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Light intensity, blood fraction, fasting and storage time affect blood biochemical metabolites in broiler chickens

A intensidade luminosa, a fração sanguínea, o tempo de jejum e armazenamento afetam os metabólitos bioquímicos sanguíneos em frangos de corte

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Highlights

Samples for AST analysis can be stored up to 120 days at -20°C.
Serum glucose, cholesterol, triglycerides, AST, ALT, and GGT analysis is indicated.
Glucose, triglyceride, AST, and ALT concentrations highest at 20 lux.
Serum cholesterol and triglyceride increased linearly with storage time.

Abstract

We determined the impacts of light intensity, blood fraction, fasting and storage time on glucose, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) concentrations in 140 broiler chickens assigned to two light intensities (5 or 20 lux/m²) and seven fasting times (0, 2, 4, 6, 8, 10, and 12 h). The collected blood was fractionated into serum or plasma and stored at -20°C for 0, 15, 30, 60, and 120 days. Serum glucose concentrations declined linearly by 1.15 mg dL^{-1} every 2 h of fasting, whereas plasma glucose responded quadratically (minimum point at 8 h and 13 min of fasting). Serum ALT concentrations showed a quadratic effect, with a maximum

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point at 3 h and 46 min of fasting. Serum GGT concentrations were not influenced by fasting time, whereas plasma decreased by 1.60 IU L^{-1} at each 2 h of fasting. The AST concentrations were influenced by fasting. Storage times influenced the glucose concentrations in a quadratic manner, with maximum points in serum and plasma at 95 and 72 days, respectively. Serum cholesterol and triglyceride concentrations increased linearly with storage time. Storage time caused a quadratic response on serum ALT, with a maximum point at 32 days, whereas there was a minimum point at 93 days in plasma. Concentrations of GGT increased linearly with storage. Glucose, triglycerides, AST, and ALT concentrations were highest at 20 lux. Samples for AST analysis can be stored up to 120 days at -20°C. Serum glucose, cholesterol, triglyceride, ALT, and GGT analysis is indicated with a 6 h fasting time, without freezing. The light intensity should be specified.
Key words: Blood components. Lux. Plasma. Poultry. Serum.

Resumo

Nós determinamos os impactos da intensidade luminosa, da fração sanguínea, do jejum e do tempo de armazenamento sobre as concentrações de glicose, colesterol, triglicérides, aspartato aminotransferase (AST), alanina aminotransferase (ALT) e gama-glutamil transferase (GGT) em 140 frangos de corte, distribuídos em duas intensidades de luz (5 ou 20 lux/m^2) e sete tempos de jejum (0, 2, 4, 6, 8, 10 e 12 h). O sangue coletado foi fracionado em soro ou plasma e armazenado a -20°C por 0, 15, 30, 60 e 120 dias. As concentrações de glicose no soro diminuíram linearmente em 1,15 mg dL^{-1} a cada 2 h de jejum, enquanto a glicose no plasma respondeu quadraticamente (ponto mínimo às 8 h e 13 min de jejum). As concentrações séricas de ALT apresentaram um efeito quadrático com um ponto máximo às 3 h e 46 min de jejum. As concentrações séricas de GGT não foram influenciadas pelo tempo de jejum, enquanto no plasma diminuiu em 1,60 UI L^{-1} a cada 2 h de jejum. As concentrações de AST foram influenciadas pelo jejum. Os tempos de armazenamento influenciaram as concentrações de glicose de forma quadrática, com pontos máximos no soro e no plasma em 95 e 72 dias, respectivamente. As concentrações de colesterol e triglicerídeos no soro aumentaram linearmente com o tempo de armazenamento. O tempo de armazenamento causou uma resposta quadrática na ALT sérica com um ponto máximo aos 32 dias, enquanto houve um ponto mínimo aos 93 dias no plasma. As concentrações de GGT aumentaram linearmente com o armazenamento. As concentrações de glicose, triglicerídeos, AST e ALT foram mais altas em 20 lux. As amostras para análise de AST podem ser armazenadas por até 120 dias a -20°C. A fração de soro para análise de glicose, colesterol, triglicerídeos, ALT e GGT é indicada com um tempo de jejum de 6 h, evitando o congelamento. A intensidade luminosa deve ser especificada.
Palavras-chave: Componentes sanguíneos. Lux. Plasma. Aves. Soro.

