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Influence of temperature and storage time on the stability of biochemical parameters in broilers

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ABSTRACT

Poultry farming is a strategic sector of Brazilian agribusiness, requiring high health standards to ensure productivity and competitiveness. Biochemical tests are essential for monitoring poultry health, but pre-analytical variables can compromise their results. This study evaluated the influence of storage time and temperature on the stability of 14 serum analytes in COBB 500 broilers, both males and females, aged 35 and 40 days. Samples were analyzed under different conditions: room temperature (27 ± 2°C), refrigeration (6 ± 2°C), freezing (−20 ± 5°C), and ultra-freezing (−80 ± 5°C), over periods of up to 365 days. Analyte stability varied according to the analyte and storage condition. Lactate was the only parameter that remained stable under all tested condition. It is concluded that the preservation of biochemical analytes depends directly on storage conditions, which must be carefully considered to ensure the reliability of results in healthy broilers.

Introduction

In Brazil, poultry farming plays a fundamental role in agribusiness, being one of the most relevant and strategic sectors for the national economy (ABPA, 2024; Santini and Pigatto, 2006). In 2023, this activity generated over 91.646 billion reais, with the production of 14.833 million tons of chicken meat, establishing the country as the world's leading exporter (ABPA, 2024). In the face of growing competitiveness in the international market, the poultry industry is constantly striving to increase production, which requires animals to reach their maximum productivity potential (Figueira et al., 2014). To achieve this goal, ensuring the welfare and health of the birds is essential, under the protocols established by the Brazilian Animal Protein Association (ABPA, 2024; Serafini and Morro, 2024).

However, broiler production faces challenges, as failures in disease prevention can lead to infectious outbreaks, consequently compromising product quality (Caldas et al., 2019; Taylor et al., 2017). To maintain poultry health and prevent production losses, laboratory tests, particularly hematological and biochemical analyses, are essential, as they provide reference parameters for the early detection of clinical diseases (Weiser, 2015). In this context, poultry medicine plays a crucial role in the collective assessment of animals, enabling early and targeted interventions (Oliveira et al., 2019).

In recent years, laboratory testing in poultry has become increasingly common, reflecting the demand for efficiency imposed by both national and international markets (Carvalho et al., 2020). Among these, biochemical analyses have been widely used in broilers to assess performance, digestibility, carcass yield, and nutrient utilization. Moreover, they enable the identification of morphological and functional changes in vital organs such as the liver and kidneys, which directly

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impact productivity and profitability for producers (Bowles et al., 2007; Carvalho et al., 2024; Gallo et al., 2015). However, information on the pre-analytical phase of laboratory testing in production animals remains scarce (Portes and Figueiredo, 2023; Rioja et al., 2018), including the influence of storage time and temperature on biochemical samples, an aspect that may compromise result reliability and the accurate interpretation of various studies (Zhou et al., 2024).

In view of this, this study aimed to investigate the influence of temperature and storage time on biochemical samples from healthy broilers. This research seeks to provide valuable information for optimizing laboratory protocols, contributing to more accurate diagnoses, and supporting poultry health management while also minimizing economic losses faced by producers.

Materials and methods

Ethical statutes

The project was approved by the Ethics Committee on Animal Use (CEUA/UFG) under protocol number 64/23. The biochemical analyses were conducted at the Veterinary Clinical Pathology Laboratory on the Veterinary Hospital of the institution (LabClinVet/HV/EVZ/UFG).

Sampling

Blood samples were collected from 140 COBB 500 broilers provided by the Broiler Teaching Aviary of the School of Veterinary Medicine and Animal Science at the Federal University of Goiás (EVZ-UFG), aged 35 and 40 days. Both male and female broilers were used in the present study; however, the influence of sex on the stability of biochemical parameters was not evaluated. This decision was based on the primary objective of the study, which was to assess the effects of time and temperature on analyte stability, regardless of intrinsic biological variations such as sex. Nevertheless, it is acknowledged that including this variable could contribute to a more comprehensive understanding of the results (Campbell, 2007). Therefore, future studies are encouraged to consider the influence of sex in their experimental designs to enhance data interpretation and the practical applicability of the findings.

Following clinical inspection, only individuals without apparent abnormalities were randomly selected for inclusion in the study. Blood collection was performed via puncture of the ulnar or metatarsal vein, obtaining 8 mL of blood per broiler. Of the total samples, 120 samples were stored in serum separator tubes (Vacutube, Biocon®, Brazil), and 20 samples in tubes containing sodium fluoride + EDTA. After collection, all birds were kept under observation to monitor potential post-procedural changes.

Sample transport

The transport of samples to the Veterinary Clinical Pathology Laboratory (LabClinVet) was carried out in insulated polystyrene boxes, avoiding direct sunlight exposure, over a route of approximately 2.6 km covered in five minutes. Initially, the tubes were centrifuged for 10 minutes at 1,644 G, and the serum/plasma from each bird was divided into 12 aliquots of at least 250 µL. The aliquots were placed in properly labeled polypropylene tubes and were either immediately processed or stored according to the designated time and temperature for each experimental group.

