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# Blood plasma biochemistry of broiler birds at slaughter age: Effect of anticoagulants and comparison with serum

**SN Abedin, V Leela, NK Roy and V Ralte**

**Abstract**
The present study was conducted on 20 broiler birds that were slaughtered at 42 days of age and blood sample was collected in clot activator, Sodium Heparin and K3EDTA tubes to study blood biochemistry viz., estimation of albumin, triglycerides, creatinine, glucose, total protein, urea, chloride and inorganic phosphorous. Sodium Heparin recorded significantly ($p$<0.05) higher values for Albumin than K3EDTA.Triglyceride and Glucose showed significant ($p$<0.01) decrease in its levels in Sodium Heparin when compared to serum. Triglyceride level was highest in K3EDTA as compared to other anticoagulants. On the other hand, Glucose level was lowest in K3EDTA. Inorganic Phosphorous levels showed significant ($p$<0.05) increase in serum when compared against both the anticoagulants. Urea, total protein, creatinine and chloride levels were non-significant among the groups. In conclusion, heparinized plasma could be useful and preferable over K3EDTA for the measurement of biochemical parameters of the blood plasma of poultry birds viz., Albumin and Chloride. Phosphorous estimation being doubtful in heparinized plasma. K3EDTA causes little changes in the triglyceride levels. Glucose and total protein values seem to be less erroneous if measured in serum.

**Keywords:** Serum, plasma biochemistry, sodium heparin, broiler, EDTA

## Introduction
Anticoagulants are the additives that inhibit the clotting of blood or plasma, thereby ensuring that the concentration of the substance to be measured changes as little as possible before the chemical analysis and estimation processes (Guder, 2001) [1]. Anticoagulation can be achieved either by inhibition of thrombin (heparin) or by limiting thrombin production, or both.

Serum separated from coagulated blood is the preferred specimen for clinical chemistry analysis of different blood parameters. But plasma obtained in an appropriate anticoagulant tube may act as an equally valid specimen and in certain conditions preferable to the serum counterpart. In addition, studies demonstrate that whole blood obtained on appropriate anticoagulant is the sample of choice for measurement of some trace elements such as ammonia, blood pH and blood gas estimation (Young and Bermes, 1999) [2]. In recent years, Sodium/Lithium Heparin has been widely used as an anticoagulant for clinical chemistry analysis. On the other hand, EDTA is particularly useful for hematological examination. In emergency situations, analysis of the plasma expedites serum because harvesting of serum requires 15–30 minutes for the completion of coagulation process. Furthermore, the yield of plasma from a given volume of whole blood is always greater than that of serum (Young and Bermes, 1999) [2]. If the serum depot runs out, it is better to obtain another sample for serum harvesting, which is practically not possible for all patients especially poultry birds. Thus analysis must be performed on plasma anticoagulated with various types of anticoagulants, EDTA being the most commonly used anticoagulant. In the past, serum analyte measurement and stability were a major concern because serum was preferred in most laboratories for clinical chemistry. However, in recent years many laboratories are switching to plasma because serum has several inherent problems: First, the time necessary for clot formation increases the turnaround time and secondly, the risk of fibrin clot interference on automated analyzers, especially those with sample probe without clot detection ability (Boyanton and Blick, 2002) [5].

The effects of various types of anticoagulants on blood plasma biochemistry were studied in man and various animal species but there is little information published for poultry plasma

biochemistry (Young and Bermes, 1999; Jones, 1985a; Jones, 1985b; Boyanton and Blick, 2002; Stokol *et al*., 2001; Ceron *et al*., 2004 and Harr *et al*., 2005) [2-8]. The purpose of the present study was to determine and compare how the commonly used anticoagulants may affect the results of routine biochemistry in poultry blood specimens.

## 2. Materials and Methods

### 2.1 Sample Collection

Approximately 3ml of blood sample was taken each time from each bird before slaughter (42 days) from the wing vein into three different blood collection tubes (EDTA: 0.1 ml of 10% K3EDTA solution for 5 ml of blood, Sodium heparin: 100 units for 5 ml of blood, and into clot activator tubes for serum harvesting). All samples were collected between the same hours to restrict any variation arising in the blood biochemical parameters which may occur due to circadian rhythm. The blood collection tubes were kept on ice in cool containers to avoid denaturation of proteins and were taken to the laboratory within 2 hours of blood withdrawal.