Introduction

Advances in animal production research improve productivity through nutrition, environment, health, and management. However, there is a need for accurate tools and less invasive methods. In this context, the analysis of blood biochemical constituents assists researchers in identifying metabolic disorders without euthanizing the animals.

Biochemical analyses represent a research tool that aims to assess health (Islam et al., 2012; Wang et al., 2012) and nutritional patterns in poultry (Andrade et al., 2017; I. M. Silva et al., 2019). Determinations of the concentrations of glucose, total cholesterol, triglycerides, and the activities of AST, ALT, and GGT are widely used in experimental assessments involving broiler chickens. However, to date, few studies have evaluated the effects of factors such as light intensity, blood fraction, fasting and storage time on blood biochemical profiles.

Diet directly influences the blood profile (Oke et al., 2017) because blood carries nutrients to the body tissues. Thus, fasting time is used in most blood analyses. However, there is a wide interstudy methodological variability in blood collection, without the need to use prior fasting (Córdova-Noboa et al., 2018) or with variations from 2 to 12 h of fasting (Behboudi et al., 2016; Sadeghi et al., 2014; Swarna et al., 2018; Zakaria et al., 2017). Moreover, the storage time for the blood samples to be processed should be determined so as not to alter the concentrations of blood metabolites. In the scientific literature, there are studies only with other species, such as humans (Cuhadar et al., 2013), dogs (É. P. D. Silva et al., 2017; Thoresen et al., 1995), lambs (Oliveira et al., 2011), goats (Divya & Jayavardhanan, 2010), and rats (Spinelli et al., 2012).

The use of serum or plasma for blood biochemical analyses in broiler chickens is another questionable doubt due to studies that have compared these blood fractions with those of other species (Burtis et al., 2012; B. R. Silva et al., 2015). In addition, there is disagreement between studies that used serum (Gilani et al., 2018; Subhani et al., 2018) or plasma (Kim et al., 2019; Yang et al., 2017), and to date, studies on broiler chickens are scarce. In addition, light intensity influences broiler chicken's behavior at a physiological level, including altering the concentrations of blood metabolites (Fidan et al., 2017). In this sense, conducting studies aiming to specify this effect is also important because blood analyses of broiler chickens housed at different light intensities largely do not take into account this source of variation.

Here, our hypothesis was that both independent variables tested can significantly alter blood metabolite concentrations and consequently affect the results in broiler chicken studies. Therefore, we aimed to determine the impacts of light intensity, blood fraction, fasting, and storage time on the concentrations of glucose, cholesterol, triglycerides, AST, ALT, and GGT in broiler chickens.

The present study was conducted at the Universidade Estadual do Oeste do Paraná (Unioeste), Marechal Cândido Rondon, PR, Brazil. All experimental procedures were approved by the Ethics Committee on Animal Use of Unioeste (protocol no. 23/20).

Animals, housing, diets

A total of 140 male broiler chickens (45 days old, Cobb 500® commercial line, 3,123 ± 654 g) were raised from 1 to 42 days of age under the same environment, receiving diet and water *ad libitum.* The animals were housed in a climate-controlled aviary according to each growth phase, with a masonry floor covered with pine wood shavings.

The broiler chickens were randomly assigned to two environments at 42 days of age, based on the light intensity provided (5 or 20 lux/m^2 for 3 consecutive days), measured with the aid of a digital lux meter (Instrutherm brand, LD-209 model, São Paulo, SP, Brazil). Each environment housed 70 broiler chickens in 1.96 m² pens (7 replicate pens of 10 birds each), equipped with nipple drinkers and tubular feeders containing the same diet. The diet was fed in mash form based on corn and soybean meal, supplemented with industrial amino acids and formulated to meet the requirements of each phase, following the nutritional requirements proposed by Rostagno et al. (2017).