Experimental groups

Samples were divided into five groups: (1) control group (**CG**), analyzed immediately upon arrival at the lab; (2) room temperature (**RT:** 27 ± 2 °C), analyzed one (**D1**) and two days (**D2**) after collection; (3) refrigeration (**RF**: 6 ± 2 °C), analyzed on days D1, D2, and **D8**; (4) freezing (**FZ**: −20 ± 5 °C), analyzed on D8, **D14**, and **D30**; and (5) ultra-freezing (**UF**: −80 ± 5 °C), analyzed on D30, **D180**, and **D365** after collection.

For each biochemical parameter analyzed, serum samples from 10 broilers were used, totaling 12 aliquots per analyte. One aliquot was processed and analyzed immediately (control group, CG), while the remaining aliquots were distributed among the different temperature and time conditions described. The analyses were performed in duplicate using an automated biochemistry analyzer (CM 250, Wiener®, Argentina), with commercial kits (Bioclin®, Brazil), calibrator (Biocal), and controls (Biocontrol N and P) from the same brand. The CM 250 Wiener® analyzer was calibrated at the beginning of each analysis time point, as described in Table 1.

A total of 14 biochemical analytes were evaluated: (1) alanine aminotransferase (**ALT**), (2) aspartate aminotransferase (**AST**), (3) alkaline phosphatase (**ALP**), (4) gamma-glutamyl transferase (**GGT**), (5) albumin (**Alb**), (6) total serum proteins (**TSP**), (7) uric acid (**UA**), (8) total cholesterol (**Chol**), (9) glucose (**Glu**), (10) lactate (**Lac**), (11) triglycerides (**Trig**), (12) calcium (**Ca**), (13) phosphorus (**P**), and (14) magnesium (**Mg**). Since the volume of blood collected from each chicken is low, which generates a reduced volume of serum for biochemical analyses, even in automated equipment, it was decided to take samples from different times and temperatures of a single analyte. Therefore, each parameter was assessed using samples from 10 different individuals. It is recommended that future studies be conducted with larger samples volumes to allow for the simultaneous analysis of multiple parameters per individual (Fig. 1).

Data analysis

Collected data were tested for normality using the Shapiro-Wilk test. For parameters with normally distributed data, Student's t-test, analysis of variance (ANOVA), and Tukey's post hoc test were applied using the means of the results. For variables without normal distribution in the residuals, the Kruskal-Wallis test was used, followed by the Friedman test and Dunn's test for multiple comparisons, using the medians. The significance level was set at $P < 0.05$. All statistical analyses were performed using *R software, version 2023.09*.

Results

Comparison of storage times by Analyte

The analysis of storage time at room temperature showed that at time points D1 and D2, there were no significant differences ($P > 0.05$) for the parameters ALT, ALP, GGT, Alb, TSP, UA, Glu, Lac, Ca, and Mg. However, an increase in AST activity and Trig values was observed on D2 ($P < 0.05$), while Chol and P showed a significant reduction ($P < 0.05$) at the same time point. The data regarding the influence of RT after one and two days of storage are presented in Table 2.

Table 1
Methods used for the biochemical analyses of broilers (*Gallus gallus domesticus*).

Parameters	Methods
Uric Acid (UA)	Enzymatic Colorimetric (UOD-PAP)
Albumin (Alb)	Bromocresol Green (BCG)
Alanine Aminotransferase (ALT)	Kinetic UV
Aspartate Aminotransferase (AST)	Kinetic UV
Calcium (Ca)	Endpoint Colorimetric (Arsenazo III)
Total Cholesterol (Chol)	Enzymatic Colorimetric (COD-PAP)
Alkaline Phosphatase (ALP)	IFCC Kinetic
Phosphorus (P)	Endpoint UV
Gamma-Glutamyl Transferase (GGT)	Modified SZASZ (IFCC)
Glucose (Glu)	Enzymatic Colorimetric (GOD-PAP)
Lactate (Lac)	Enzymatic UV
Magnesium (Mg)	MANN YOE
Total Serum Proteins (TSP)	Biuret
Triglycerides (Trig)	Enzymatic Colorimetric

