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INFLUENCE OF BLOOD SAMPLE HEMOLYSIS ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN COWS DURING EARLY LACTATION*

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Summary: *The aim of this study is to investigate the influence of hemolysis degrees on the values of hematological and biochemical parameters in the blood samples of cows in early lactation. The study included a total of 45 blood samples of cows in early lactation. Out of the total number of samples analyzed, 15 indicated no signs of hemolysis, whereas 15 indicated moderate hemolysis and 15 severe hemolysis. The influence of hemolysis was examined by determining the bias (%) and by means of the Wilcoxon signed-rank test. The results showed that the hemolyzed samples had decreased values of the HCT, RBC, MCV, and increased values of the MCH, MCHC, and PLT. The total number of LEU showed a declining trend with increasing degrees of hemolysis. The changes in the HGB values were not detected despite the visual identification. The hemolysis degrees showed a significant influence on the values of biochemical parameters. The variations in biochemical parameters were less significant in the samples with moderate hemolysis compared to the samples with severe hemolysis. The most hemolysis-sensitive biochemical parameters were the AST, TBIL, TGC, and NEFA as great variations of their values were found even in moderate hemolysis. A slight deviation was noted in the BHB and total protein values. With respect to all the parameters examined, the Wilcoxon test showed significant differences in the samples with moderate and severe hemolysis in comparison with the control group. In conclusion, hemolysis significantly influenced the values of hematological and biochemical parameters in the blood samples analyzed.*

Key words: *cow, metabolite, hematology, pre-analytical factor, hemolysis.*

INTRODUCTION

The evaluation of metabolic and hematologic status of cows in early lactation is an important part of herd health control. In addition, the estimates of animal welfare on farms can be performed (Đoković et al., 2014; Belić et al., 2015; Cincović et al, 2015; Kovačević et al., 2015). Most parameters are determined by standard laboratory methods and their values can be modified by the influence of numerous pre-analytical factors even in well-controlled environments (Belić et al., 2014). There are numerous factors that can influence the values of hematological and biochemical parameters in blood samples. They can be divided into: pre-analytical, analytical, and post-analytical. In some instances, the standards from human medicine can be utilized in veterinary medicine in order to reduce potential errors (such as the International Council for Standardization in Hematology (ICSH, 1993) or the Clinical Laboratory Standard Institute (CLSI, 2007)). In farming animals such as cattle, pre-analytical errors can be caused by the inadequate preparation of animals, inadequate sampling, and inadequate sample handling. Humann-Ziegank and Ganter (2012) described important pre-analytical factors and corrective measures that should be taken in order to reduce pre-analytical errors. In our previous study, a significant influence of different vacutainers and errors pertaining to their use was found on the values of blood parameters (Belić et al., 2015).

The most common consequence of numerous errors and inflowing procedures in the pre-analytical phase is hemolysis. During hemolysis, hemoglobin is released, which can interfere with other biochemical parameters and cause systemic errors during measurements. Consequently, deviations in the measured parameter values can occur in

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comparison with their real results. This definition is provided by the Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The aim of this study is to determine how different degrees of hemolysis affect the values of hematological and biochemical parameters in early lactation cows.

MATERIAL AND METHODS

The experiment included 45 blood samples taken from cows in early lactation. Out of the total number of samples analyzed, 15 indicated no signs of hemolysis, whereas 15 indicated moderate hemolysis (orange to red colored serum) and 15 severe hemolysis (red colored serum).

The values of hematological parameters (HCT-hematocrit, RBC-red blood cells, HGB-hemoglobin, MCV-mean cellular volume, LEU-leukocytes, MCH-mean cellular hemoglobin, MCHC-mean cellular hemoglobin concentration, PLT-platelets) were determined by standard hematological procedures and by a Hemavet counter.

The following values of biochemical parameters were determined: glucose, NEFA, BHB, TGC, cholesterol, albumin, total protein, AST. Standard biochemical reagents were used (Randox, UK; Pointe Scientific, USA), and photometer Rayto.

The statistical value of bias was determined respective to the relative changes in hematological and biochemical parameter values of the hemolyzed samples compared to the control group samples which indicated no hemolysis. The following formula was used: [(Ch – Cc)/ Cc)] x 100; Cc: the concentration of non-hemolyzed control sample, Ch: the concentration of hemolyzed sample. The Wilcoxon signed-rank test analysis was used for the difference analysis between groups. The Microsoft Excel software (USA) was used for the statistical analysis.

RESULTS AND DISCUSSION

The results show that the hemolyzed samples had decreased hematocrit values (HCT), the number of RBC, and the mean cell volume (MCV), whereas the MCH, MCHC, and platelet count were increased. The total number of leukocytes showed a declining trend according to the degree of hemolysis. It is noteworthy that the hematological equipment did not indicate changes in hemoglobin concentrations despite the visual identification.