Blood sampling, preparation, and analysis

Broiler chickens were fasted for 1 h (on day 45) and then fed for 30 min. This procedure was performed so that all birds had the same nutritional or feeding patterns postprandial. After the feeding period, the first blood sample was collected at time zero via brachial puncture in the ulnar vein. Blood was collected from 70 broiler chickens per light intensity (*n* = 10 birds/fasting time randomly selected) in the lateral decubitus position, using 4-mL vacuum needles and tubes with adapters and 25 × 0.8-mm needles at each fasting time (0, 2, 4, 6, 8, 10, and 12 h). Every 10 birds were used only for a single sampling at a given fasting time. Tubes without anticoagulants were used for serum, tubes for blood collection with clot activator sprayed on the tube wall were used to accelerate the clotting process, tubes with sodium fluoride were used to obtain plasma, and the tubes for blood collection contained the glycolytic inhibitor sodium fluoride at 5 mg.

Two blood samples (4 mL) per broiler chicken were collected within the allowable limit for species and body weight (Kelly & Alworth, 2013). Two collections were performed with the same needle, in which the sample for the serum was obtained first. These two draws collected per bird were consecutive. After collection, the tubes were left to rest in a horizontal decubitus position for 15 min at room temperature. Subsequently, they were centrifuged (Kasvi brand, K14-4000, São Paulo, SP, Brazil) at 2,500 g for 10 min to separate serum or plasma, placed in 2-mL microtubes, and stored in a freezer at -20ºC.

One aliquot was immediately analyzed in the blood laboratory of Unioeste. The remaining aliquots (n = 4 microtubes) were stored for 15, 30, 60, and 120 days for further analysis. During the storage time, the freezer temperature was monitored frequently to avoid possible oscillations. Before the readings were taken, the samples were thawed at 2°C to 8°C and then centrifuged in an Eppendorf microcentrifuge (Eppendorf® brand, Minispin®, Hamburg, Germany) at 2,500 g for 10 min to remove the fibrin.

The biochemical parameters were analyzed using an automatic biochemical analyzer equipped with a spectrophotometer (Elitech® brand, Flexor EL200 model, Puteaux, France) with reagents, calibrators, and calibration standards for birds. The readings were performed for glucose [glucose PAP, Trinder's method, enzymatic colorimetric

kinetic (Trinder, 1969)], cholesterol [Trinder's method, enzymatic colorimetric endpoint (Allain et al., 1974)], triglycerides [triglycerides SL New, enzymatic colorimetric endpoint (Fossati & Prencipe, 1982)], alanine aminotransferase [ALT, IFCC method without pyridoxal phosphate, kinetic, UV (Schumann et al., 2002a)], aspartate aminotransferase [AST, IFCC method without pyridoxal phosphate, kinetic, UV (Schumann et al., 2002b)], and gamma-glutamyl transferase [GGT, GAMMA GT plus, Glupa-C substrate method, kinetic (Schumann et al., 2002c)].

Study design and statistical procedures

The experiment was conducted in a factorial scheme considering blood fraction, fasting and storage time, and light intensity as fixed effects. The residual error was considered as a random effect. All data were submitted to normality and homogeneity of variance tests for further analysis of variance (ANOVA). When significant in two-way ANOVA ($p < 0.05$), regression analysis was performed for fasting and storage times. Light intensity effect was tested via ANOVA using the F-test. Triple and quadruple interactions were not analyzed. Double interactions for blood fraction × fasting time, blood fraction × storage time, and the single factors were assessed. All statistical procedures were performed using the GLM package of SAS.

Results and Discussion

Energy pathway metabolites

There was an interaction effect ($p < 0.05$) between fasting time × blood fraction on glucose, cholesterol, and triglyceride concentrations (Table 1). There was an interaction effect ($p < 0.05$) of storage time × blood fraction on glucose and triglyceride concentrations, but no effect was observed on cholesterol concentrations.

Serum glucose concentrations declined linearly by 1.15 mg dL^{-1} every 2 h of fasting (Figure 1), and plasma glucose responded in a quadratic manner, with a minimum glucose point of 217 mg dL^{-1} at 8 h and 13 min of fasting. Serum and plasma cholesterol concentrations declined linearly by 0.39 and 1.25 mg dL^{-1}, respectively, every 2 h of fasting. Triglyceride concentrations showed a quadratic effect, with minimum points of 30.25 mg dL^{-1} at 7 h and 49 min of fasting on serum and 35.91 mg dL^{-1} at 8 h and 17 min of fasting on plasma.