### 2.2 Processing and analysis of samples

All blood samples were transferred onto ice, to the laboratory and centrifuged at 2,000×g for 10 minutes providing serum and plasma, which were then refrigerated until measurement (approximately 90 minutes after harvesting). No haemolysis was detected in any of the samples analysed. The concentrations of glucose (GLU, GOD-PAP method), urea (UREA, urease/glutamate dehydrogenase method), creatinine (CREA, Kinetic Modified Jaffe's method), total protein (TP, Direct Biuret method), albumin (ALB, bromcresol green method), chloride (Cl, Modified Thiocyanate Method), Inorganic Phosphorous (Pi, Phosphomolybdate Method) and triglycerides (TG, GPO-TOPS method) were measured by commercial kits (Agappe Diagnostics Ltd, Kerala,INDIA) using colorimeter. Statistical analysis was done by one-way analysis of variance (ANOVA) using SPSS software package, version 23.00. Post-hoc Tukey's test was used to compare the studied parameters between the groups, p values <0.05 were considered as significant.

## 3. Results and Discussions

**Table 1:** Mean±SEM of different biochemical parameters in poultry blood

| Parameters | Serum (Mean±SEM) (N=20) | Sodium Heparin (Mean±SEM) (N=20) | K3 EDTA (Mean±SEM) (N=20) | p-value |
|---|---|---|---|---|
| Albumin(mg/dL) | $1.15\pm0.11^{ab}$ | $1.27\pm0.07^{b}$ | $0.90\pm0.05^{a}$ | 0.013* |
| Triglycerides (mg/dL) | $54.88\pm5.82^{b}$ | $40.19\pm2.38^{a}$ | $56.61\pm5.33^{b}$ | 0.039* |
| Creatinine (mg/dL) | 0.35±0.02 | 0.35±0.03 | 0.32±0.01 | $0.557^{NS}$ |
| Glucose (mg/dL) | $315.56\pm9.16^{b}$ | $175.86\pm11.65^{a}$ | $161.94\pm10.00^{a}$ | 0.000** |
| Total Protein (g/dL) | 2.32±0.08 | 2.19±0.05 | 2.29±0.07 | $0.355^{NS}$ |
| Urea (mg/dL) | 0.67±0.05 | 0.59±0.04 | 0.49±0.04 | $0.062^{NS}$ |
| Chloride (mEq/L) | 90.41±5.28 | 91.19±5.05 | 85.61±4.40 | $0.690^{NS}$ |
| Inorganic Phosphorous (mg/dL) | $11.43\pm1.08^{b}$ | $7.05\pm0.62^{a}$ | $10.38\pm1.59^{ab}$ | 0.032* |

*- $p < 0.05$
**- $p < 0.01$
NS- Not Significant
$^{a,b,c}$ superscripts in the same row indicate significant differences between groups.

**3.1 Albumin:** Albumin concentration significantly ($p < 0.05$) increased in heparinized plasma (1.27±0.07 mg/dL) when compared against EDTA treated plasma (0.90±0.05 mg/dL). Sodium/ Lithium Heparin was generally recommended as the most suitable anticoagulant for plasma biochemical measurements by Young and Bermes, (1999) [2], although in previous reports significant differences in albumin levels were found between heparinized plasma and serum (Thorensen *et al*., 1992; Mohri *et al*., 2007a and Mohri *et al*., 2007b) [12-14]. An artifactual increase in albumin concentration in heparinized plasma, compared with serum and other anticoagulants, using a bromcresol green assay (BCG) was described in canine by Stokol *et al*., (2001) [6], Ceron *et al*., (2004) [7] and in sheep samples by Laborde *et al*., (1995) [15]. This difference was suggested to be partly due to the combination of heparin with fibrinogen as discussed by Stokol *et al*., (2001) [6]. Significant increase in total protein and glucose amounts with significant decrease in urea concentration were reported in sheep heparinized plasma in comparison to serum (Laborde *et al*., 1995 and Morris *et al*., 2002) [15, 16], but the present study do not confirm these reports.

**3.2 Triglycerides:** The results of this study indicated a statistically significantly ($p < 0.05$) reduced mean level of triglycerides when sodium heparin (40.19±2.38 mg/dL) was used as an anticoagulant as compared to serum (54.88±5.82 mg/dL). The reduced concentration of Triglycerides in heparin is consistent with a cross-sectional study by Yang *et al*., (1998) [23] who observed a significant reduction in triglyceride levels when low molecular weight heparin was administered to diabetic patients on haemodialysis. Another study by Katopodis *et al*., (2007) [24] has shown that there occurs a reduction in Triglyceride levels in first, second and third hour after administration of intravenous heparin in human subjects during renal replacement therapy. The mechanism that links to heparin induced decrease in Triglyceride level is not well understood and is still debatable. However, Katopodis *et al*., (2007) [24] explained that heparin directly stimulates lipoprotein lipase (LPL) release into plasma from epithelial cells. Such transient increase in LPL enhances the body's ability to remove Triglyceride, thereby lowering its plasma concentrations.

