inflammatory syndrome. According to the specialty literature, we have mild leukocytosis when the white blood cells count is between 9.8-12 thousands/mm³ (Parvu, 2003).

Table 4. The result of WBC, LY, MO, NE parameters in newborn calves

Groups/Breed ¹	Parameters ²	$\bar{X} \pm s_x$	sd	V%
E ₁ : F ₁ CH x BNR	WBC, thousands /mm ³	10.99±0.60	1.91	17.38
E ₂ : F ₁ BBB x BNR		12.45±1.99	1.96	15.74
E ₃ : F ₁ AA x BNR		10.47±0.72	2.29	21.87
E ₄ : F ₁ LI x BNR		10.05±0.12	0.38	3.78
M: BNR		11.92±1.00	3.19	26.76
$F_{critical}=1.93; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.124).$				
E ₁ : F ₁ CH x BNR	LY, %	72.68±3.24	10.32	14.20
E ₂ : F ₁ BBB x BNR		66.81±3.84	12.22	18.29
E ₃ : F ₁ AA x BNR		67.33±3.83	12.20	18.12
E ₄ : F ₁ LI x BNR		61.24±4.62	16.38	26.75
M: BNR		59.60±4.91	6.55	10.99
$F_{critical}=1.72; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.164).$				
E ₁ : F ₁ CH x BNR	MO, %	4.95±0.73	2.34	47.27
E ₂ : F ₁ BBB x BNR		8.21±0.27	2.71	33.01
E ₃ : F ₁ AA x BNR		4.97±1.14	3.63	73.04
E ₄ : F ₁ LI x BNR		4.41±1.11	3.55	80.50
M: BNR		6.17±4.30	4.06	65.80
$F_{critical}=1.89; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.131).$				
E ₁ : F ₁ CH x BNR	NE, %	22.37±3.07	9.78	43.72
E ₂ : F ₁ BBB x BNR		23.51±3.58	11.40	48.49
E ₃ : F ₁ AA x BNR		27.70±4.45	14.16	51.12
E ₄ : F ₁ LI x BNR		34.32±5.58	17.77	51.78
M: BNR		30.31±1.30	4.10	13.53
$F_{critical}=1.43; F_{0.05}(8; 44)=2.00; F_{critical} < F_{0.05}; (p=0.241).$				

¹CH=Charolaise, BBB=Blanc Blue Belgique, AA=Aberdeen Angus, LI=Limousine, BNR=Romanian Black Spotted.

²WBC=leukocytes/white blood cells count, LY=lymphocytes percentage, MO=monocytes percentage, NE=neutrophil percentage.

The lymphocyte leukocytosis can be detected when lymphocytes are more than 60%, found in hematois, septicaemia and virosis. In cattle, the total number of WBC decreases with age. The total WBC count were higher in newborn calves than adult reference intervals in some studies, but in others reports, the mean of WBC counts in newborn calves were within adult reference intervals (Mohri, 2007; Knowles, 2000). The difference may be attributed variability between individual calves. Calves have a NE:LY ratio greater than 1.0 at birth, with a rapid decrease in NE and increase in LY

resulting in an NE:LY ratio similar to that of adult cattles at seven days of age (Jones, 2007). Neutrophils provide the first line of defense against any inflammatory process-microorganisms, tissue trauma, etc. (Appelberg, 2006). The recorded average values for NE were situated in normal physiological limits (means from 21.42%±4.11% to 33.02%±7.31%) with a coefficient of variation between 14.49%-55.39% for all groups. In the present study, we observed a congenital anemia in calves at birth

which are in agreement with results observed in other studies (Parvu, 2003).

CONCLUSIONS

The haematological examination used for health status assessment in newborn calves revealed a slight anemia of these. The incidence of subclinical anemia is, often, found in newborn calves. The obtained results showed no statistical differences between experimental studied groups. However, in case of mean corpuscular hemoglobin concentration, significant differences between the experimental groups were observed. To explore the haematological profile in newborn calves, larger scale studies with more animal's are needed to correlate the results with clinical data.

ACKNOWLEDGEMENTS

This study was supported by Sectoral Project ADER 5.1.10/2015.