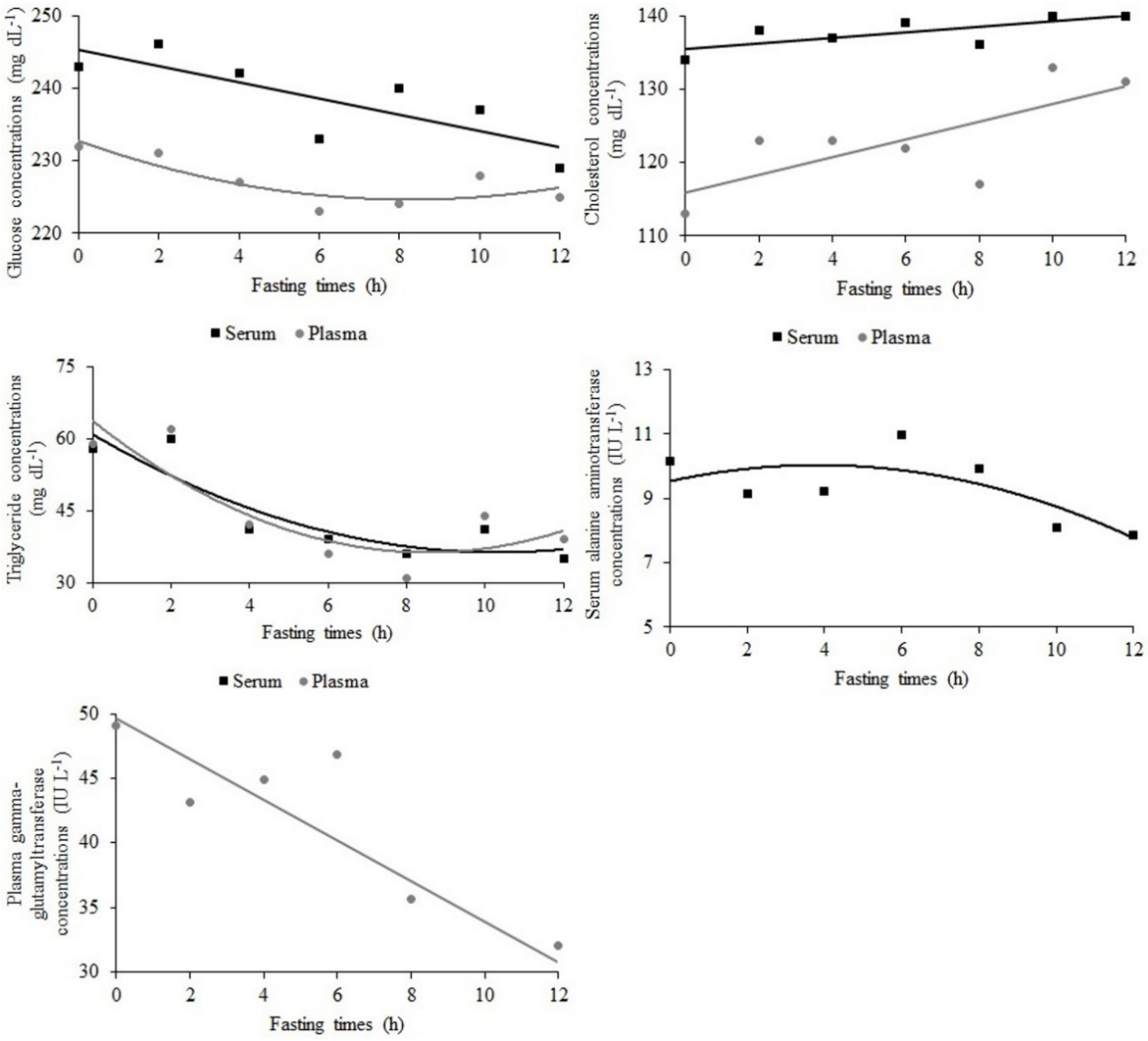

Figure 1. Serum and plasma concentrations of glucose, cholesterol, triglycerides, alanine aminotransferase and gamma-glutamyl transferase activity in broiler chickens at 45 days subjected to different fasting times.