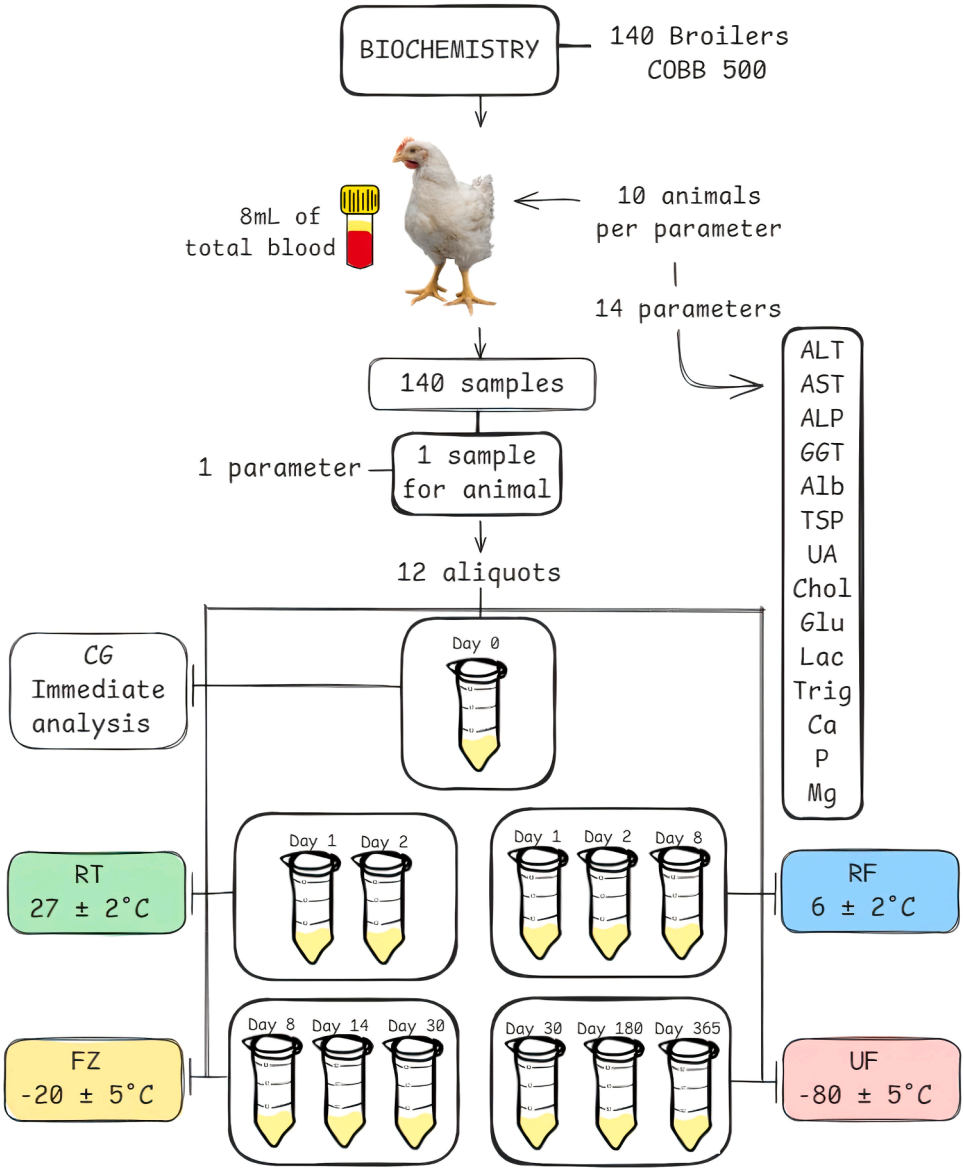

Fig. 1. Experimental groups. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium; CG, control group; RT, ambient temperature; RF, refrigeration temperature; FZ, freezing temperature; UF, ultra-freezing temperature.

Regarding the storage time at refrigeration temperature, ALP, GGT, Alb, Glu, Lac, Trig, and Ca showed no significant variations ($P > 0.05$) at any of the analyzed time points. However, an increase in ALT activity and TSP values was detected on D8 ($P < 0.05$). AST activity increased from D1, while Mg levels decreased, remaining different from the CG ($P < 0.05$). UA also showed a significant increase ($P < 0.05$) on D8. Chol decreased on D2 ($P < 0.05$), whereas P remained stable compared to the CG ($P > 0.05$), despite showing an increase ($P < 0.05$) between D1 and D8. The results of the influence of RF (6 ± 2°C) after one, two, and eight days of storage are presented in Table 3.

Under freezing conditions, an increase in ALT, AST, and UA activity was observed, along with a reduction in Ca levels on D8 ($P < 0.05$). ALP and Lac levels showed no significant differences ($P > 0.05$) throughout the analyzed period. GGT and Alb values decreased, while Chol and Mg increased on D8 ($P < 0.05$). TSP remained stable ($P > 0.05$) but showed an increase between D14 and D30 ($P < 0.05$). Trig levels significantly decreased on D30 ($P < 0.05$). P showed a reduction on D8 ($P < 0.05$), indicating its instability at this time point. The data on the influence of FZ (-20 ± 5°C) after eight, 14, and 30 days of storage are presented in Table 4.

At ultra-freezing temperature, ALT, AST, and Mg did not remain stable over the analyzed periods, with enzyme levels increasing and mineral values decreasing compared to the CG ($P < 0.05$). ALP showed a decrease, while TSP increased on D180 ($P < 0.05$). GGT, Alb, UA, Chol, Glu, Lac, Trig, Ca, and P remained stable ($P > 0.05$) throughout the storage period. The biochemical data regarding the influence of UF (-80 ± 5°C) after 30, 180, and 365 days of storage are presented in Table 5.

