Research Article

Effect of Blood Collection Site and Samples Freezing Cycles on the Biochemical Parameters of Poultry Blood Serum

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Abstract | This study was conducted on healthy adult female poultry, comprising two experiments. The first involved drawing blood from two groups of broiler chickens (Ross 308) and laying hens (Lohmann), each containing 10 chickens, with 10 replicates. Blood was collected from four locations on each chicken's body: the jugular vein, the wing vein, the heart, and the carpal vein. The second experiment involved collecting additional blood samples from two groups of broilers, with 30 blood samples collected from the brachial vein. Serum was collected from these samples to test the effect of continuous freezing for the first group and to study the impact of freezing and thawing for the second group on the studied parameters over the following periods (0, 7, 14, 21, and 28) days. The results of the first experiment indicated that the levels of cholesterol, triglycerides, glucose, uric acid, and calcium in the blood serum of broiler and laying hens remained relatively stable when blood was drawn from the studied sites. However, some other parameters, such as total protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), did show significant differences at the ($P \leq 0.05$) and ($P \leq 0.01$) levels. Blood samples drawn directly from the heart recorded the highest concentrations compared to the other groups. The results of the second experiment indicated that the continuous freezing storage period had no significant effect on the studied parameters. The freezing and thawing cycles significantly influenced ($P \leq 0.05$ and $P \leq 0.01$) the total protein, albumin, glucose, AST, and ALT, whereas, the other parameters remained unaffected.

Keywords | Blood physiology, Biochemical parameters, Brachial vein, Heart, Jugular vein, Metatarsal vein

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INTRODUCTION

Blood in poultry is a liquid connective tissue composed of erythrocytes, leukocytes, thrombocytes, and plasma, it serves as the principal medium for delivering oxygen and minerals to all body cells while facilitating the removal of waste, and it is crucial in protecting the body against pathogens through the operation of the immune system (Wickramasuriya *et al*., 2022; Zheng *et al*., 2024). The locations of blood collection from poultry vary, and their ability to provide sufficient blood for biochemical examinations depends mainly on the size and visibility of the veins, as well as their location in the bird's body (Livingstone, 2020). Perhaps the most prominent of these, which are used as indicators of poultry disease or safety, include: Glucose, cholesterol, triglycerides, total protein, albumin, liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), uric acid, and calcium, as they are essential in assessing the health status of poultry.

Because it provides a comprehensive view of the poultry physiological state, it will therefore aid in the early detection of diseases or health problems (Kokore *et al*.,

2021; Al-Salhi and Al-Shatty, 2023). Scientific research in the field of poultry is expanding on a massive scale, and standard practices related to preserving blood serum samples by freezing have emerged. This could be because the researcher lacks the necessary tools, the settings are incorrect, or they can only collect and analyze a small number of samples at a time. All of these things can change the absorbance values of biological samples on a spectrophotometer. Some studies in previous experiments have shown the effect of serum freezing on some blood biochemical parameters (Cuhadar *et al.*, 2013; Kawasaki *et al.*, 2020; Du *et al.*, 2023). Therefore, our study, unlike previous ones, aimed to conduct an in-depth study on the extent to which these parameters are affected when serum is stored at -20 °C for specific periods. We had examined the impact of different storage conditions on serum over various periods, employing statistical analysis at 0.05 and 0.01 probability levels. We also investigated the impact of freeze-thaw cycles on these qualities by freezing samples, thawing them, and then freezing them again at specific intervals, and recorded the results. This attempt aimed to simulate certain conditions, including those experienced by laboratories in some countries where power outages occur during sample storage. This study also addresses the common practice of sample storage, which researchers and graduate students often employ in scientific laboratories at certain universities.

MATERIALS AND METHODS

Collecting and distributing blood samples

This study was conducted on healthy adult female poultry, including broiler chickens (Ross 308, 35 days old and weighing 2,150 g ± 57.55 S.E) and laying hens (Lohmann, 45 weeks old and weighing 1,950 g ± 25.15 S.E), which were characterized by clear veins and abundant blood.

The first experiment involved drawing blood from two groups of poultry (broiler chickens and laying hens), each group containing 10 birds, with 10 replicates (each chicken representing one replicate). Blood was collected from four locations on each chicken's body, with 3 ml from each area, resulting in a total of 12 ml drawn from each chicken. Blood was drawn using a medical syringe as follows: The jugular vein, the pterygoid vein, the heart, and the medial metatarsal vein (also known as the carpal vein). A total of 40 blood samples were drawn from each group.

