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Effect of Anticoagulants on Some Biochemical Indicators of Cow Blood

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Abstract

This study presents the effect that some anticoagulants have on some biochemical indicators of the blood of cows. Blood samples for the purposes of this study are taken in the area of Elbasan, in the administrative unit of Paper. Approximately 20 ml of blood was taken from each of the cows, which was stored as follows: (i) 5ml stored in test tube without any anticoagulant substances, (ii) 5ml stored in test tube containing lithium heparin, (iii) 5ml stored in test tube containing K2EDTA, and (iii) 5 ml stored in test tube containing Na Citrate. The values obtained from measurements taken with serum have served as reference values in order to compare the figures obtained from samples mixed with other anticoagulants. The metabolites measured are bilirubin total, proteins total, creatinine, glucose, ALT, AST, GGT, potassium, chlorine and calcium. The evaluation of metabolic indicators is achieved using the semiautomatic Biochemical Analyzer EMP-168, through commercial kits "Human".

The results have shown that the values of metabolites, enzymes, and macro-microelements measured in the plasma containing heparin are comparable to the values obtained from measurements with serum. Meanwhile, values of plasma metabolite stored with EDTA have shown a significant difference in elements as urea, protein total, and creatinine ($p<0.01$). Furthermore, a higher concentration of potassium was observed in samples stored with EDTA ($p<0.001$), while calcium demonstrated a significant decrease for samples stored with EDTA, and Na Citrates ($p<0.001$). Values of plasma metabolites stored with citrate have stated a significant reduction ($p>0.001$) for all the assessed metabolites, compared such with their values measured in serum.

In conclusion, based on the results obtained by this study and also other authors' findings, it is important to state that, for routine biochemical analysis of cow blood, in addition to those in serum, the most reliable references are the samples when blood is stored with anticoagulant elements as heparin.

Keywords: Biochemistry, EDTA, Lithium heparin, Citrate Na, Cows

1. Introduction

The evaluation of blood biochemical indicators is obtained by direct measurement with serum or plasma. Even though the methods of serum are considered the most preferred for biochemical analysis, the plasma stored with suitable anticoagulants may lead to exact benefits under certain conditions [18]. Furthermore, taking into consideration that the process of separating the serum takes a long time to achieve the clotting stage before centrifugation and the overall volume of the serum taken from a certain volume of blood is smaller, it makes the plasma methodology the most recommended rather than serum for a group of certain metabolites [26]

Routine blood tests, both hematological and biochemical require a high accuracy and precision. Therefore, there are developed and recommended different methodologies and protocols to enable the most accurate results. In order to avoid further errors in obtaining the results, all pre-analytical and post-analytical factors that might affect these assessments should be well known [4, 20]. More in regard, the para-analytical factors constitute the main group with around 46-68% of the total factors that influence such analysis. In this group are also included the inaccurate identification of the sample, sample collection accuracy (hemolysis, clotting, insufficient volume,

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etc.), the type of anticoagulant used for the analysis, unsuitable storage containers, storage during transport, etc. Another important factor is considered the type of the anticoagulant used for the analysis [24]. Having said this, the anticoagulants depending on their type, have specific effects in the blood chemistry. Anticoagulants are additives that inhibit blood clotting, ensuring so that the concentration of the substance measured to have as little difference as possible [10]. They should be selected based on the type of indicator to be examined and, in all cases, should not affect the integrity of the sample. Not all anticoagulants can be used to measure the serum or plasma metabolites, since some of them can cause alterations in the results [4]. The effects of different types of anticoagulants in the animal blood chemistry are stated by many authors [5, 16, 17, 18, 11, 9, 15]. In our country, it has not come to our attention any kind of these conclusions, which is the main reason to undertake this study, in order to conclude how different anticoagulants, affect some biochemical indicators in cow blood.