Comparison between storage temperatures by Analyte

When comparing storage temperatures and the CG for each biochemical parameter, ALT and AST values differed significantly ($P < 0.05$) between TR (6 ± 2°C), FZ (-20 ± 5°C), and UF (-80 ± 5°C). ALP, TSP, UA, Chol, and Lac did not show variations ($P > 0.05$) across all tested temperatures. GGT activity in serum remained stable ($P > 0.05$) across all temperatures compared to CG; however, its concentrations decreased in FZ (-20 ± 5°C) and increased in UF ($P < 0.05$). Alb did not show differences ($P > 0.05$) when comparing all temperatures with CG,

Table 2
Effects of room temperature (RT: 27 ± 2°c) on the analyzed parameters in broilers (*Gallus gallus domesticus*) at different time points: control group (CG – immediate analysis - D0), day 1 (D1), and day 2 (D2) (n = 10 broilers per analyte).

Parameters	CG	RT	
	D0	D1	D2
ENZYMES			
ALT (U/L)	5 ± 1	7 ± 2	6 ± 2
	5a	7a	7a
AST (U/L)	322 ± 54A	347 ± 69A	424 ± 76B
	307	316	456
ALP (U/L)	3602 ± 1769	3609 ± 1681	3570 ± 1813
	2086a	3023a	3061a
GGT (U/L)	17 ± 3A	17 ± 3A	18 ± 2A
	17	18	17
PROTEINS			
Alb (g/dL)	1,6 ± 0,3	1,5 ± 0,2	1,6 ± 0,2
	1,7a	1,6a	1,7a
TSP (g/dL)	2,8 ± 0,3	2,8 ± 0,2	2,9 ± 0,3
	2,9a	2,8a	2,9a
METABOLITES			
UA (mg/dL)	5 ± 1	5 ± 1	5 ± 1
	5a	5a	6a
Chol (mg/dL)	137 ± 14	134 ± 14	121 ± 11
	139a	134ab	121b
Glu (mg/dL)	281 ± 20A	273 ± 20A	278 ± 17A
	288	274	275
Lac (mg/dL)	52 ± 10	53 ± 10	52 ± 10
	54a	54a	54a
Trig (mg/dL)	128 ± 26	130 ± 25	178 ± 40
	126a	130a	178b
MINERALS			
Ca (mg/dL)	10 ± 1	10 ± 1	9 ± 1
	10a	10a	9a
P (mg/dL)	7 ± 2	6 ± 1	5 ± 1
	7a	6ab	5b
Mg (mg/dL)	2,1 ± 0,3A	2,5 ± 0,4A	2,0 ± 0,7A
	2,0	2,5	2,0

Data with homogeneity ($P > 0.05$): Means (rows) with the same uppercase letter (A and B) are statistically equal (T-Test, ANOVA, and Tukey). Otherwise ($P < 0.05$), medians (rows) with the same lowercase letter (a and b) are statistically similar (Kruskal-Wallis, Friedman, and Dunn).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl Transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium.

Table 3
Effects of refrigeration temperature (RF: 6 ± 2°C) on the analyzed parameters in broilers (*Gallus gallus domesticus*) at different time points: control group (CG – Immediate analysis - D0), Day 1 (D1), Day 2 (D2), and Day 8 (D8) (n = 10 broilers per analyte).

Parameters	CG	RF		
	D0	D1	D2	D8
ENZYMES				
ALT (U/L)	5 ± 1	7 ± 2	7 ± 2	20 ± 9
	5a	6a	6a	18b
AST (U/L)	322 ± 54A	558 ± 155B	516 ± 138B	496 ± 132B
	307	590	527	493
ALP (U/L)	3602 ± 1769	3089 ± 1241	3248 ± 1340	3352 ± 1476
	3086a	2866a	2948a	2999a
GGT (U/L)	17 ± 3A	17 ± 2A	17 ± 3A	16 ± 3A
	17	17	17	16
PROTEINS				
Alb (g/dL)	1,6 ± 0,3	1,8 ± 0,3	1,6 ± 0,2	1,6 ± 0,2
	1,7a	1,8a	1,7a	1,6a
TSP (g/dL)	2,8 ± 0,3	2,8 ± 0,4	2,9 ± 0,2	3,2 ± 0,2
	2,9a	2,9a	3a	3,2b
METABOLITES				
UA (mg/dL)	5 ± 1	5 ± 1	4 ± 0,4	6 ± 1
	5a	5ab	4a	6b
Chol (mg/dL)	137 ± 14	145 ± 19	112 ± 10	139 ± 14
	139a	152a	107b	140a
Glu (mg/dL)	281 ± 20A	286 ± 24A	291 ± 24A	281 ± 25A
	288	287	284	277
Lac (mg/dL)	52 ± 10	54 ± 9	52 ± 10	47 ± 12
	54a	56a	55a	52a
Trig (mg/dL)	128 ± 26	121 ± 17	143 ± 42	145 ± 24
	126a	119a	149a	144a
MINERALS				
Ca (mg/dL)	10 ± 1	10 ± 1	10 ± 1	10 ± 1
	10a	10a	10a	11a
P (mg/dL)	7 ± 2	6 ± 1	7 ± 2	10 ± 3
	7ab	6a	6ab	11b
Mg (mg/dL)	2,1 ± 0,3A	1,8 ± 0,2AB	1,7 ± 0,3B	1,7 ± 0,3B
	2,0	1,8	1,7	1,7