Figure 1 shows a detailed design diagram of the first experiment for the first group (broiler chickens). The second group (laying hens) followed the same design as the first group. Blood was then poured directly into tubes containing no anticoagulant to measure blood biochemical parameters.

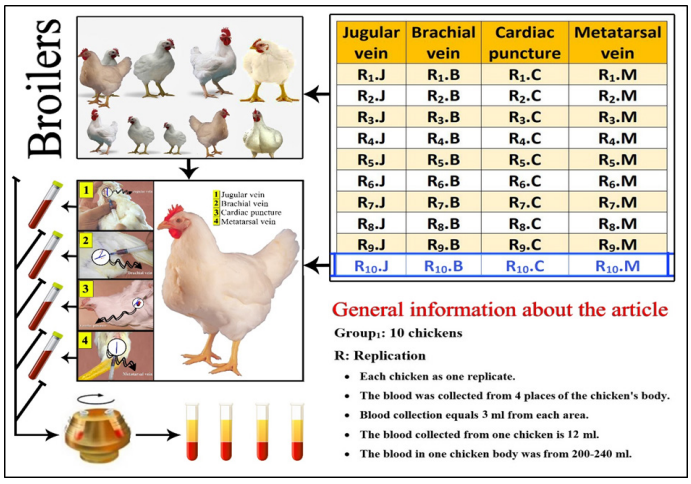

Figure 1: Diagram illustrating study design.

The second experiment included collecting additional blood samples from female broiler chickens, totaling 30 samples from the brachial vein. These samples were divided into two groups (each group containing 15 chickens). Blood was drawn and serum collected to test the effect of continuous freezing on serum at -20 °C biochemical parameters over the following periods (0, 7, 14, 21, and 28) for the first group, and to study the effect of freezing and thawing on serum biochemical parameters for the second group, over the same periods.

Figures 2 and 3 illustrate the design of the second experiment for the continuous freezing and freeze-thawed samples, respectively.

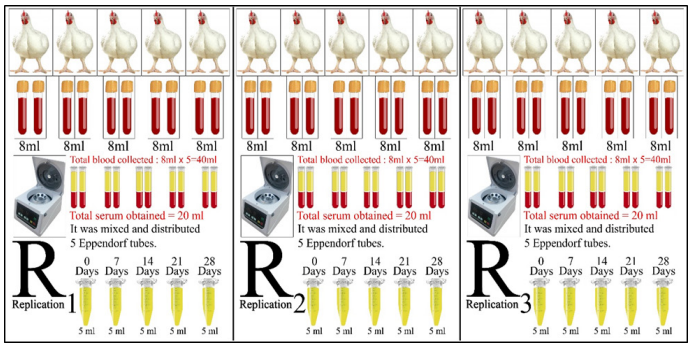

Figure 2: Diagram illustrating the continuous freezing process of samples.

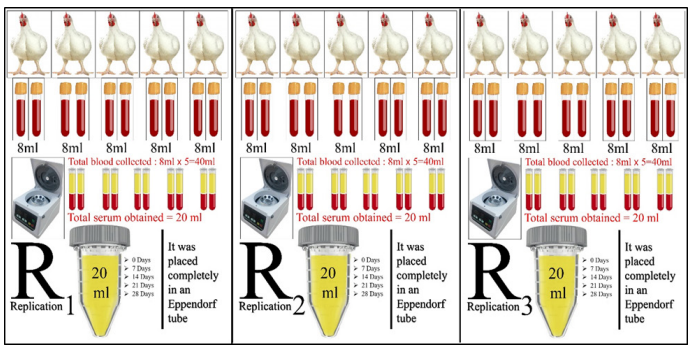

Figure 3: Diagram illustrating the freeze–thaw process for samples.

Table 1: Effect of blood collection area on biochemical parameters of broiler serum (mean ± SE).

Test Type	Blood collection area				Sig. level
	Jugular vein	Brachial vein	Cardiac puncture	Metatarsal vein	
Glucose (mg/100 ml)	245.49±15.20	251.76±11.16	253.76±8.91	247.43±13.31	N. S
Cholesterol (mg/100 ml)	131.22±8.78	136.88±14.40	140.86±11.84	130.53±13.17	N. S
Triglyceride (mg/100 ml)	112.56±2.42	116.22±2.54	118.51±3.48	114.85±5.60	N. S
Total protein (g/100 ml)	4.16±0.12^{b}	4.24±0.25^{ab}	4.25±0.18^{a}	4.19±0.22^{ab}	*
Albumin (g/100 ml)	2.21±0.03^{ab}	2.23±0.02^{ab}	2.27±0.04^{a}	2.20±0.02^{b}	*
Uric acid (mg/100 ml)	9.25±1.27	9.26±2.22	9.26±1.19	9.25±1.14	N. S
AST (U/L)	23.41±1.25^{b}	24.33±2.51^{b}	41.32±1.43^{a}	23.65±1.75^{b}	*
ALT (U/L)	10.00±3.67^{b}	10.96±2.33^{b}	16.66±4.54^{a}	10.92±3.24^{b}	**
Calcium (mg/100 ml)	9.12±0.87	9.14±0.42	9.78±1.11	9.12±0.65	N.S