2. Material and Methods

The study included 12 clinically healthy cows of farms in the district of Elbasan. The determination of health conditions is made through anamnesis of the owners and measurements of parameters such as: pulse, body temperature and respiratory rate. The average age of the cows was 6.7 years. The ones selected for the study were not pregnant or newborns. The blood samples were collected in the jugular vein with the most suitable syringe available with a needle of 18G. From each cow, 20 ml of blood was taken and stored as follows: (i) 5 ml in test tubes without any anticoagulants, (ii) 5ml in test tubes with anticoagulants as lithium heparin 100 UI, (iii) 5 ml in test tubes with anticoagulants as K2 EDTA where 0,1 ml of 10% K2EDTA was measured, and (iv) 5 ml in test tubes with anticoagulants as Na citrates 3.8%. The anticoagulant test tubes belonged to the brand FL- medical Italy. Blood samples were stored in refrigerated containers to the Laboratory of Diagnosis, at the Faculty of Veterinary Medicine in Tirana. Prior to centrifugation, the samples were observed in order to avoid any hemolyzed samples, and the hemolyzed ones were removed from the study. The centrifugation was performed with the Heraeus Labofuge 400 centrifuge with 1500 rotations per 15 minutes. The plasma and serum were separated in the respective containers and the indicators were measured under appropriate methodologies. The biochemical parameters included in the study are: (i) Total Bilirubin, Glucose, Urea, Total Protein, Creatinine, ALT, AST, GGT, Chlorine, Calcium and Potassium. The measurements were performed with a semi-automatic Biochemical Analyzer EMP-168. The preparation of the reagents was achieved in the same period of time that their measurement was performed. In order to calibrate the device, a quality control sample was used, and specific standards were used to adjust the parameters of each measured indicator. The measurements are conducted with the following methodologies: (i) Bilirubin total with the DCA-photometric-colorimetric methodology; (ii) Urea with the enzymatic-colorimetric methodology, (iii) Creatinine with the Jaffe-Reaction, methodology (iv) Protein total with the Biruet methodology (photometric-colorimetric), (v) Glucose with the GOD-PAP methodology (enzymatic-colorimetric without deproteinization), (vi) AST (aspartate transaminase), ALT (alanine transaminase), GGT (gamma glutamate transferase) with the IFC (kinetic) methodology, (vii) Chlorine (Cl) with the TPTZ (photometric-colorimetric methodology), (viii) Calcium (Ca) with the OPC (photometric) methodology, (ix) Potassium with the enzymatic methodology (Fix-time). Calculations and statistical analysis are processed using R Studio, as (i) the average ± δ, (ii) the fluctuation limits, (iii) the veracity change of two means (tD). Results are statistically evaluated using t-test, taking as significant the $P<0.05$.

3. Results and Discussion

In table 1 are presented the mean values and standard deviations of the values for each of the metabolites according to the anticoagulants used in the study. The values of metabolites in serum are considered as comparable values to their plasma values containing heparin, K_2EDTA and Citrate Na.

Heparin is used in the form of the salt of sodium, potassium, lithium, ammonium and simple heparin. Moreover, heparin is considered as the most universal anticoagulant and is used widely in the evaluation of biochemical indicators of blood [4]. Meanwhile, the results obtained from the measurements of metabolites in serum and plasma containing lithium heparin did not show any significant diversity in terms of biochemical indicators.

Table 1. The values of measured metabolits in serum and various types of cows blood plasmas

	Serum	Heparinized plasma	EDTA plasma	Citrated plasma
Total bilirubin (mg/dl)	0.33 ± 0.11	0.39± 0.11	0.36±0.11	0.22 ± 0.08*
Urea (mg/dl)	22.1 ± 1.96	20.2 ± 1.8	19.71±1.8*	18.0 ± 2.02***
Creatinine (mg/dl)	1.25 ± 0.21	1.1 ± 0.21	1.02±0.20*	0.99 ± 0.22**
Total protein (mg/dl)	6.46 ± 0.57	6.34 ± 0.54	5.78 ± 0.5**	5.38 ± 0.66**
Glucose (g/dl)	62.0 ± 6.8	60.15 ± 6.8	64.3 ± 5.7	51.4 ± 6.7***

