

Effect of Centrifugation Speed and Blood Tubes with Different Contents on Certain Serum/Plasma Biochemical Parameters in Cocks

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Article Info	ABSTRACT
<p>Received: 03.07.2024 Accepted: 08.11.2024 Online first: Published:</p> <p>Keywords: Centrifugation speed, EDTA, Lithium heparin, Plasma, Serum.</p>	<p>This study was carried out to investigate the effect of blood tubes with different anticoagulants and different spinning speed applications in the blood centrifuge on biochemical analyses. The study conducted with a total of 16 Gerze cocks, a native breed of Türkiye. In the first part of the study, 8 roosters were slaughtered and the blood taken was transferred to lithium heparinized tubes only. Then, the blood was centrifuged at 3000, 4000 and 5000 <i>rpm</i> for 10 minutes. Blood samples were taken into plain-tubes, K₃EDTA and lithium heparin tubes, and centrifuged at 3000 <i>rpm</i> for 10 minutes. The study results indicated that, centrifugation speed had no statistically significant effect on the levels of plasma glucose ($p = 0,691$), triglyceride ($p = 0,451$), total cholesterol ($p = 0,993$), high-density lipoprotein [(HDL-cholesterol) ($p = 0,917$)], low-density lipoprotein [(LDL-cholesterol) ($p = 0,990$)], total protein ($p = 0,860$), inorganic phosphorus ($p = 0,885$), calcium ($p = 0,980$), amount of plasma ($p = 0,903$) and, aspartate aminotransferase activities [(AST) ($p = 0,692$)]. In the second part of the study 8 different cocks apart from first part of the study, were slaughtered and the blood taken into tubes with different contents as plain tubes (serum), lithium heparinized tubes (plasma) and K₃EDTA tubes (plasma). Same parameters with first part of the study were investigated. The results showed that no statistical difference found between serum or plasma obtained from blood tubes with different contents ($p > 0.05$). Additionally, the calcium levels could not be analyzed with K₃EDTA plasma. Consequently, we observed that centrifugation speeds up to 5000 <i>rpm</i> did not affect the parameters frequently investigated in scientific research and routine analysis in poultry. Similarly, in the tube content experiment, no statistical difference was found in other parameters.</p>

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INTRODUCTION

Blood plasma tests are widely used in the laboratory to diagnose of various diseases, and monitor treatments. Plasma is isolated from whole blood by using centrifugation (Anderson & Anderson, 2003). The purpose of centrifugation is to remove blood cells without changing the plasma composition. Cadamuro et al., (2018) reported that centrifugation speed, centrifugation time, and temperature were found to affect sample quality. Prolonged centrifugation at high speed can lead to hemolysis or structural damage to the measured sample, while short centrifugations at low speed can lead to insufficient separation of plasma or serum from cellular blood components (Lippi et al., 2008).

Anticoagulants are additives that prevent blood or plasma from clotting and it has been reported that this substances don't cause a significant difference in analytical process (Guder, 2010). Anticoagulation is achieved by binding calcium ions (ethylenediaminetetraacetic acid [EDTA]) or inhibiting thrombin (heparin). It has been known that the most preferred anticoagulant in chemical analyzes is heparin. Conversely, EDTA is especially useful for hematology. Laboratory tests may also require biochemical analyses other than hemograms. Therefore, analysis should be performed with anticoagulants for plasma parameters (Mohri et al., 2007). As anticoagulants, except for EDTA, heparins (low molecular weight and standard), warfarin, and recently developed oral anticoagulants are also used (Işık & Kozak, 2024).

Heparin is a substance that prevents the conversion of fibrinogen to fibrin (Baïen et al., 2018), and used to exminate presence of electrolyte, gas, and, alcohol in blood. However, due to acidic structure of heparin, it is not recommended for use in morphologic analyzes such as white cell and platelet counts, and polymerase chain reaction (PCR) diagnosis. EDTA is recommended as an anticoagulant in hematologic tests because it best preserves cellular components and blood cell morphology (Vertiprakhov et al., 2021).