a,b: The different letters within a row indicate significant differences between the means. *: Indicates significant difference at the 0.05 probability level. **: Indicates significant difference at the 0.01 probability levels. N.S.: Indicates no significant differences between the means.

Laboratory analysis of blood samples

Blood samples were centrifuged at 3,000 rpm for 15 minutes to obtain poultry serum. The serum was then transferred to new test tubes prepared by Naugra Medical, India, and labeled with the appropriate replication codes and collection points. Biochemical marker concentrations were measured directly using kits produced by Randox (Northern Ireland), a veterinary analytical company. Analyses were performed according to the manufacturer's technical protocol, using a spectrophotometer to measure absorbance at the wavelengths specific to each assay. The analytical methodology described in Kafle (2019) and Biswas *et al*. (2020) was used, which documents methods used in poultry biochemistry and focuses on the steps of sample collection, processing, and calibration to achieve accurate results in the poultry serum matrix.

Storage experiment with blood serum samples

The samples were preserved at a temperature of -20 °C for four weeks. This aimed to facilitate weekly analysis of the preserved samples and to evaluate the effect of the standard approach (freezing preservation) on the quality of blood biochemical assays. A sample (time 0) was utilised as a control, with results documented immediately without preservation or freezing. The absorbance reading was repeated three times consecutively for each sample to reduce experimental error and ensure accurate results.

Statistical analysis

SPSS (2024) was used to perform the statistical analyses, including ANOVA and Duncan's Multiple Range Test, for mean separation at significance levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

Effect of blood collection area on the biochemical parameters of broiler blood serum

This study examined the effect of blood collection area on serum biochemical markers and found no significant differences between the mean values (Table 1). For some biochemical parameters including glucose, cholesterol, triglycerides, uric acid, and calcium, no significant differences were observed. In contrast, several other parameters showed significant variation: total protein, albumin, and AST exhibited significant differences at the probability level of (P≤0.05), while ALT showed highly significant differences at both (P≤0.05) and (P≤0.01). Notably, blood samples collected directly from the heart displayed the highest concentrations for these biochemical markers compared with the other sampling groups.

Effect of blood collection on the serum biochemical parameters of the laying hens

Table 2 illustrates the impact of the blood collection site on the concentrations of serum biochemical indicators in the laying chickens. Glucose, cholesterol, triglycerides, and uric acid levels do not exhibit any substantial variations, and calcium level was also stable. Conversely, other examined parameters exhibited significant differences, with some reaching probability levels of (P≤0.05) and (P≤0.01), such as total protein and AST concentration. In contrast, others, including albumin and ALT enzyme, showed significant differences only at the probability level of (P≤0.05). The samples collected directly from the heart exhibited the highest quantities of the biochemical markers in the blood serum.

Blood samples drawn directly from the heart showed significant elevation in the total protein, albumin, and hepatic enzymes (AST and ALT) compared to samples drawn from other areas, such as the jugular vein, brachial vein, and carpal vein. This elevation can be attributed to several physiological factors, most notably the heart's central role as the primary pump responsible for distributing blood with high force to all tissues. As a result, cardiac blood represents a more direct and less filtered

Table 2: Effect of blood collection area on biochemical parameters in hens (mean ± SE).

Test type	Blood collection area				Sig. level
	Jugular vein	Brachial vein	Cardiac puncture	Metatarsal vein	
Glucose (mg/100 ml)	217.25±13.66	224.32±15.85	230.47±11.48	215.81±17.63	N. S
Cholesterol (mg/100 ml)	122.22±11.52	124.81±12.02	130.73±15.77	124.51±23.05	N. S
Triglyceride (mg/100 ml)	112.48±2.00	115.23±2.12	116.89±1.19	113.72±3.25	N. S
Total protein (g/100 ml)	4.20±0.35 [b]	4.23±0.15 [b]	4.31±0.33 [a]	4.21±0.21 [b]	**
Albumin (g/100 ml)	2.21±0.01 [b]	2.23±0.05 [ab]	2.26±0.03 [a]	2.21±0.08 [b]	*
Uric acid (mg/100 ml)	9.21±2.23	9.23±2.12	9.25±1.84	9.22±1.37	N. S
AST (U/L)	48.74±2.33 [b]	49.65±3.12 [b]	55.92±4.23 [a]	49.33±2.44 [b]	**
ALT (U/L)	21.11±1.98 [b]	22.73±3.62 [b]	29.61±2.55 [a]	19.35±5.22 [b]	*
Calcium (mg/100 ml)	12.8±0.34	12.14±0.78	13.45±0.37	11.45±0.66	N. S