* p<0.05; ** p< 0.01; ***p< 0.001

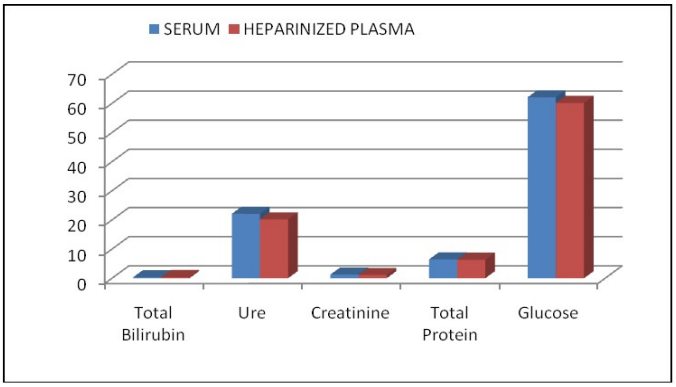
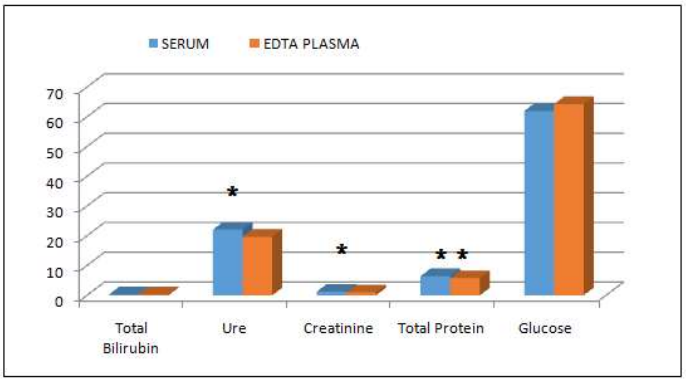


Figure 1. Comparison of metabolites values measured in serum and heparinized plasma.

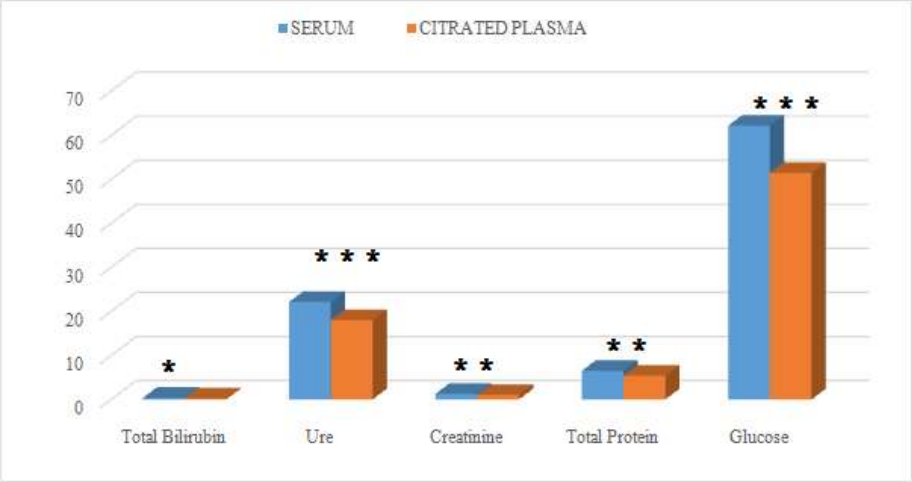
The authors Belić [2] and Braunstein [3] have published the same results in ruminants where, the values of metabolites in serum and plasma containing lithium heparin did not state any significant differences. Also, studies in cats and dogs have shown that heparin does not cause any alteration of biochemical indicators [5, 18, 11]. Lithium heparin is considered as the most preferred anticoagulant in biochemical studies since it delivers similar studies to those taken in serum [4, 11, 16]. Even though it is the most similar, there are contradictory views related to heparin have shown studies held in cows and sheep [17, 18]. According to those, heparin delivers some alteration in some indicators as urea, creatinine, protein total and bilirubin total, without knowing the cause of these alterations. The authors Stokol et al [22] and Ceron et al have shown that heparin causes an artificial increase of albumins, explaining this fact with the combination of heparin with fibrinogen, or perhaps the methodology used to evaluate the albumin (bromocresol green assay, BCG) and other unknown factors. The anticoagulant EDTA, in all its forms is considered the most suitable for hematological studies given its ability to save the integrity of blood cells. There are some controversy discussions in the studies of biochemical indicators where some of the researchers have also considered it suitable for the biochemical indicators. In the study held by us, the average values of metabolites in plasma with K2EDTA (table 1; figure 2) have shown that bilirubin total and glucose did not show any alterations in their values measured in serum, meanwhile urea and creatinine have shown slight alterations (p<0.05). The most differences (p<0.01) were evaluated in protein total in plasma containing K2EDTA, compared to its values measured in serum.



* p<0.05; ** p< 0.01;

Figure 2. Comparison of metabolites values measured in serum and EDTA plasma.