Storage times affected serum and plasma glucose concentrations in a quadratic manner, with maximum points at 95 and 72 days, respectively (Figure 2). Serum triglyceride concentrations increased linearly ($p < 0.05$), but plasma triglyceride concentrations were not influenced by storage time. In addition, the cholesterol concentrations increased linearly ($p < 0.05$) with storage time. Moreover, the concentrations were higher ($p < 0.05$) at a light intensity of 20 lux/m^2, except for cholesterol concentrations, in which broiler chickens subjected to the intensity of 5 lux/m^2 had higher concentrations (Table 1).

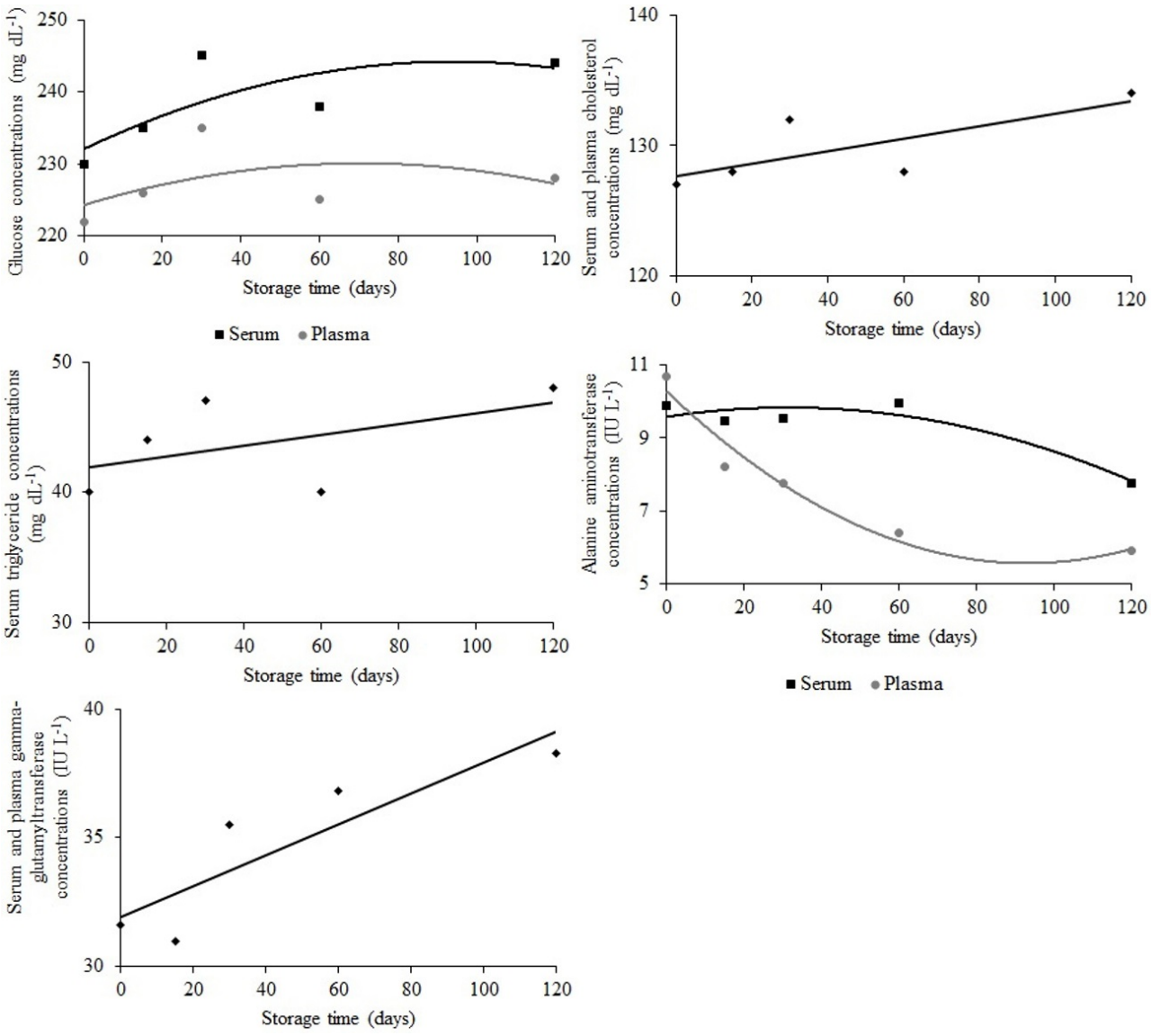

Figure 2. Glucose, cholesterol, triglycerides concentrations, and alanine aminotransferase and gamma-glutamyl transferase activity of serum and plasma fractions subjected to different storage times in broiler chickens at 45 days.