a,b: The different letters within a row indicate significant difference between the means. *: Indicates significant difference at the 0.05 probability level. **: Indicates significant difference at the 0.01 probability levels. N.S.: Indicates no significant difference between the means.

Table 3: Effect of freezing duration on biochemical parameters of broiler serum (mean ± SE).

Test name	0 Days	7 Days	14 Days	21 Days	28 Days	Sig. level
Glucose (mg/100 ml)	246.9±3.81	246.02±4.53	244.13±2.36	240.78±3.12	237.54±5.48	N. S
Cholesterol (mg/100 ml)	142.55±1.21	142.14±1.54	141.33±1.43	140.67±1.74	139.99±3.10	N. S
Triglyceride (mg/100 ml)	125.99±1.37	125.48±1.92	124.65±2.24	123.42±2.70	122.82±1.50	N. S
Total protein (g/100 ml)	4.37±0.43	4.20±0.02	3.94±0.38	3.64±0.15	3.43±0.25	N. S
Albumin (g/100 ml)	2.26±0.028	2.25±0.02	2.24±0.027	2.22±0.03	2.19±0.09	N. S
Uric acid (mg/100 ml)	9.28±0.01	9.28±0.00	9.27±0.02	9.27±0.01	9.26±0.02	N. S
AST (U/L)	22.66±1.20	22.44±0.33	22.44±0.29	22.33±1.12	22.10±0.44	N. S
ALT (U/L)	11.55±0.22	11.55±0.29	11.08±0.08	11.05±0.04	11.00±0.00	N. S
Calcium (mg/100 ml)	9.43±0.20	9.42±0.30	9.42±0.16	9.41±0.19	9.41±0.16	N. S

N.S.: Indicates no significant differences between the means.

mixture of circulating proteins, enzymes, and minerals. In contrast, blood drawn from peripheral veins has already passed through various tissues and organ systems, where filtration, metabolic activity, and nutrient exchange can alter the concentration of biochemical components. These physiological differences help explain the observed variations between sampling sites (Voss and Werner, 2010; Chloupek *et al*., 2011; Kelly and Alworth, 2013).

Another explanation relates to the distribution of proteins and enzymes secreted by the liver and other organs located near the heart within the body cavity. Because these substances enter circulation closest to the heart, the central point of distribution, blood drawn directly from the heart will contain higher concentrations compared with blood collected from more distant peripheral veins such as the pterygoid, carpal, or jugular veins. As blood travels through these peripheral vessels, it undergoes continuous exchange with surrounding tissues and organs, leading to decreases in protein and enzyme concentrations due to utilization, storage in tissues and muscles, or participation in metabolic processes. Moreover, cardiac blood is less affected by physiological fluctuations caused by tissue-level metabolic activity, helping preserve the original concentrations of circulating proteins and enzymes before they are modified by peripheral metabolism (Jang *et al*., 2019; Sauer *et al*., 2020; Kokore *et al*., 2021; Morishita and Porter, 2024).

Effect of cryopreservation on the serum biochemical parameters of broilers

The effect of freezing duration on the concentration of biochemical markers in broiler blood serum is presented in Table 3. Parameters including total protein, albumin, triglycerides, glucose, cholesterol, uric acid, AST, ALT, and calcium showed no significant changes ($P > 0.05$) across all experimental groups when comparing initial values (time 0) with those measured after 7, 14, 21, and 28 days of continuous freezing. Although minor fluctuations were observed, they were not statistically significant. These results indicate that continuous freezing for up to 28 days did not adversely affect the stability of these biochemical parameters.

Effect of freeze-thaw cycles on the serum biochemical parameters of broilers

Table 4 shows how freezing cycles affect the serum biochemical characteristics in broilers. The average levels of calcium, triglycerides, uric acid, and cholesterol among

Table 4: Effect of sample freezing cycles on biochemical parameters of broiler serum (mean ± SE).