Hemolysis exerted significant effects on the biochemical parameter values. However, the variations in the biochemical parameters were less significant in the samples with moderate hemolysis compared to the samples with severe hemolysis, which indicated greater variations. The AST, TBIL, NEFA, and TGC were the most sensitive to hemolysis because they indicated a significant decrease even in the samples with moderate hemolysis. A slight decrease was found in the values of BHB and total proteins. Moreover, it was recorded that the glucose concentrations showed a decrease compared to the control group, but no value change in comparison with the samples with severe hemolysis.

The Wilcoxon signed-rank test showed a statistically significant decrease in the evaluated parameter values of the samples with moderate and severe hemolysis compared to the control group without hemolysis.

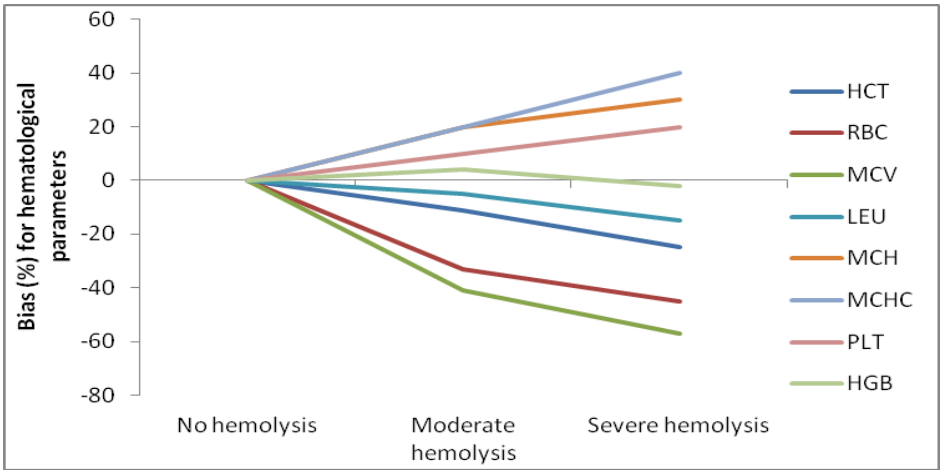

Figure 1. Bias (%) for hematological parameters in the hemolysis function

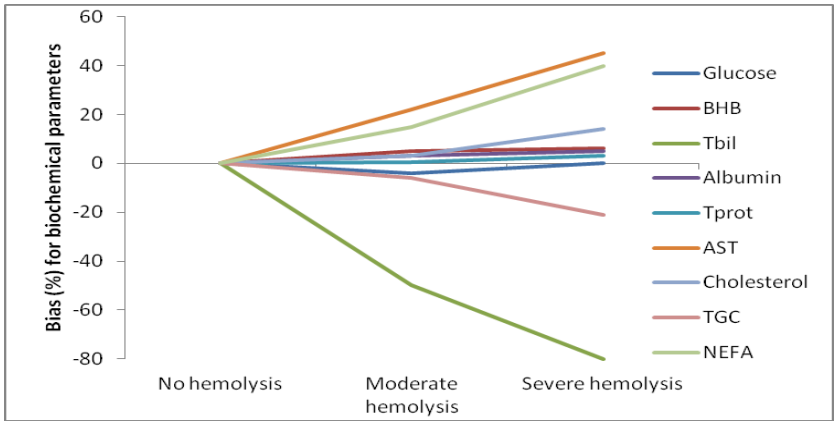

Figure 2. Bias (%) for biochemical parameters in the hemolysis function

Table1. Median value of the hematological and biochemical parameters in the control and experimental group, and the statistical difference between the groups

Parameter (unit)	Control group – no hemolysis (Median)	Moderate hemolysis (Median)	Severe hemolysis (Median)	Statistical difference		
				Moderate vs. control	Severe vs. control	Moderate vs. severe
HCT (%)	26.4	23.5	19.8	p<0.01	p<0.01	p<0.01
RBC ($\times 10^{12}$/L)	6.8	4.6	3.7	p<0.01	p<0.01	p<0.01
MCV (fl)	54.5	32.2	23.4	p<0.01	p<0.01	p<0.01
LEU ($\times 10^{9}$/L)	8.9	8.5	7.6	p<0.1	p<0.1	p<0.1
MCH (pg)	18.3	21.9	23.8	p<0.01	p<0.01	p<0.01
MCHC (g/dL)	33.8	40.6	47.3	p<0.01	p<0.01	p<0.01
PLT ($\times 10^{9}$/L)	355	390	426	p<0.01	p<0.01	p<0.01
HGB (g/dL)	10.1	10.5	9.9	NS	NS	NS
Glucose (mmol/l)	2.8	2.69	2.8	NS	p<0.05	NS
BHB (mmol/l)	0.89	0.93	0.94	p<0.05	p<0.05	NS
Total bilirubin (µmol/l)	7.55	3.78	1.51	p<0.01	p<0.01	p<0.01
Albumin (g/l)	33.6	34.61	35.28	NS	p<0.05	NS
Total protein (g/l)	75.1	75.5	77.3	NS	p<0.05	NS
AST (U/L)	84.5	103.1	122.5	p<0.01	p<0.01	p<0.01
Cholesterol (mmol/l)	2.3	2.4	2.6	p<0.05	p<0.01	p<0.01
TGC (mmol/l)	0.11	0.1	0.09	p<0.01	p<0.01	p<0.01
NEFA (mmol/l)	0.59	0.68	0.83	p<0.01	p<0.01	p<0.01