**3.3 Creatinine:** The creatinine level at slaughter age was non-significant among the groups. This catabolite is directly related to increased muscle activity and volume. Creatinine is a breakdown product of creatine phosphate from muscle and protein metabolism, anticoagulants seems to have no effect on its concentration. Creatinine results agreed with previous reports by Jones, (1985a) [3] and Mohri *et al*., (2007b) [14] who found no differences in its concentration between EDTA-

treated plasma and serum in cattle samples, unlike a decrease in creatinine activity in EDTA-treated samples was reported by Young and Bermes, (1999) [2] in humans and Mohri *et al*., (2007a) [13] in horses. Distribution of specific isoenzymes could be responsible for inter-species variations.

**3.4 Glucose:** Current study revealed a significant ($p$<0.01) increase in the glucose levels in serum (315.56±9.16 mg/dL) when compared against blood collected in heparin (175.86±11.65 mg/dL) and EDTA (161.94±10.00 mg/dL). Stressful conditions inherent during slaughter time advent a marked rise in blood glucose levels as reported by Thrall, (2007) [10]. Broiler chickens have blood glucose levels within the range 200 to 500 mg/ dL which differs with the strain, age and geographic locations in which the birds are reared. Gambino *et al*., (2009) [18] reported higher plasma glucose values when paired blood samples were collected from serum and heparinized plasma and stored at same ambient temperature. On the other hand, Turchiano *et al*., (2013) [19] reported higher glucose levels in serum when serum gel separator tubes were used against those of fluoride tubes. This could be attributed to the fact that glycolysis is inhibited quickly due to the separation of serum from the cellular components when serum is collected in tubes with a clot activator and then centrifuged (Turchiano *et al*., 2013 and Szabo *et al*., 2005) [9,11]. Such explanation implies that the practice of using serum sample for blood glucose estimation could be leading to many wrong reports as reported by Gupta and Kaur, (2014) [20]. Currently, the best practice is by putting the plasma glucose samples immediately on ice slurry and then centrifuging it within 30 minutes to avoid overestimation or underestimation of the mean glucose levels (Sacks *et al*., 2011) [21]. So, as to obtain a reliable result, it is therefore suggested that analysis of blood glucose concentrations should be carried out immediately after collection of the specimen or within the shortest possible time after storage in an anticoagulant.

**3.5 Total protein**: Use of different anticoagulants for routine blood biochemistry had no significant effect on Total protein concentrations. The mean values obtained in this study are little lesser than the values cited by Thrall, (2007) [10] that is 2.5 to 4.5 g / dL. In a similar study in horses, measurements of Total protein values were unaltered and were not affected by either anticoagulant. But it was also reported that if higher doses of anticoagulants were used, a small dip in underestimation of Total protein values were noted with both K3EDTA and lithium heparin, when biuret method was used for estimation. Thus, the underestimation in Total protein values of samples with high doses of anticoagulant may be explained by the dilutional effect of the anticoagulants as illustrated by Estepa *et al*., (2006) [22]. The use of K3EDTA as an anticoagulant in comparison to sodium heparin may result in slight overestimation of Total protein values as reported in Table 1. This overestimation is likely to be variable depending on the commercial solution of K3EDTA used and is bound to increase when the dose of K3EDTA is very high. In contrast, our current results reveal that the use of clot activator tubes can be routinely used for more accuracy in TP estimations.

**3.6 Urea:** The lower concentrations of urea, albeit not significant in EDTA-treated plasma than serum are in contrast with previous reports in dog and in humans as reported by Young and Bermes, (1999) [2] and Ceron *et al*., (2004) [7]. Although the exact mechanism that causes such differences was not clear but Dubin and Hunt, (1978) [17] stated that the osmotic fluid shift from red cells to plasma and/or differences between species may be contributory factors.

**3.7 Chloride and Inorganic phosphorous:** Chloride levels when measured showed no significant difference among different groups. However, as far our knowledge there are no literatures to support the significant ($p$<0.05) increase in Phosphorous levels in serum (11.43±1.08 mg/dL) when compared against sodium heparin (7.05±0.62 mg/dL).

## 4. Conclusion

In conclusion, heparinized plasma could be used for the measurement of biochemical parameters of the blood plasma of poultry birds viz., albumin and chloride. Phosphorous estimation being doubtful in heparinized plasma and needs further investigation with larger sample size. K3EDTA causes unfavorable changes in the concentrations of some biochemical parameters of poultry blood plasma but however causes little changes in the Triglyceride levels. Glucose and total protein values seem to be less erroneous if measured in serum despite adding anticoagulants. The statistically significant differences for some biochemical parameters between serum and blood plasma with different type of anticoagulants may not have clinically significant difference because most variations remained in the reference range. Thus, it is of utmost importance to interpret the changes of the amounts of measured parameters in plasma based on the type of anticoagulant and to study the level of variation from the reference value standards. In normal and healthy poultry birds with the values of biochemical parameters ranging lower or higher than normal levels, the slightest changes in plasma due to addition of anticoagulant might result in abnormal levels and misinterpretation.