The reduction in serum glucose concentrations and the initial decrease in plasma glucose are similar to those reported by Rodrigues et al. (2017), who observed that fasting reduced the circulating glucose concentration due to an insufficient glycogen content.

The linear increase in circulating concentrations of cholesterol and triglycerides occurs because the broiler chickens are in a fasting state. This promotes the mobilization of triglycerides from adipose tissue to be directed to the bloodstream for transport via intermediate density lipoproteins, which represent a cholesterol fraction (Lumeij, 2008). The reduced glucose concentrations, with a subsequent minimum point at 92 days of storage on serum and 75 days on plasma, are explained by the fact that storage at -20ºC did not maintain the analyte stable. This finding corroborates that reported by Clark et al. (1990), who assessed the stability of plasma glucose with sodium fluoride in human blood. However, Cuhadar et al. (2013) reported stable glucose concentrations in humans throughout the storage time. This suggests that the stability of the glucose concentration depends on the storage time and the initial glucose content, as well as an inevitable and constant loss in the presence of degradative enzymes, as reported by Clark et al. (1990). Cholesterol concentrations showed a linear increase related to storage time under freezing at -20°C. This is similar to the findings of Pini et al. (1990) and a result of changes in circulating lipoprotein compositions due to thermal reasons. This causes the analyte values to increase with storage time; further, according to the authors, the colorimetric enzymatic method also underestimates the cholesterol concentrations in samples that are not frozen.

The increasing linear response presented by the triglyceride concentrations according to storage time is explained by lipid redistribution through the physical breakdown of lipoproteins, as described by Evans et al. (1995). Also, variations in the concentrations of this analyte are described in a study involving humans (Cuhadar et al., 2013).

The fact that broiler chickens subjected to the highest light intensity (20 lux) had higher glucose concentrations than those kept at the lowest intensity (5 lux) may be related to welfare. Puvadolpirod and Thaxton (2000) reported increased circulating glucose concentrations as indicative of greater stress in broiler chickens. This effect supports one of the goals of using lower light intensities to reduce stress in broiler chickens, according to Fidan et al. (2017), who also found increased triglyceride concentrations in broiler chickens kept at higher light intensities.

A higher concentration of circulating glucose may occur due to increased physical activity (Olanrewaju et al., 2010) and stress-associated metabolic changes in broiler chickens subjected to higher light intensity (Puvadolpirod & Thaxton, 2000). This promotes increased energy mobilization for maintenance and a consequent reduction in production performance (Olanrewaju et al., 2010).

Liver enzyme

There was an interaction effect ($p < 0.05$) between fasting time × blood fraction on ALT and GGT activities, but there was no interaction effect on AST activity (Table 2). There was an interaction effect ($p < 0.05$) of storage time × blood fraction on ALT activity, but no effect was observed on AST and GGT activity.

Fasting time had a quadratic effect ($p < 0.05$) on serum ALT activity, with a maximum point of 10.02 IU L^{-1} at 3 h and 46 min of fasting (Figure 1), where as for plasma activities, the linear and quadratic regression models did not adjust suitably to the data. Aspartate aminotransferase activity was influenced by fasting time ($p < 0.05$; Table 2); however, it did not adjust suitably to linear and quadratic regression models. Moreover, serum GGT activity was not influenced by fasting time, whereas the plasma GGT concentrations decreased linearly by 1.60 L^{-1} every 2 h of fasting.

The storage time influenced ($p < 0.05$) the serum ALT activity in a quadratic manner, with a maximum point at 32 days (Figure 2); however, there was a quadratic effect on plasma, with a minimum point at 93 days. In addition, the GGT concentrations increased linearly ($p < 0.05$). Alanine aminotransferase activity was higher ($p < 0.05$) in the serum fraction compared to the plasma fraction. The concentrations were higher ($p < 0.05$) at a light intensity of 20 lux/m^2, except for GGT activity, in which broiler chickens subjected to the intensity of 5 lux/m^2 had higher concentrations (Table 2).