Data with homogeneity ($P > 0.05$): means (rows) with the same uppercase letter (A and B) are statistically equal (T-test, ANOVA, and Tukey). Otherwise ($P < 0.05$), medians (rows) with the same lowercase letter (a and b) are statistically similar (Kruskal-Wallis, Friedman, and Dunn).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl Transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium.

but its values decreased ($P < 0.05$) in UF (-80 ± 5°C) compared to RF (6 ± 2°C). Glu levels decreased ($P < 0.05$) in FZ (-20 ± 5°C) compared to RF (6 ± 2°C). Trig concentrations remained stable ($P > 0.05$) across all temperatures compared to CG; however, samples stored at RT (27 ± 2°C) and RF (6 ± 2°C) differed significantly ($P < 0.05$) from those stored in FZ (-20 ± 5°C) and UF (-80 ± 5°C), showing a reduction in the latter. Ca levels decreased in FZ (-20 ± 5°C) ($P < 0.05$). P values showed a reduction ($P < 0.05$) in samples stored in FZ (-20 ± 5°C). Mg remained stable ($P > 0.05$) across all analyzed temperatures compared to CG; however, Mg levels in RF (6 ± 2°C) were lower ($P < 0.05$) than in RT (27 ± 2°C), FZ (-20 ± 5°C), and UF (-80 ± 5°C). The biochemical data regarding the influence of storage temperature are presented in Table 6.

Discussion

The characterization of biochemical parameters in production birds, particularly in broilers, remains limited in the scientific literature, which highlights the relevance of the findings of this study. Some of the discoveries, such as the feasibility of analyzing samples subjected to ultra-freezing for up to one year, represent important advancements in the field. Given the scarcity of species-specific data, the discussion was based on studies involving other species which, despite physiological differences, provided valuable insights for interpreting the results.

The alteration of the ALT enzyme observed in this study is consistent with findings reported in goats (Divya and Jayavardhanan, 2010) and humans (Schimizu and Ichihara, 2019). ALT activity was unstable FZ (−20 ± 5°C) and UF (−80 ± 5°C) conditions. Therefore, it can be concluded that freezing and ultra-freezing temperatures cause structural changes in this enzyme in broilers. On the other hand, the instability of ALT compared to other biochemical parameters, as noted by Hochleithner et al. (2005a), may be attributed to the inherent fragility of this marker in avian species. Additionally, studies in dogs and humans indicate that ALT remains stable at RF and FZ for varying periods suggesting that its activity requires further investigation to optimize storage and analytical conditions (Pineli et al., 2017; Silva et al., 2017). The stability of biochemical parameters under different storage conditions is essential to ensure the reliability of laboratory analyses in production birds. In the present study, AST activity was significantly affected by storage, and it is recommended that analyses be performed on fresh samples within 24 hours of collection. This instability, previously reported in humans (Ikeda et al., 2015), may be related to the enzyme's sensitivity to protein degradation, aggregation, pH variations, and the dissolution of substrates and cofactors (Herrmann et al., 2001).

In contrast, ALP activity demonstrated stability at all tested temperatures (RT, RF, and FZ), in agreement with findings in different species, such as humans (Ikeda et al., 2015; Schimizu and Ichihara,

Table 4
- Effects of freezing temperature (FZ: -20 ± 5°C) on the parameters analyzed in broilers (*Gallus gallus domesticus*) at different time points: control group (CG – Immediate analysis - D0), Day 8 (D8), Day 14 (D14), and Day 30 (D30) ($n = 10$ broilers per analyte).