Test name	0 Days	7 Days	14 Days	21 Days	28 Days	Sig. level
Glucose (mg/100 ml)	249.19±0.28^{a}	247.87±1.16^{ab}	244.67±0.51^{bc}	244.00±1.83^{bc}	243.7 ±1.52^{b}	*
Cholesterol (mg/100 ml)	140.12±1.01	140.28±1.32	139.47±1.56	139.58±1.23	139.02±2.24	N. S
Triglyceride (mg/100 ml)	121.12±1.24	121.41±1.85	120.15±1.47	122.77±1.58	122.49±1.34	N. S
Total protein (g/100 ml)	5.45±0.24^{a}	5.05±0.39^{ab}	4.51±0.00 ^{b}	4.44±0.09 ^{b}	3.69±0.11^{c}	**
Albumin (g/100 ml)	2.62±0.24^{a}	2.48±0.23^{ab}	2.36±0.15 ^{ab}	2.20±0.04 ^{ab}	1.80±0.25^{c}	*
Uric acid (mg/100 ml)	9.33±0.15	9.27±0.06	9.15±0.12	9.12±0.08	9.01±0.19	N. S
AST (U/L)	55.4±0.03^{a}	52.44±0.00^{b}	47.36±0.19 ^{c}	22.44±0.10 ^{d}	20.88±0.29^{e}	**
ALT (U/L)	23.77±0.11^{a}	21.32±1.20^{a}	17.99±1.20 ^{b}	12.66±1.52^{c}	8.99±0.38^{d}	**
Calcium (mg/100 ml)	9.80±0.15	9.77±0.34	9.45±0.29	9.34±0.35	9.21±0.24	N. S

a,b,c,d and e: The different letters within a row indicate significant difference between the means. *: Indicates significant differences at the 0.05 probability level. **: Indicates significant differences at the 0.01 probability levels. N.S.: Indicates no significant differences between the means.

the experimental groups showed no discernible variations at 0.05 and 0.01 probability levels. This suggests that these parameters remain unaffected by freezing cycles, despite a minor reduction in their concentrations that does not achieve statistical significance. The same table also shows that certain biochemical parameters were affected by the freezing and thawing process, as evidenced by significant differences in their mean values at probability levels of 0.05 and 0.01. Specifically, glucose, total protein, albumin, AST, and ALT exhibited variability in their concentrations during absorbance measurements. Statistical analysis confirmed these fluctuations, indicating that repeated freezing and thawing cycles significantly influenced these serum biochemical markers ($P \leq 0.05$ and $P \leq 0.01$).

The results of the study showed that keeping serum samples frozen at -20 °C for up to 28 days did not cause noticeable changes in most biochemical measures, like cholesterol, triglycerides, uric acid, and calcium. This agrees with that reported by others (Cuhadar *et al*., 2013; Kawasaki *et al*., 2020). However, notable changes were observed in glucose, total protein, albumin, and the liver enzymes AST and ALT. These alterations likely occurred due to the degradation of proteins, sugars, and enzymes, as well as damage to cell membranes caused by repeated freezing and thawing. Such damage can lead to leakage of cellular contents and a reduction in the activity and biological function of these molecules due to structural alterations. These findings are consistent with previous studies investigating the effects of freezing, thawing, and storage on the biochemical properties of blood serum in both humans and animals (Mansto *et al*., 2007; Nardid *et al*., 2009; Hekal and Shun, 2018; Freiburghaus *et al*., 2020; Du *et al*., 2023).

CONCLUSIONS

The study showed that the levels of cholesterol, triglycerides, glucose, uric acid, and calcium in the serum of broiler and laying hens stayed relatively stable regardless of where the blood was taken from. On the other hand, some parameters like total protein, albumin, and the liver enzymes AST and ALT showed significant noticeable differences. It was also observed that keeping samples frozen continuously did not have a significant effect on the measured values, but repeated cycles of freezing and thawing did affect several of these parameters at similar levels of significance. In general, parameters such as cholesterol, triglycerides, uric acid, and calcium mainly remained unchanged during the study.

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NOVELTY STATEMENT

This study provides a comprehensive evaluation of how blood collection sites influence poultry serum biochemical parameters, highlighting their importance in accurate interpretation and decision-making.

It further distinguishes the effects of continuous freezing versus repeated freeze–thaw cycles, offering practical guidance for sample handling and storage in laboratory.

Ethical approval

After following all national and international guidelines for animal care before and after experimental procedures, the Scientific Committee of the Department of Animal Production, College of Agriculture, Marshes, University of Thi-Qar, approved this study and granted it ethical approval No. 12, dated March 19, 2024.

Generative AI and AI-assisted technology statement

Generative AI and AI-assisted technologies were not used in the preparation or analysis of this research.

Conflict of interest

The author has declared no conflict of interest related to the publication of this work.

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