Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

COMPARATIVE STUDY ON METABOLIC BIOMARKERS IN LACTATING DAIRY COWS AND BUFFALOES

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Abstract

The knowledge of haematological and urine biomarkers is useful to diagnose various metabolic and pathological disorders, which have a negative impact on the overall performances of dairy species. The aim of this study was to investigate the haematological and urine parameters in lactating dairy cows comparative with lactating buffaloes. The study was carried out on sixty-eight Romanian Black and Spotted cattle and on fifty Romanian buffalo. The obtained values for hemoglobin concentration (HGB), hematocrit percentage (HCT), white blood cells count (WBC), lymphocytes percentage (LY), monocytes percentage (MO), and neutrophil percentage (NE), varied significantly ($p<0.01$) between cows and buffaloes. For other haematological indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), statistical differences were recorded. Urine examination of cows and buffaloes showed that all the parameters studied were within the normal physiological limits, with minor fluctuations for protein level (30 mg/dl). The obtained results could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of dairy herd's.

Key words*: buffaloes, cows, haematological profile, urine profile*.

INTRODUCTION

Haematological studies are useful in the diagnosis and prognosis of many diseases in farm animal's (Olafedehan et al., 2010; Togun et al., 2007; Parvu et al., 2003) and it plays a vital role in the physiological, nutrition and pathological status of an organism (Doyle, 2006). According to Xie et al. (2013), significant variations in the blood metabolic profile depend on genetic (breed and genotype) and non-genetic factors (age, sex, physiological status). Seasons and diet, also, influence the blood parameters of farm animal's (Knaus, 2013; Varra et al., 2017; Garkal et al., 2016; Radkowska and Herbut, 2014). Changes in haematological parameters are often used to determine status of the body and to determine stresses due to environmental, nutritional and pathological factors (Afolabi et al., 2010). Urinalysis is a useful and inexpensive tool to detect metabolic diseases such as diabetes mellitus, liver diseases, glomerulonephritis and urinary tract infections (Finco, 1997). There exists difference between the cattle and buffaloes regarding utilization of feed. Buffaloes have better digestive ability than cattle to utilize poor quality roughage (Agarwal et al., 2008) and better degrade crude protein and protein free dry matter (Terramoccia et al., 2000). With this background, the aim of the present study was to investigate the haematological and urine parameters of apparently healthy Romanian Black and Spotted cows and Romanian buffalo and, also, to compare the metabolic profile between both groups. The determination of these parameters could then aid in exploring the differences in the metabolism of this and their organ function.

MATERIALS AND METHODS

Sixty-eight multiparous Romanian Black and Spotted dairy cows and fifty multiparous Romanian buffalo cows, clinically healthy, were screened for metabolic profile (haematological and urine indicators) during the autumn season (October, 2019). The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine

Balotesti, and at the Experimental Farm of the Research and Development Station for Buffalo Sercaia, Romania. The cows and buffaloes were housed under tied stanchion barn conditions. Feeding system was differentiated, for cows, the diet/head/day consisted of 4 kg alfalfa hay, 20-25 kg corn silage and 3-4 kg concentrates, and, for buffaloes, the diet/head/day consisted of 4 kg sedge, 7 kg marsh hay and 28 kg corn silage. The lactating dairy cows and buffaloes received salt and water *ad libitum*. For the haematological examinations, blood samples (1-2 ml) were collected aseptically from the jugular vein of each animal, 2-4 hours after the morning feeding, in vacutainer tubes with disodium-ethylene diamine tetra acetic acid ($EDTA/Na_2$). After harvesting, the samples were chilled to +4 °C. Urine samples were collected in 50 ml sterilized vials from both groups studied as free catch (mid stream voided) during micturition. Haematological parameters (RBC, HGB, HTC, MCV, MCH, MCHC, WBC, LY, MO, NE) were determined using automated hematology analyzer Abacus Junior Vet 5. Urine examination (bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrites, leukocytes, specific gravity) were determined with the DocUReader urine analyzer, used for *in vitro* diagnostics. Means ± (Standard Deviations) and coefficients of variation (CV) of blood indicators were calculated. Data were analyzed to compare the mean values of cows and buffaloes by applying independent mean t-test and 95% confidence intervals at 0.05 significance level using a descriptive statistical tool (computer software Microsoft Excel). The experimental procedures were performed in accordance with the *Romanian Law no. 43/2014* and the *Council Directive 2010/63/EU* on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSIONS

The Table 1 shows means values of blood parameters in lactating dairy cows and buffaloes studied, and the Table 2 shows haematology reference intervals in bovine.