The results are consistent with earlier results obtained in other animal species and humans (Lippi et al., 2008; Koseoglu et al., 2011; Anđelić et al., 2013; Braun et al., 2015). The changes in the HCT, PCV, and RBC numbers occur because the lysed RBC are not included in the count or measurement of the PCV (the HCT is dependent on the RBC count). Hemolysis results in a higher free hemoglobin result (which gives color to the blood serum) relative to the HCT and RBC count, and the indices related to these measurements, the mean cell hemoglobin (MCH) and mean

cell hemoglobin concentration (MCHC), will be falsely high and may be cancelled. The mean cell volume (MCV) is directly measured by automated analyzers and is unaffected by hemolysis (unless it is a calculated value from the PCV). In hemolysis, collapsed RBCs may be counted by the analyzer erroneously as platelets, falsely increasing the platelet count. Ultimately, because automated analyzers deliberately lyse RBC to measure hemoglobin, the hemoglobin measurement is the same with or without hemolysis. Our previous results (Belić et al., 2015a) showed that the most common error in the vacutainers sent for biochemical analyses was the hemolysis of blood samples. The coagulation of samples was the most common error in the samples sent for hematological analysis. A high percentage of errors were a consequence of insufficiently filled vacutainers. Vacutainer issues are major issues in hematological analyses (Belić et al., 2015a). The sample hemolysis is a significant problem in the colorimetrical evaluation of biochemical blood parameters owing to interference. Interference can be a consequence of the dilution effect of the released content from erythrocytes, as well as a consequence of the chemical interference of the released hemoglobin with biochemical parameters and reagents. In addition, interference occurs during measurements as hemoglobin can absorb waves that are passing through the samples (Guder, 1986; Sonntag 1986; Jay and Proveasek, 1993; Kroll and Elin, 1994; Steen et al, 1996). Moreover, erythrocytes contain many other components that can be released and take part in interference (Guder, 1986).

Although hemolysis is one of the main factors which can affect the values of biochemical blood parameters, there are results indicating that the degrees of hemolysis (evaluated on the concentrations of free hemoglobin) do not correlate with the degree of deviation in the values of some parameters. Therefore, the changes in the concentrations of enzymes and potassium are interpreted by the dilution effect and leaking content form erythrocytes (Lippi et al., 2006). These results are consistent with ours as a significant correlation between the hemoglobin concentration and numerous biochemical parameters was not recorded.

CONCLUSION

Based on the results obtained, it can be concluded that hemolysis exerts significant effects on the values of hematological and biochemical blood parameters in cows. In further research, it is necessary to determine the clinical importance of obtained variations.

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UTICAJ HEMOLIZE UZORKA NA RELATIVNE PROMENE U VREDNOSTI HEMOATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KOD KRAVA U RANOJ LAKTACIJI

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Izvod: Cilj ovog rada bio je da se ispita uticaj hemolize na vrednost hematoloških i biohemijskih parametara u krvi krava u ranoj laktaciji. U ogled je uključeno 45 uzoraka krvi krava u ranoj laktaciji, tako da kod 15 uzoraka nije postojala hemoliza, kod 15 je detektovana umerena hemoliza (narandžasto do crveno prebojavanje seruma), a kod 15 jaka hemoliza (crveno prebojavanje seruma). Uticaj hemolize ispitan je određivanjem bijasa (%) i pomoću Wilxcohon-ovog testa ranga. Rezultati pokazuju da u hemoliziranim uzorcima opada vrednost hematokrita (HCT), broj eritrocita i njihova srednja zapremina, a raste vrednost MCH i MCHC indeksa kao i broj trombocita . Ukupan broj leukocita pokazuje trend opadanja. Koncentracija hemoglobina je bila nepromenjena uprkos različitim vizuelnim pokazateljima hemolize. Variranje vrednosti biohemijskih parametara je značajno slabije ukoliko postoji umerena hemoliza, dok značajnije variranje postoji kod izrazito jake hemolize. Od biohemijskih parametara najosetljiviji na hemolizu su AST, TBIL, TGC i NEFA jer imaju veliko odstupanje u vrednosti čak i u umerenoj hemolizi. Izuzetno malo odstupanje nađeno je za vrednost BHB, glukoze i ukupnih proteina. Wilcoxon-ov test ranga pokazuje da su dobijena odstupanja vrednosti kod hemolize statistički značajna. Na osnovu dobijenih rezultata zaključujemo da hemoliza ima značajan uticaj na vrednost hematoloških i biohemijskih parametara u krvi krava.

***Ključne reči*:** *krave, metaboliti, hematologija, preanalitički faktori, hemoliza.*

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