## 5. References


1. Guder WG. The quality of diagnostic samples. Blood Gas News. 2001; 10:18-24.
2. Young DS, Bermes EW. Specimen collection and processing: sources of biological variation. In: Burtis, C.A., Ashwood, A.R. (Eds.), Tietz Textbook of Clinical Chemistry, third ed. W.B. Saunders, Philadelphia, 1999, 42-72.
3. Jones DG. Stability and storage characteristics of enzymes in sheep blood. Research in Veterinary Science. 1985a; 38:307-311.
4. Jones DG. Stability and storage characteristics of enzymes in cattle blood. Research in Veterinary Science. 1985b; 38:301-306.
5. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clinical Chemistry. 2002; 48:2242-2247.
6. Stokol T, Tarrant JM, Scarlett JM. Overestimation of canine albumin concentration with the bromcresol green method in heparinized plasma samples. Veterinary Clinical Pathology. 2001; 30:170-176.
7. Ceron JJ, Martinez-Subiela S, Hennemann C, Tecles F. The effects of different anticoagulants on routine canine plasma biochemistry. Veterinary Journal. 2004; 167:294-301
8. Harr KE, Raskin RE, Heard DJ. Temporal effects of 3 commonly used anticoagulants on hematologic and

biochemical variables in blood samples from macaws and Burmese pythons. Veterinary Clinical Pathology. 2005; 34:383-388
9. Sandhu BS, Singh B, Brar RS. Haematological and biochemical studies in broiler chickens fed ochratoxin and inoculated with inclusion body hepatitis virus, singly and in concurrence. Veterinary Research Communications. 1998; 22:335-346.
10. Thrall MA. Hematologia e bioquímica clínica veterinária. Philadelphia, Lippincott, Williams &Wilkins, São Paulo: Roca. 2007, 582.
11. Szabó A, Mezes M, Horn P, Sütő Z, Bázár GY, Romvari R. Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). Acta Veterinaria Hungarica. 2005; 53(4):397-409.
12. Thorensen SI, Havre GN, Morberg H, Mowinckel P. Effects of storage time on chemistry results from canine whole blood, heparinized whole blood, serum and heparinized plasma. Veterinary Clinical Pathology. 1992; 21:88-94.
13. Mohri M, Allahiari L, Sardari K. Effects of common anticoagulants on routine plasma biochemistry of horse and comparison with serum. Journal of Equine Veterinary Science. 2007a; 27:313-316.
14. Mohri M, Shakeri H, Lotfollah Zadeh S. Effects of common anticoagulants (heparin, citrate, and EDTA) on routine plasma biochemistry of cattle. Comparative Clinical Pathology. 2007b; 16:207-209.
15. Laborde CJ, Chapa AM, Burleigh DW, Salgado DJ, Fernandez JM. Effects of processing and storage on the measurement of nitrogenous compounds in ovine blood. Small Ruminant Research. 1995; 17:159-166.
16. Morris JD, Fernandez JM, Chapa AM, Gentry LR, Thorn KE, Weick TM. Effects of sample handling, processing, and hemolysis on measurements of key energy metabolites in ovine blood. Small Ruminant Research. 2002; 43:157-166.
17. Dubin S, Hunt P. Effect of anticoagulants and glucose on refractometric estimation of protein in canine and rabbit plasma. Laboratory Animal Science. 1978; 28:541-544.
18. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clinical Chemistry. 2009; 55(5):1019-1021.
19. Turchiano M, Nguyen C, Fierman A, Lifshitz M, Convit A. Impact of blood sample collection and processing methods on glucose levels in community outreach studies. Journal of Environmental and Public Health. 2013.
20. Gupta S, Kaur H. Inhibition of glycolysis for glucose estimation in plasma: recent guidelines and their implications. Indian Journal of Clinical Biochemistry. 2014; 29(2):262-264.
21. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clinical Chemistry. 2002; 48(3):436-472.
22. Estepa JC, Lopez I, Mayer-Valor R, Rodriguez M, Aguilera-Tejero E. The influence of anticoagulants on the measurement of total protein concentration in equine peritoneal fluid. Research in Veterinary Science. 2006; 80(1):5-10.
23. Yang CW, Wu TH, Huang CC. Low molecular weight heparin reduces triglyceride, VLDL and cholesterol/HDL levels in hyperlipidemic diabetic patients on hemodialysis. American Journal of Nephrology. 1998; 18:384-390.
24. Katopodis KP, Koliousi E, Gouva C, Balafa O, Bairaktari E. Acute effect of heparin on lipid parameters in patients on renal replacement therapy. ASAIO Journal. 2007; 53:46-49.