Table 1 - Average concentrations of glucose, total cholesterol and triglycerides in serum and plasma of broiler chickens at 45 days subjected to different fasting and storage times, and light intensities (5 or 20 lux/m²)

Item	Glucose (mg dL⁻¹)			Total cholesterol (mg dL⁻¹)			Triglycerides (mg dL⁻¹)		
	Serum	Plasma	μ²	Serum	Plasma	μ	Serum	Plasma	μ
Fasting times (h)									
0	243 ± 15	232 ± 14	237 ± 15	134 ± 10	113 ± 9	122 ± 14	58 ± 15	59 ± 20	58 ± 18
2	246 ± 16	231 ± 15	237±14	138 ± 14	123 ± 11	130 ± 15	60 ±19	62 ± 17	61 ± 18
4	242 ± 16	227 ± 13	233±16	137 ± 18	123 ± 16	129 ± 18	41 ± 12	42 ± 13	41 ± 12
6	233 ± 13	223 ± 13	228 ±14	139 ± 13	122 ± 13	130 ± 15	39 ± 8	36 ± 7	37 ± 8
8	240 ± 14	224 ± 16	231 ± 17	136 ± 13	117 ± 12	126 ± 16	36 ± 6	31 ± 7	33 ± 7
10	237 ± 15	228 ± 17	232 ± 16	140 ± 12	133 ± 13	136 ± 13	41 ± 7	44 ± 9	43 ± 8
12	229 ± 13	225 ± 17	227 ± 15	140 ± 13	131 ± 15	135 ± 15	35 ± 9	39 ± 13	38 ± 12
Storage times (days)									
0	230 ± 14	222 ± 14	225 ± 15	134 ± 14	120 ± 14	127 ± 16	40 ± 15	42 ± 17	41 ± 16
15	235 ± 13	226 ± 13	230 ± 14	135 ± 14	122 ± 14	128 ± 15	44 ± 15	43 ± 15	43 ± 15
30	245 ± 15	235 ± 15	239 ± 16	141 ± 12	125 ± 15	132 ± 16	47 ± 14	45 ± 16	46 ± 15
60	238 ± 15	225 ± 15	231 ± 17	136 ± 13	122 ± 14	128 ± 15	40 ± 12	44 ± 17	42 ± 15
120	244 ± 15	228 ± 15	235 ± 17	143 ± 13	126 ± 14	134 ± 16	48 ± 16	44 ± 17	46 ± 16
Light intensities (lux)									
5	238 ± 15	225 ± 14	231 ± 16B	141 ± 14	124 ± 13	131 ± 16A	42 ± 13	41 ± 15	42 ± 14B
20	238 ± 16	229 ± 16	233 ± 16A	135 ± 13	123 ± 16	128 ± 16B	46 ± 16	46 ± 17	46 ± 16A
μ	238 ± 15	227 ± 15	232 ± 16	138 ± 13	123 ± 14	130 ± 0.45	44 ± 15	44 ± 16	44 ± 16
SEM[1]	0.47			0.45			15.76		
p fasting times	<0.001			<0.001			<0.001		
p blood fraction	<0.001			<0.001			0.649		
p storage times	<0.001			<0.001			<0.001		
p light intensities	0.026			<0.001			<0.001		
p fasting times × blood fraction	<0.001			<0.001			0.001		
L	<0.001	0.004		0.006	<0.001		<0.001	<0.001	
Q	<0.021	<0.001		0.012	<0.001		<0.001	<0.001	
p storage times × blood fraction	0.021			0.554			0.002		
L	<0.001	0.078		<0.001			0.006	0.117	
Q	<0.002	0.001		0.974			0.527	0.292	

[1]SEM: pooled standard error of the mean.
p: significance level.
Q: quadratic effect.

[2]μ: average.
L: linear effect.
A–BCapital letters in the same column differ by the F-test ($p < 0.05$).