Table 1. Haematological parameters in lactating dairy cows and buffaloes

Parameters	Group I Cows		Group II Buffaloes		P	Reference limits
	X ± SD	CV, %	X ± SD	CV, %		
RBC, $10^6/\mu L$	6.56±1.34	20.43	6.79±1.24	18.26	0.114	5-8
HGB, g/dL	9.48±1.38	14.56	12.29±2.27	18.47	0.000	9-11
HCT, %	28.50±3.21	11.26	37.69±7.10	18.83	0.000	32-38
MCV, fl	43.98±4.41	10.03	55.46±2.83	5.10	0.000	40-60
MCH, pg	14.9±1.65	11.08	18.14±1.37	7.55	0.000	11-17
MCHC, g/dL	33.93±2.03	5.98	32.71±2.40	7.33	0.012	30-36
WBC, $10^3/\mu L$	8.37±2.27	27.12	9.86±2.14	21.70	0.000	6.5-9.5
LY, %	54.10±10.14	18.74	45.93±7.41	16.13	0.000	45-61
MO, %	7.03±2.81	39.97	5.21±2.00	38.39	0.003	0-4
NE, %	38.74±8.97	23.15	48.84±7.86	16.09	0.000	15-41

RBC - red blood cells, HGB - hemoglobin, HCT - hematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, WBC - total white blood cells, LY - lymphocytes, MO - monocytes, NE - neutrophils

Table 2. Haematology reference interval in bovine (source Roland et al., 2014)

Parameters	Wood and Quiroz-Rocha, 2010	George et al., 2010	Kraft and Dürr, 2005
RBC, $10^6/\mu L$	4.9-7.5	5.1-7.6	5-10
HCT, %	21-30	22-33	28-38
HGB, g/dL	8.4-12.0	8.5-12.2	9-14
MCV, fl	36-50	38-50	46-65
MCH, pg	14-19	14-18	11-17
MCHC, g/dL	38-43	36-39	31-34

RBC - red blood cells, HCT - hematocrit, HGB - hemoglobin, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration

The results revealed no significant variation ($P>0.05$) in RBC count for the group I and for the group II studied. A significant variation ($p<0.01$) for HGB, HCT, WBC, LY, MO, and

NE, between the group I and the group II, was observed. The MCV, MCH, MCHC indices were, also, found to be significantly ($P<0.01$ for MCV and MCH; $P<0.05$ for MCHC) between groups studied, this erythrocyte indices are usually used for morphological classification of anaemia. The recorded mean values for RBC (6.56±1.3410^6/µL), HGB (9.48±1.38 g/dL), MCV (43.98±4.41 fl), MCH (14.9±1.65 pg), MCHC (33.93±2.03 g/dL), WBC (8.37±2.27 10^3/µL), LY (54.10±10.14%), and NE (38.74±8.97%) parameters were situated in the normal physiological limits in lactating dairy cows. However, in the present study, a lower mean value for HCT (28.50±3.21%) and a high mean value for MO (7.03±2.81%) than normal physiological limits were recorded. According to Research Animal Resources (2009), the reference values for cow are situated between 8-15 g/dL for HGB, 40-60 fl for MCV, 11-17 pg for MCH, 30-36 mg/dL for MCHC, 4-12 10^3/µL for WBC, 40-70 % for LY, and 1-6 % for MO. Merck Manual (2012) related the following rage of values for cow: 5-10 10^6/mm^3 for RBC 10-15 g/dL for HGB, 39-55 fl for MCV, 13-17 pg for MCH, and 30-36 mg/dL for MCHC. The recorded mean values for RBC (6.79±1.24 10^6/µL), HCT (37.69±7.10 %), MCV (55.46±2.83 fl), and LY (45.93±7.41 %), parameters were situated in the normal physiological limits in lactating buffaloes with slight increase for HGB (12.29±2.27 g/dL), WBC (9.86±2.14 10^3/µL), MCH (18.14±1.37 pg), MO (5.21±2.00%) and high increase for NE (48.84±7.86%) mean values. Mahmood et al. (2013) related means values of 6.73±1.28 10^6/µL for RBC, 11.20±2.06 g/dL for HGB, 31.69±5.39% for HCT, 47.97±4.03 fl for MCV, 17.39±1.73 pg for MCH, and 36.02±0.97 for MCHC. Shahzadi et al. (2014) reported the following values in lactating buffaloes: 5.38±0.25 10^6/µL for RBC, 9.94±0.47 g/dL for HGB, 8.32±0.78 10^3/µL for WBC, 27.17±1.27 % for HCT, 51.75±2.08 fl for MCV, 19.85±0.63 pg for MCH, and 37.85±0.45 for MCHC. The obtained values for the coefficient of variation, for MCV and MCHC, were below that critical threshold of 10%, indicated a very homogeneous population, in cows. The coefficient of variation calculated for RBC, HGB, HCT, MCH, and LY was lower than 20%, expressing a homogeneous population. For the WBC and NE, the coefficient of variation was 27.12%, respectively 23.15%. However, for the MO, the coefficient of variation was 39.97% expressing a heterogeneous population. The coefficient of variation calculated for MCV, MCH, MCHC was lower than 10%, expressing a very homogeneous population, in buffaloes. For the RBC, HGB, HTC, WBC, LY, and NE the coefficient of variation was lower than 20%, expressing a homogeneous population. For the MO, the coefficient of variation was 38.39% expressing a heterogeneous population. Results of the urinalysis in lactating dairy cows and buffaloes are shown in Table 3.