Parameters	CG	FZ		
	D0	D8	D14	D30
ENZYMES				
ALT (U/L)	5 ± 1 5a	15 ± 6 15b	20 ± 6 21b	22 ± 7 24b
AST (U/L)	322 ± 54A 307	442 ± 125AB 405	490 ± 137B 509	502 ± 134B 513
ALP (U/L)	3602 ± 1769 3086a	4078 ± 1843 5214a	2840 ± 963 2875a	2756 ± 1338 2650a
GGT (U/L)	17 ± 3A 17	13 ± 2B 13	13 ± 5AB 14	16 ± 3AB 16
PROTEINS				
Alb (g/dL)	1,6 ± 0,3 1,7a	1,0 ± 0,2 1,1b	1,7 ± 0,2 1,7a	1,6 ± 0,2 1,6a
TSP (g/dL)	2,8 ± 0,3 2,9ab	2,9 ± 0,3 2,9ab	2,8 ± 0,2 2,9a	3,1 ± 0,1 3b
METABOLITES				
UA (mg/dL)	5 ± 1 5a	5 ± 1 5ab	6 ± 1 6bc	7 ± 1 6c
Chol (mg/dL)	137 ± 14 139a	230 ± 14 233b	123 ± 8 126a	125 ± 9 125a
Glu (mg/dL)	281 ± 20A 288	270 ± 19AB 270	256 ± 15B 252	261 ± 17AB 257
Lac (mg/dL)	52 ± 10 54a	51 ± 10 53a	47 ± 10 48a	50 ± 10 51a
Trig (mg/dL)	128 ± 26 126a	143 ± 25 151a	110 ± 19 102ab	78 ± 28 71b
MINERALS				
Ca (mg/dL)	10 ± 1 10a	9 ± 1 9ab	8 ± 1 9bc	7 ± 1 7c
P (mg/dL)	7 ± 2 7a	4 ± 1 4b	4 ± 1 4b	5 ± 1 5ab
Mg (mg/dL)	2,1 ± 0,3A 2,0	2,6 ± 0,3B 2,7	2,0 ± 0,3A 2,0	2,1 ± 0,2A 2,1

Data with homogeneity ($P > 0.05$), means (rows) with the same uppercase letter (A and B) are statistically equal (T-Test, ANOVA, and Tukey). Otherwise ($P < 0.05$), medians (rows) with the same lowercase letter (a and b) are statistically similar (Kruskal-Wallis, Friedman, and Dunn).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl Transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium.

Table 5
Effects of ultra freezing temperature (UF: -80 ± 5°C) on the analyzed parameters in broilers (*Gallus gallus domesticus*) at different time points: control group (CG – Immediate analysis - D0), Day 30 (D30), Day 180 (D180), and Day 365 (D365) ($n = 10$ broilers per analyte).

Parameters	CG	UF		
	D0	D30	D180	D365
ENZYMES				
ALT (U/L)	5 ± 1 5a	10 ± 2 9b	25 ± 4 26b	4 ± 3 4a
AST (U/L)	322 ± 54A 307	511 ± 143B 525	579 ± 155B 573	463 ± 117AB 473
ALP (U/L)	3602 ± 1769 3086a	2870 ± 653 3009a	1777 ± 575 1780b	2690 ± 725 2550ab
GGT (U/L)	17 ± 3A 17	20 ± 2A 20	21 ± 6A 19	18 ± 7A 18
PROTEINS				
Alb (g/dL)	1,6 ± 0,3 1,7a	1,5 ± 0,1 1,5a	1,4 ± 0,2 1,5a	1,4 ± 0,2 1,5a
TSP (g/dL)	2,8 ± 0,3 2,9ab	2,8 ± 0,1 2,9a	3,1 ± 0,2 3,1bc	3,2 ± 0,2 3,2c
METABOLITES				
UA (mg/dL)	5 ± 1 5ab	6 ± 1 6a	4 ± 1 4b	5 ± 1 5ab
Chol (mg/dL)	137 ± 14 139a	125 ± 17 126a	144 ± 22 148a	123 ± 12 124a
Glu (mg/dL)	281 ± 20AB 288	271 ± 21AB 269	285 ± 20A 288	257 ± 30B 259
Lac (mg/dL)	52 ± 10 54a	49 ± 8 50a	52 ± 12 52a	51 ± 11 53a
Trig (mg/dL)	128 ± 26 126a	106 ± 9 104a	118 ± 25 120a	101 ± 22 101a
MINERALS				
Ca (mg/dL)	10 ± 1 10a	10 ± 1 10a	10 ± 1 9a	10 ± 1 10a
P (mg/dL)	7 ± 2 7a	6 ± 1 6a	7 ± 1 8a	6 ± 1 6a
Mg (mg/dL)	2,1 ± 0,3A 2,0	1,8 ± 0,3B 1,9	2,4 ± 0,2C 2,4	2,3 ± 0,5AC 2,3

Data with homogeneity ($P > 0.05$), means (rows) with the same uppercase letter (A and B) are statistically equal (T-Test, ANOVA, and Tukey). Otherwise ($P < 0.05$), medians (rows) with the same lowercase letter (a and b) are statistically similar (Kruskal-Wallis, Friedman, and Dunn).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl Transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium.

2019), rats (Singh et al., 2015), horses (Oliveira et al., 2016), dogs (Pineli et al., 2017), and turtles (Camargo et al., 2020). Similarly, UF maintained activity up to 30 days, with a decrease only at D180, which may be attributed to the structural thermal resistance of the enzyme (Dong et al., 2021; Fernandez and Kidney, 2007; Zander et al., 2014).