REFERENCES

- Anton A., Pavel G., Solcan Gh., Boghian V., 2009. The effect of copper deficiency on haematological profile of neonatal black pie dairy calves, *Bulletin UASVM, Veterinary Medicine*, 66 (2), 19-25.
- Appelberg R., 2006. Neutrophils and intracellular pathogens: beyond phagocytosis and killing, *Trends Microbiology*, 15, 87-92.
- Blaxter K.L., Sharman G.A., MacDonald A.M., 1957. Iron deficiency anaemia in calves, *British Journal Nutrition*, 11, 234-246.
- Brockus C.W., 2011. Erythrocytes, *In: Duncan and Prasse's veterinary laboratory medicine: clinical pathology*, Latimer KS, 5th ed., Wiley, Chichester, UK, 3-44.
- Brun Hansen H.C., Kampen A.H., Lund A., 2006. Hematologic values in calves during the first 6 months of life, *Veterinary Clinical Pathology Journal*, 35, 182-187.
- Botezatu A., Vlagioiu C., Codreanu M., Oraşanu A., 2014. Biochemical and hematological profile in cattle effective, *Bulletin UASVM Veterinary Medicine*, 71 (1), 27-30.
- Boudreaux M.K., Spangler E.A., Welles E.G., 2011. Hemostasis. *In: Duncan and Prasse's veterinary laboratory medicine: clinical pathology*, ed. Latimer KS, 5th ed., 107-144. Wiley, Chichester, UK.
- Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes, OJEU L 276/33 IA.
- Gherariu S., Pop A., Kadar L., 1985. *Clinical veterinary laboratory guide*, Ceres Publishing, Bucharest, 82.
- Glader B., 2004. Anemia, General considerations. *In Wintrobe's Clinical Hematology*, Philadelphia, 948-975.
- John A.C., 2010. Erythrokinetics and Erythrocyte, *Erythrocytes-section III*, 138, *Schalm's veterinary hematology-6th ed.*, Chap. 22, 138.
- Jones J.L., Allison R.W., 2007. Evaluation of the ruminant complete blood cell count, *Veterinary Clinics of North America. Food Animal Practice*, 23, 377 - 402.
- Kincaid R.L., 1999. Assessment of trace mineral status of ruminants, A review, *Journal of Animal Science*, 77, 1-10.
- Knowles T.G., Edwards J.E., Bazeley K.J., Brown S.N., A. Butterworth, Warriss P.D., 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age, *Vet Record*, 147, 593 - 598.
- Kumar B., Pauchaura S.P., 2000. Haematological profile of crossbred dairy cattle to monitor herd health status at medium elevation in central Himalayas, *Research in Veterinary Science*, 69, 141-145.
- Law no. 43/2014 regarding handling and protection of animals used for scientific purposes, OM no.326/Parth I.
- Means R., 2004. Erythrocytosis. *In Wintrobe's Clinical Hematology*. Philadelphia, 1495-1505.
- Mohri M., Sharifi K., Eidi S., 2007. Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults, *Research in Veterinary Science*, 83, 30 - 39.
- Parvu Gh., Costea M., Pirvu M., Nicolae B., 2003. *Treaty by animal nutrition*, Coral Sanivet Publishing, Bucharest, 840.
- Perkins S., 2004. Examination of the Blood and Bone Marrow. *In Wintrobe's Clinical Hematology*, Philadelphia, 3-21.
- Roland L., Drillich M., Iwersen M., 2014. Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation*. www.sagepub.com/journals, 26(5), 592-598.
- Russell K.E., 2010. Platelet kinetics and laboratory evaluation of thrombocytopenia. *In: Schalm's veterinary hematology*, ed. Weiss DJ, Wardrop KJ, 6th ed., Wiley, Ames, 576-585.
- Stancioiu N., 1999. *Animal physiology*. Publishing Coral Sanivet, Bucuresti, 207.
- Terzano G.M., Allegrini S., Borghese A., Ranconi C., Alfieri L., 2005. Metabolic and hormonal parameters in buffaloes, Italy, 230.
- Yaqub L.S., Kawu M.U., Ayo J.O., 2013. Influence of reproductive cycle, sex, age and season on haematologic parameters in domestic animals: A review, *Journal of Cell and Animal Biology, Nigeria*, 7(4), 37-43.