Synchronous to our results where the effect of K2EDTA anticoagulant in values of bilirubin total does not have any significant affect, have also been published by the authors Mohri [17] and Belić [2]. Results close to the values obtained by our study have been also published for studies undertaken in sheep [18] and cats [11]. Meanwhile the authors, Mohammadi [15] and Ceron [5] in studies undertaken in buffalos and dogs have shown that the K2EDTA does not affect the values of bilirubin total, and overall, other metabolites have stated values comparable to those in serum, with the exception, of its influence in values of albumin, fructose-amine and bile acids, not knowing though the exact mechanism that causes these differences [17]. The sodium citrate is not used frequently in clinical biochemistry, but it is a standard anticoagulant in tests of coagulations, function of platelet, and assessment of erythrocyte sedimentation. The results obtained from the measurements of metabolites in plasma stored with Na citrate (table 1, figure 3) have shown a significant difference in the measured metabolites, where the most reduction compared to their values in serum, are introduced in urea and glucose ($p<0.01$). Meanwhile, the values of the total bilirubin stored with Na citrate have undergone mild changes ($p<0.05$) compared to the values measured in serum.

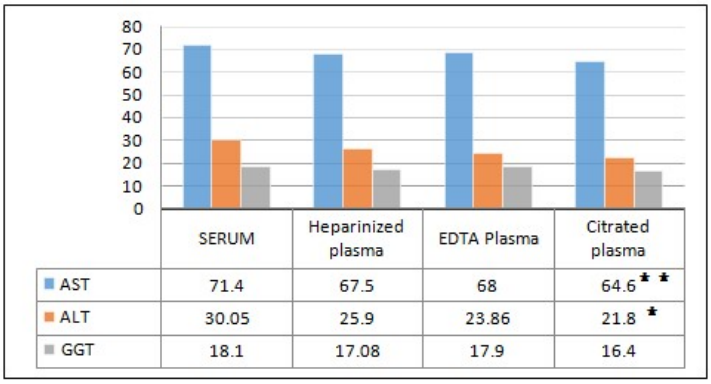


* p<0.05; ** p< 0.01; ***p< 0.001

Figure 3. Comparison of values of metabolites measured in serum and citrated plasma

In figure 4 are presented the values of enzymes (AST, ALT and GGT) obtained by measurements in serum, plasma stored with heparin, EDTA and Na citrates. The values of GGT did not suffer any alterations compared to the values of GGT measured in serum. Results comparable with the values obtained in terms of GGT in our study, are also supported and published by various authors in other studies undertaken in dogs, cats, horses, etc. [5, 11, 2, 16, 18].

The values of AST and ALT stored in heparinized plasma and EDTA plasma did not show any significant alterations, while in citrated plasma were significantly lower than their values obtained by the measurements in serum ($p < 0.01$, $p < 0.05$). Regarding the values of AST, Mohammadi et al [15] support the data of our study, while other authors show that AST decreases significantly in both citrated and EDTA plasmas [16, 2, 17, 18], linking such with the effect that gelatin plays in anticoagulants as EDTA, and high degree of dilatation (1:9) of blood with liquid citrate.

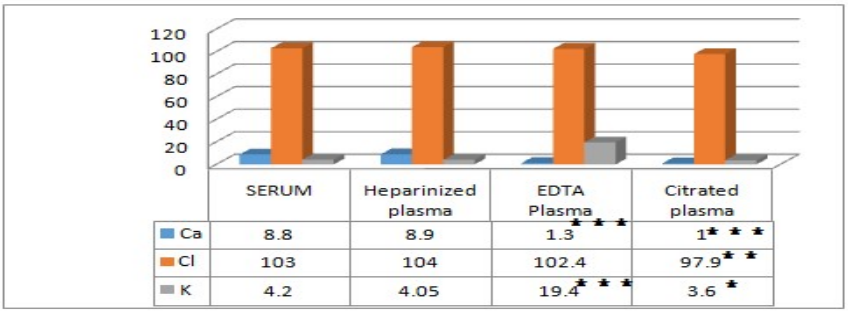


	SERUM	Heparinized plasma	EDTA Plasma	Citrated plasma
AST	71.4	67.5	68	64.6**
ALT	30.05	25.9	23.86	21.8*
GGT	18.1	17.08	17.9	16.4

Figure 4. Comparison of enzyme values measured in serum and heparinized, EDTA and citrated plasma.