GGT exhibited a distinct behavior: despite the stability reported in humans and dogs over extended freezing periods (Pineli et al., 2017; Schimizu and Ichihara, 2019; Tanner et al., 2008), this study observed a significant decrease in its activity starting from D8. A similar finding was reported in turtles (Camargo et al., 2020), suggesting a possible greater sensitivity of this enzyme in birds.

It was observed that Alb values at RT and RF remained stable throughout the analyzed time points, similar to what has been reported in humans (Oddoze et al., 2012), as well as under UF conditions. Under FZ conditions, a decrease in Alb values was observed, which is consistent with findings in turtles (Camargo et al., 2020) and dogs (Pineli et al., 2017) under the same temperature conditions due to prolonged storage.

TSP showed stability at room and refrigerated temperatures for up to two days, and complete stability under FZ conditions throughout the evaluated period, a result consistent with observations in several species such as lambs (Oliveira et al., 2011), humans (Cuhadar et al., 2013), turtles (Camargo et al., 2020), and cattle (Kovačević et al., 2021; Megerssa and Gari, 2024). Additionally, UF remained stable for up to 30 days of sample storage. The method used, biuret, is described as a reliable technique for determining TSP (Kaplan et al., 2015). However, changes in TSP values in samples stored for one year may be related to the physicochemical effects of serum components during the thawing process after an extended period (Cuhadar et al., 2013; Nagyová et al., 2016).

Regarding UA, early instability was detected at RT and FZ, possibly due to variations in the solubility of the compound in acidic environments (Boyanton and Blick, 2002), corroborating findings in human serum (Heins et al., 1995).

Chol, in turn, increased in the first days of freezing, as described by Wachholz et al. (2023), an effect likely related to changes in circulating lipoproteins and interference from specific biochemical methods (Pini et al., 1990).

Glu levels were stable at RT and RF, a result consistent with the literature and attributed to the action of sodium fluoride in inhibiting glycolysis (Chan and Cockram, 1989; Oddoze et al., 2012). However, at FZ, instability was observed starting from the second week, diverging from some reports showing a linear increase (Wachholz et al., 2023), reinforcing that fluoride is not effective in preserving glucose at −20°C (Astles et al., 1994; Clark et al., 1990). In frozen human serum samples, glucose has shown stability for up to 3 months (Cuhadar et al., 2013).

Lac, on the other hand, remained stable at all temperatures and time

Table 6
Effect of ambient temperature (RT: 27 ± 2°C), refrigeration temperature (RF: 6 ± 2°C), freezing temperature (FZ: -20 ± 5°C), and ultra-freezing temperature (UF: -80 ± 5°C), compared to the control group (CG – immediate analysis) for each biochemical analyte in broilers (*Gallus gallus domesticus*) (n = 10 broilers per parameter).

Parameters	CG	RT	RF	FZ	UF
ENZYMES					
ALT (U/L)	5 ± 1 5a	6 ± 2 7ab	11 ± 8 8bd	19 ± 7 20c	13 ± 9 9d
AST (U/L)	322 ± 54A 307	386 ± 81AC 385	523 ± 139B 529	478 ± 130BC 485	518 ± 143B 516
ALP (U/L)	3602 ± 1769 3086a	3589 ± 1702 3049a	3229 ± 1313 2893a	3225 ± 1508 2820a	2446 ± 797 2434a
GGT (U/L)	17 ± 3ABC 17	18 ± 2AC 17	17 ± 3AB 16	14 ± 4B 14	20 ± 5C 20
PROTEINS					
Alb (g/dL)	1,6 ± 0,3 1,7ab	1,6 ± 0,2 1,6ab	1,7 ± 0,2 1,7a	1,5 ± 0,3 1,5ab	1,4 ± 0,2 1,5b
TSP (g/dL)	2,8 ± 0,3 2,9a	2,9 ± 0,2 2,9a	2,9 ± 0,3 3,0a	2,9 ± 0,2 2,9a	3,0 ± 0,2 3,0a
METABOLITES					
UA (mg/dL)	5 ± 1 5a	5 ± 1 5a	5 ± 1 5a	6 ± 1 6a	5 ± 1 5a
Chol (mg/dL)	137 ± 14 139a	128 ± 14 128a	132 ± 20 128a	159 ± 52 132a	130 ± 20 129a
Glu (mg/dL)	281 ± 20AB 288	275 ± 18AB 274	286 ± 24A 284	263 ± 17B 258	271 ± 26AB 274
Lac (mg/dL)	52 ± 10 54a	53 ± 10 54a	51 ± 11 54a	50 ± 10 50a	51 ± 10 51a
Trig (mg/dL)	128 ± 26 126ab	154 ± 41 152a	140 ± 32 128a	110 ± 36 105b	108 ± 21 106b
MINERALS					
Ca (mg/dL)	10 ± 1 10a	10 ± 1 9a	10 ± 1 10a	8 ± 1 8b	10 ± 1 10a
P (mg/dL)	7 ± 2 7ab	5 ± 1 6ac	8 ± 3 7b	4 ± 1 4c	7 ± 1 6b
Mg (mg/dL)	2,1 ± 0,3AB 2,0	2,2 ± 0,6A 2,3	1,8 ± 0,2B 1,7	2,2 ± 0,6A 2,1	2,2 ± 0,4A 2,3