It has been reported that blood samples for coagulation studies in birds should be collected in plastic or silicone tubes containing 3.8% sodium citrate. The principle is based on inhibiting clotting in the blood sample by binding calcium with sodium citrate. (Kaneko et al., 2008).

There is sufficient information in the literature about the effect of different types of anticoagulants on blood parameters in humans (Li et al., 2013) or animals (Rezaei et al., 2022), but there is limited information about the use of tubes for analyzing bird blood samples (Guzman et al., 2008). Keskin (2020) reported that he separated the serum by applying the centrifugation speed at 3.000 *rpm* for 5 minutes in his study in rats. Kara et al., (2024) used K₂EDTA as a coagulant in their study with Kangal Dogs for hematological analyses. It is known that, unlike mammals, mature erythrocytes in birds have nuclei and are larger than mammalian erythrocytes (Jones, 2015). In this context, in order to obtain accurate analysis results, it is essential to select the right tubes during blood sample collection. Therefore, the present study was aimed to compare the effect of different blood tubes and increasing centrifugate speeds on blood parameters in poultry.

MATERIAL and METHOD

Animals and Keeping

In the present study, 16 Gerze breed cocks at the age of 35 weeks were bred at Bahri Dağdaş International Agricultural Research Institute were used.

The decision of the ethics committee of the study was obtained from the Bahri Dağdaş

International Agricultural Research Institute Animal Experiments Local Ethics Committee, with the decision dated 31.03.2023 and numbered 155. The animals were kept in a cage system measuring in 4 m width × 3 m length × 1.5 m height, and 80 cm from the ground for 6 weeks. The cocks were fed *ad libitum* with the ration given in Table 1 and had uninterrupted access to water with a nipple system.

Table 1. Ration content given to cocks

Raw materials	%	Nutrients	Diet
Wheat	27.5	DM, %	90
Corn	32.5	CP,%	22
Sunflower oil	1.2	ME,kcal/kg	2800
Soybean meal, %48	30	Ca,%	1
Marble powder	1.35	Ash %	0.43
Dicalcium phosphate	1.6	Na,%	0.2
Salt	0.35	Cl	0.26
Vitamin-mineral mixture	0.25	Met+Sis,%	0.75
		Lysine,%	1.12
		Threonine,%	0.81
		Tryptophan,%	0.3

Tube Content

In this part of the study, it was investigated the effects of blood tubes with different contents on biochemical parameters. For this, 8 cocks were slaughtered. Only the left *vena jugularis* was cut with a sterile lancet to avoid mixing with arterial blood. In this study, 6 ml capacity tubes with plain-tubes, 6 ml capacity Lithium Heparin tubes, and 6 ml capacity K₃EDTA tubes were used. Approximately 3 ml of blood was taken into each tube. The obtained blood was rapidly centrifuged at 3000 *rpm* for 10 minutes, and, the obtained serums and plasmas were sent for biochemical analysis rapidly.

Centrifugation Speed

In this part of the study, 8 cocks, which were different from the first part, were slaughtered. Only the left *vena jugularis* was cut with a sterile lancet to avoid mixing arterial blood, and blood was taken from each animal into 6 ml lithium-heparinized tubes only, to not exceed 3 ml. The excess blood in the tubes was then removed with an Ependorf pipette. Thus, a net of 3 ml of blood was obtained in each tube. To investigate the effect of centrifugation speeds on the samples obtained, plasmas were obtained by centrifugation at 3000 *rpm*, 4000 *rpm*, and 5000 *rpm* for 10 minutes. Then the plasmas were collected with an insulin syringe, and the amount of plasma was determined and sent for biochemical analysis rapidly.