Table 3. Urine parameters in lactating dairy cows and buffaloes (twenty heads/group)

Parameters	Group I Cow	Group II Buffalo	Reference limits	References values	
				Kim et al., 2010	Zanetti et al., 2008
Bilirubin, mg/dL	Negative	Negative	Negative	Negative	
Urobilinogen, mg/dL	Normally	Normally	Normally	Negative	
Ketones, mg/dL	Negative	Negative	Negative	Negative	
Glucose, mg/dL	Negative	Negative	Negative	Negative	
Protein, mg/dL	30	30	Negative	Negative	
Blood, Ery/µL	Negative	Negative	Negative	Negative	
pH	7-8	7-8	5-7	7.0-8.4	
Nitrite	Negative	Negative	Negative	-	
Leukocytes, Leu/µL	Negative	Negative	Negative	Negative	
Specific gravity	1.020-1.030	1.010-1.025	1.015-1.025	1.020-1.040	

Urine examination of healthy cows and buffaloes showed that all the parameters were within the normal physiological limits with minor fluctuations for urine proteins (30 mg/dL). The obtained results are in agreement with the obtained results by Kim et al., 2010 and Zanetti et al., 2008. Normally, no bilirubin is detectable in the urine. Values of 0.5-1 mg/dL bilirubin are indicated. Higher values of bilirubin are associated with early liver damage

or disease. Urobilinogen is normally present in the urine in small quantity. Concentrations of > 2 mg/dL are considered to be pathological. Raised levels may be due to cirrhosis, hepatitis, hepatic necrosis and pernicious anaemia. The presence of ketones in urine is called ketonuria. Normally, the urine is free of ketones. Animals in late pregnancy and early post parturition may develop ketosis (pregnancy toxaemia), a severe and sometimes fatal disorder. Ketonuria occurs in diabetes mellitus and starvation, vomiting and diarrhoea may also result in ketosis. Values of 50 mg/dL acetone are indicated. Glucose cannot be detected in the urine although small amounts are secreted by the healthy kidney. The presence of urine glucose is called glucosuria. Pathologic glucosuria is associated with milk fever in cattle. Values of 40 mg/dL glucose are indicated. Protein in urine is most frequently evaluated, which primarily assesses the albumin content. Normally, no protein is detectable in the urine (Radostis et al., 2008). The presence of protein in urine is called proteinuria. Pathological causes of proteinuria include renal disease, urinary tract infections and haematuria. Many diseases can contribute to proteinuria because the inflammatory response can cause glomerulonephritis (Darling et al., 2009). Proteinuria may result from glomerulonephropathy, tubular transport defects, inflammation or infection within the urinary tract. The increased protein level in the urine might be due to acute nephritis or inflammatory exudation resulting from pyelitis, urethritis, cystitis and urolithiasis (Naghy, 2009). Values of 15 mg/dL albumine are indicated. Blood (erythrocytes) in the urine can be associated with haematuria. Values of approximative 5 erythrocytes/µL are indicated.
Urine pH is a measurement of the kidneys ability to conserve hydrogen ions. It is influenced by diet, recent feeding, bacterial infection, metabolic alkalosis, and urinary retention. The urinary pH in normal cattle is usually on the alkaline side and may range from 7.4 to 8.4 (Mavangira et al., 2012). In bovine obstructive urolithiasis, urine is usually alkaline (Sharma et al., 2006). Nitrites are formed by the breakdown of urinary nitrates. The presence of nitrites suggests bacterial infection such as *Escherichia coli*, *Staphylococcus* and *Klebsiella*. Values of 0.05-0.1 mg/dL nitrite are indicated. Urine of healthy animal's do not contain any leucocytes. Leucocytes in the urine are associated with urinary tract infections. Values of 10-20 leuocytes/µL are indicated. Specific gravity is a valuable test for renal disease (Parrah et al., 2013). Urine with a specific gravity outside the range 1.020-1.040 suggests alteration by the renal tubules. High specific gravity (>1.045) is associated with nephrotic syndrome, dehydration, acute glomerulonephritis, heart failure, liver failure. Low specific gravity (<1.005) is associated with diabetes insipidus, nephrogenic diabetes insipidus, acute tubular necrosis, or pyelonephritis (Kraft and Dürr, 2005). Specific gravity in health varies with the level of hydration and fluid intake. The range of specific gravity of urine in normal cattle is between 1.025-1.045 with an average of 1.035 (Kannan and Lawrence, 2010), and in obstructive urolithiasis it ranges from 1.008 to 1.025 (Braun, 2006).

CONCLUSIONS

The obtained data revealed significant differences for most blood parameters ($p<0.01$) studied. Urine examination of lactating dairy cows and buffaloes showed that all the parameters studied were within the normal physiological limits, with minor fluctuations for protein level (30 mg/dl). These differences are likely due to different feeding habit, diet and metabolism. The obtained results could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of dairy herd's. Monitoring the health of farm animal's is useful to the assessment of animal body status health and welfare. The changes in blood and urine constituents can reflect the physiological condition, nutritional and pathological status of dairy herds.

ACKNOWLEDGEMENTS

This study was supported by Research Internal Thematic Plan of the Research and Development Institute for Bovine Balotesti, Project No. 4466/2018.

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