Data with homogeneity ($P > 0.05$), means (rows) with the same uppercase letter (A and B) are statistically equal (T-test, ANOVA, and Tukey). Otherwise ($P < 0.05$), medians (rows) with the same lowercase letter (a and b) are statistically similar (Kruskal-Wallis, Friedman, and Dunn).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; Alb, albumin; TSP, total serum proteins; UA, uric acid; Chol, total cholesterol; Glu, glucose; Lac, lactate; Trig, triglycerides; Ca, calcium; P, phosphorus; Mg, magnesium.

points, possibly due to the structural robustness of the molecule (Pederiva et al., 2022), in line with findings in horses (Oliveira et al., 2016).

Trig measurement remained stable at RT until D1, and at RF and UF throughout the period. Although the literature indicates stability even after freeze-thaw cycles (Cuhadar et al., 2012; Paltiel et al., 2008), this study observed a decrease in levels starting from the second week at FZ, indicating sensitivity to temperature (Megerssa and Gari, 2024). This instability was also evidenced in broilers by Wachholz et al. (2023), who described a linear increase in values over time.

Ca remained stable for two days at RT and for up to one year under UF, but decreased at FZ, as observed in horses (Schumacher et al., 2017) and felines (Souza et al., 2012). This instability may be associated with acidification caused by CO_2 diffusion (Constable et al., 2018; Stanford, 2005b). Refrigeration kept levels stable for up to eight days, a result consistent with other studies in horses (Sanmartí et al., 2023; Schenck et al., 1996; Szenci et al., 1994).

P exhibited instability at RT, similar to the findings of Moe et al. (2018) in pigs, and at FZ; however, it remained stable at RF and UF. As observed by Wagner, 2023, the presence of phosphorus in the form of inorganic phosphates or bound to macromolecules provides greater structural resistance to degradation.

Mg levels remained stable at RT and RF until D1. The instability observed at FZ and UF is consistent with findings in lambs (Oliveira et al., 2011), and may impact diagnostic accuracy, as discussed by Viveros et al. (2002).

It is important to note that the handling of biochemical samples is subject to significant alterations caused by freeze-thaw cycles (Guglielmo et al., 2002). For this reason, in this study, each sample aliquot was discarded after processing, and subsequent analyses were performed using previously separated aliquots from the same sample. This approach indicates that each aliquot was analyzed only once, with no repeated measurements afters freeze-thaw cycles. Consequently, the study did not assess the impact of such cycles on analyte stability from the same sample, which represents a relevant methodological limitation from a practical laboratory perspective. Each cycle may compromise the integrity of biochemical compounds. Therefore, future studies are recommended to investigate the stability of analytes subjected to multiple freeze thaw cycles to enhance the practical applicability of the findings.

Finally, it is worth highlighting that fresh samples processed immediately after collection (D0) were used as controls, providing greater accuracy in evaluating changes over time and under different temperatures. Although some observed values remained within the reference ranges for broilers (Paterlini et al., 2023), the use of specific controls for each sample (CG – D0) strengthens the methodological validity of the study, allowing for a more accurate measurement of the storage effects on biochemical analyses.

Conclusion

The storage times and temperatures influence the stability of enzymatic activities and values according to biochemical analytes in healthy broilers. This should be taken into consideration by field veterinarians when it is necessary to store samples for biochemical analyses in production broilers. Under the storage conditions evaluated in this study, the parameters that remained stable for the longest periods were: ALT - RT or RF up to 2 days post-collection; AST - RT up to 1 day; ALP and TSP - FZ or UF up to 30 days; Mg - RT up to 2 days; GGT, Alb, UA, Chol, Glu, Lac, Trig, Ca, and P - UF up to one year after blood collection. It is noteworthy that Lac was the only parameter that remained stable under all evaluated conditions and time points. One of the challenges of this study was the difficulty in explaining the variations in the levels of some biochemical parameters, highlighting the need for further research to understand the internal or external factors influencing these changes.

Disclosures

We, Katalina Cifuentes Ruiz, Andriele Ferreira Qualhato, Luis Fernando Duarte Albuquerque, Thays de Campos Trentin, Carmen Elena Barragán Ruiz, Gonzalo Marin Oviedo, Marcos Barcellos Café, Ana Flávia Machado Botelho, and Danieli Brolo Martins, authors of the manuscript entitled "*Influence of Temperature and Storage Time on the Stability of Biochemical Parameters in Broilers*", declare that there is no conflict of interest related to the preparation and submission of the aforementioned work to the journal *Poultry Science*.

We affirm that there are no financial, professional, or personal relationships that could have influenced the data, analyses, or conclusions presented in this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105568.

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