Blood Analysis

Glucose, triglyceride, total cholesterol, HDL- cholesterol, LDL- cholesterol, total protein, inorganic phosphorus, calcium levels and, ALT (alanine aminotransferase), AST (aspartate aminotransferase) activities were analyzed in the serum and plasma samples. In the study, attention was paid to the presence of carbohydrate, lipid, protein, lipoprotein, enzymes, and mineral substances in the parameter set, which is commonly investigated in poultry studies. Analyses were performed with an auto analyzer. (Abbott-Architect C8000- USA).

Statistical Analysis

The SPSS 25.0 package program was used for statistical analysis. The distribution of the data set was analyzed by Shapiro-Wilk and it was determined that the data set was not normally distributed. For this reason, a nonparametric Kruskal-Wallis test was applied to determine the differences. The pairwise comparisons of groups were made using Mann-Whitney U test with Bonferonni correction.

RESULTS

In the effects of tube contents on biochemical parameters, no statistical difference was observed in the parameters value ($p>0.05$). Although there was no statistically significant difference in other parameters; the total protein value was found to be lowest in the plain-tube; glucose, HDL-cholesterol, inorganic phosphorus levels, and AST activities were found to be lowest in the K₃EDTA tube; triglyceride, total cholesterol, and LDL- cholesterol levels were found to be lowest in the lithium heparin tube (Table 2).

Table 2. Effect of tube content on biochemical parameters (n=8)

	Plain Tube (Serum)	K ₃ EDTA (Plasma)	Lithium Heparin (Plasma)	p value
Total Protein (mg/dl)	44.47 ± 1.28	47.08 ± 1.42	47.32 ± 1.58	0,267
Glucose (mg/dl)	275.25 ± 10.19	273.50 ± 10.74	280.75 ± 10.66	0,696
Triglyceride (mg/dl)	48.62 ± 8.53	57.37 ± 8.58	40.75 ± 5.40	0,177
Total Cholesterol (mg/dl)	99.37 ± 4.19	96.12 ± 4.20	94.25 ± 4.60	0,357
LDL-cholesterol (mg/dl)	203.12 ± 31.08	177.12 ± 29.94	174.01 ± 26.24	0,727
HDL-cholesterol (mg/dl)	69.33 ± 2.80	66.93 ± 2.65	68.70 ± 2.83	0,605
Inorganic phosphorus (mg/dl)	3.85 ± 0.24	3.75 ± 0.23	3.78 ± 0.26	0,75
Calcium (mg/dl)	10.63 ± 0.14	< 2	11.07 ± 0.19	0,792
AST (U/L)	240.50 ± 8.66	219.12 ± 7.61	235.75 ± 11	0,226
ALT(U/L)	< 6	< 6	< 6	-

Although there was no statistically significant result on the effect of centrifugation speed on biochemical parameters; the total protein value was found to be the lowest at 4.000 *rpm*; with glucose, total cholesterol, LDL-cholesterol, inorganic phosphorus, and calcium values at 3.000 *rpm*, and triglyceride, HDL-cholesterol, and AST activities at 5.000 *rpm* (Table 3).

Table 3. Effect of centrifugation speed on biochemical parameters (n=8)

	3000 rpm	4000 rpm	5000 rpm	p value
Total Protein (mg/dl)	47.0 ± 1.52	46.22 ± 1.38	46.52 ± 1.29	0,86
Glucose (mg/dl)	246.5 ± 6.18	250.62 ± 5.51	252.0 ± 7.36	0,691
Triglycerid (mg/dl)	34.0 ± 4.15	32.12 ± 4.66	31.25 ± 4.81	0,451
Total cholesterol (mg/dl)	93.50 ± 3.79	93.87 ± 3.80	94.12 ± 3.03	0,993
LDL-cholesterol (mg/dl)	197.0 ± 27.08	205.37 ± 27.10	214.25 ± 30.88	0,99
HDL-cholesterol (mg/dl)	67.0 ± 3.41	66.91 ± 3.49	66.45 ± 3.34	0,917
Inorganic phosphorus (mg/dl)	3.32 ± 0.2	3.41 ± 0.19	3.41 ± 0.2	0,885
Calcium (mg/dl)	10.36 ± 0.13	10.65 ± 0.06	10.75 ± 0.12	0,98
AST (U/L)	245.0 ± 14.12	237.12 ± 12.77	229.37 ± 10.93	0,692
ALT (U/L)	< 6	< 6	< 6	-
Amount of Plasma (ml ⁻²)	126.62 ± 5.73	128.75 ± 6.55	129.50 ± 7.10	0,903
Visible Hemolysis	-	-	-	