The fact of reduction of values of ALT in EDTA plasma, heparinized plasma and citrated plasma is also supported in the study undertaken in horses [16], while as per the case of buffalos, the ALT is decreased in both citrated and EDTA plasmas [15]. Compared to our study, the values of ALT seem to decrease only in citrated plasma. Contrary to the results, studies undertaken in Struthio camelus and dogs have not found any changes as per AST and ALT values in both EDTA and citrated plasma [14,5].

Results obtained in our study (figure 5) testify that the values of Ca and Cl measured in plasma stored with heparin are comparable to their values measured in serum, and consistent to results presented by different authors [5, 2, 11].



	SERUM	Heparinized plasma	EDTA Plasma	Citrated plasma
Ca	8.8	8.9	1.3***	1***
Cl	103	104	102.4	97.9**
K	4.2	4.05	19.4***	3.6*

Figure 5. Comparison of CA, CL, K values measured in serum and heparinized, EDTA and citrated plasma.

Significant reductions ($p < 0.001$) were experienced in values of Ca in plasma stored with EDTA and Na citrate, supported also by publications of numerous authors for different animals [2, 11, 14, 5, 16]. The decrease of Ca in plasma stored with EDTA and Na citrates is related to the ability of the anticoagulants to forms stable complexes with calcium ions, making Ca an undetectable element in samples stored with these anticoagulants [11, 8, l6]. Kerr MG [12] in his book cites that calcium in samples taken with the

anticoagulants EDTA, citrates and oxalates is undetectable and can lead to false results as well as misinterpret and misdiagnose the health of animals and humans. The attentions of clinicians and laboratory staff should be driven to not take blood sample with any of the aforementioned coagulants for the assessment of Calcium. The chlorine found in plasma stored with EDTA did not show any significant differences compared to its values measured in serum, whereas the plasma stored Na citrate, the chlorine values are lower than those measured in serum ($p<0.01$). Other similar findings are published also by Ceron et al [5] and Kamali et al [11], considering so the lithium heparin and EDTA as trusted anticoagulants for the chlorine measurements. The Potassium values in heparinized plasma have shown a slight decrease compared to values measured in serum. The values of Potassium measured in serum are higher, and according to Kerr [12] and Steven [21] this may be related to the fact that, formation of blood clots to obtain the serum is accompanied with the release of potassium and other blood elements and increase in terms of extracellular environment. Therefore, the most preferred methodology is measuring the potassium in plasma rather than serum [12]. In cases when blood stays for a long time in test tubes, the release of potassium from the blood cells occurs and consequently there is a false increase of potassium levels in plasma [23, 8]. In order to avoid this false increase of potassium, researchers suggest separating the cells from the serum and plasm at least 8 hours form sampling [12].

In blood samples where plasma is stored with EDTA, potassium has shown to be many times higher (figure 4) than its values measured in serum ($p<0.001$), while in blood samples stored with Na citrates, the values have resulted lower that those measured in serum ($p<0.05$). The expectation of these results is related to the ability of K2-K3 EDTA to increase (false) in the concentrations of potassium and to lower the concentration of Ca, Mg and Zn in plasma [8, 6, 20,7]. It should be noted that the EDTA contained in the test tubes is potassium salt so the samples collected with this anticoagulant will give an absurdly high K reading [12]. According to the concrete data and sources of the authors, the most suitable anticoagulant for measuring potassium is recommended heparin.

4. Conclusions

Heparinized plasma is more suitable for the measurements of biochemical metabolites, micro and macro elements and bile enzymes. Plasma metabolite values containing EDTA showed significant changes for urea, protein total, and creatinine, however EDTA is considered appropriate for the measurement of total bilirubin, glucose, ALT, AST, and GGT. Sodium citrate causes high alteration in terms of values of metabolites, ALT, AST, but has no effect in the values of GGT. The values of chlorine remained the same in the EDTA and heparinized plasma suffer a decrease in citrated plasma. The values of Ca in EDTA and citrated plasma have marked a significant decrease in values of Ca measured in serum and in the heparinized plasma. The false decrease of such values is related to the effect that EDTA and citrates build calcium complexes, making it undetectable in plasma. Sodium citrate and EDTA are unsuitable for Ca measurements. Measurement of Ca is most convenient to be undertaken in serum, in the impossibility to be taken with plasma stored with heparin. The values of potassium in the EDTA plasma had a significant increase and decrease in citrated plasma. More in regard, the lithium heparin is considered the most suitable anticoagulant for measuring the potassium values.

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