DISCUSSION

Ethylenediaminetetraacetic acid (EDTA) is an organic chemical compound used in biochemical analysis, especially in whole blood counts, which prevents clotting by binding calcium ions in plasma. EDTA also has the function of chelating metallic ions. EDTA is the preferred anticoagulant because it does not change erythrocyte morphology, so ideal for use in hematology (Riba et al., 2020). Calcium and phosphorus levels in poultry are very important parameters in fertility, egg production, and shell quality. In the present study, centrifugation speed did not affect calcium, and phosphorus levels. However, it was observed that K₃EDTA binds calcium, and the calcium analysis could not be performed (Table 2). Therefore, tubes that contain EDTA should not be preferred for the measurement of cations such as calcium, zinc, iron, and copper which have +2 valence in reactions. In the current study, ALT activities could not be measured by the biochemistry autoanalyzer, which is used in routine analysis, in different centrifugation speed trials, both in tubes with different contents. For this reason, ELISA or RIA methods which, are more sensitive, can be used for ALT analyzes. Additionally, ALT analysis can also be performed by spectrophotometric method which is more economic.

For glucose, total protein, total cholesterol, HDL-cholesterol, inorganic phosphorus levels, and ALT activities were analyzed in different tube contents (Table 2), it was observed that the levels were very close to each other, and it can be said that these measurements were not affected by the tube content. However, when triglyceride and LDL-cholesterol levels were examined, some numerical differences were observed, although there was no statistical difference. It is thought that the numerical difference between EDTA-tube and lithium heparinized-tube measurements in triglyceride levels may be important for scientific research. Also, in LDL-cholesterol levels, although there was no statistical difference between plain-tube and Lithium-Heparin tube measurements, it was thought that the numerical difference may affect scientific research.

In the present study, it was investigated whether blood tubes and centrifugation speed have an effect on routine analyses commonly used in scientific studies in poultry. It is known that mature erythrocytes in birds, unlike mammals, have nuclei and are larger than mammalian erythrocytes (Jones, 2015). The disintegration of erythrocytes and the spread of hemoglobin content outside the cell is called hemolysis (Türkmen et al., 2007). In the present study, it was thought that hemolysis might increase with increasing centrifugation speed, but according to the results, visible hemolysis was not observed at increasing centrifugation speeds ($p>0.05$). Except for the biochemical parameters, the increase in centrifugation speed did not affect the amount of plasma.

CONCLUSION

As a result, it was observed that the parameters frequently investigated in scientific research and routine analysis in poultry were not affected by centrifugation speeds up to 5000 *rpm* (Table 3). Similarly, except for calcium, no effect of tube content on other parameters was observed. The study's findings suggest that centrifugation speeds more than 3000 *rpm* are unnecessary. Blood tubes containing lithium heparin are approximately three times more expensive than plain-tubes. The using of plain-tubes in routine biochemical assays would be more cost-effective.

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Ethical Statement

Since retrospective data were used in this study, there was no need to obtain ethical permission.

Author Contributions

Research Design: Halil HARMAN (%40) Abdullah BİLİR (%30), Halil YAVUZ (%30)

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Conflict of Interest

There is no conflict of interest between the authors.

Sustainable Development Goals (SDG)

9 Industry, Innovation and Infrastructure

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