Table 2 - Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) activity in serum and plasma of broiler chickens at 45 days subjected to different fasting and storage times, and light intensities (5 or 20 lux/m²)

Item	AST (IU L⁻¹)			ALT (IU L⁻¹)			GGT (IU L⁻¹)		
	Serum	Plasma	μ[2]	Serum	Plasma	μ	Serum	Plasma	μ
Fasting times (h)									
0	384 ± 107	320 ± 112	348 ± 114	10.14 ± 3.17	8.23 ± 3.44	9.08 ± 3.45	29.01 ± 9.55	49.10 ± 12.21	38.53 ± 14.80
2	345 ± 92	299 ± 107	319 ± 103	9.14 ± 3.15	6.83 ± 3.64	7.85 ± 3.61	30.88 ± 9.80	43.13 ± 15.49	36.93 ± 14.28
4	395 ± 137	347 ± 132	368 ± 136	9.21 ± 3.29	8.07 ± 3.84	8.57 ± 3.64	30.34 ± 9.11	44.89 ± 11.54	38.29 ± 12.78
6	365 ± 102	320 ± 122	340 ± 115	10.97 ± 3.18	7.87 ± 3.24	9.23 ± 3.56	29.49 ± 5.48	46.87 ± 12.53	37.90 ± 12.92
8	376 ± 107	338 ± 127	355 ± 120	9.94 ± 3.30	8.25 ± 4.03	8.97 ± 3.82	28.97 ± 8.92	35.63 ±12.95	32.57 ± 11.73
10	365 ± 122	358 ± 128	361 ± 125	8.08 ± 3.13	8.11 ± 3.47	8.10 ± 3.30	28.99 ± 7.15	29.52 ± 12.48	29.28 ± 10.39
12	350 ± 92	307 ± 94	326 ± 95	7.85 ± 2.77	6.77 ± 2.39	7.25 ± 2.62	26.68 ± 7.00	32.00 ± 15.28	29.59 ± 12.49
Storage times (days)									
0	359 ±102	319 ± 115	337 ± 111	9.87 ± 4.03	10.69 ± 3.56	10.32 ± 3.79	24.95 ± 7.38	37.21 ± 15.65	31.60 ± 13.96
15	360 ± 103	334 ± 133	346 ± 121	9.48 ± 2.68	8.20 ± 3.33	8.77 ± 3.13	25.95 ± 7.70	35.50 ± 13.23	30.95 ± 11.93
30	365 ± 109	337 ± 119	350 ± 115	9.54 ± 3.16	7.76 ± 3.14	8.55 ± 3.27	30.12 ± 7.78	40.28 ± 14.94	35.48 ± 13.10
60	376 ± 121	314 ± 118	342 ± 123	9.96 ± 3.07	6.41 ± 2.80	8.00 ± 3.41	31.60 ± 8.63	42.61 ± 16.43	36.81 ± 14.02
120	381 ± 116	329 ± 111	352 ± 116	7.75 ± 3.04	5.90 ± 2.66	6.72 ± 2.98	33.64 ± 7.40	42.57 ± 13.72	38.26 ± 11.97
Light intensities (lux)									
5	362 ± 109	311 ± 119	334 ± 117[B]	9.00 ± 3.28	7.47 ± 3.55	8.15 ± 3.51[B]	30.60 ± 8.55	42.97 ± 13.23	36.82 ± 12.75[A]
20	375 ± 112	342 ± 118	357 ± 116[A]	9.63 ± 3.29	7.99 ± 3.45	8.73 ± 3.47[A]	27.79 ± 7.87	36.46 ± 15.86	32.44 ± 13.49[B]
μ	368 ± 110[a]	327 ± 119[b]	345 ± 117	9.31 ± 3.30	7.73 ± 3.51	8.43 ± 3.50	29.19 ± 8.33	39.50 ± 15.04	34.55 ± 13.31
SEM[1]	3.30			0.10			0.39		
p fasting times	0.002			<0.001			<0.001		
p blood fraction	<0.001			<0.001			<0.001		
p storage times	0.593			<0.001			<0.001		
p light intensities	0.008			0.004			<0.001		
p fasting times × blood fraction	0.394			<0.001			<0.001		
L	0.963			<0.001	0.357		NS	<0.001	
Q	0.146			<0.001	0.156		NS	<0.001	
p storage times × blood fraction	0.288			<0.001			0.452		
L	0.215			<0.001	<0.001		<0.001		
Q	0.640			<0.008	<0.001		<0.019		

[1]SEM: pooled standard error of the mean. [2]μ: average. p: significance level. L: linear effect. Q: quadratic effect. NS: non-significant.
[A–B or a–b]Capital letters in the same column or lowercase in the same row differ by the F-test (p < 0.05).

The effect of fasting on AST activity and the peak result at approximately 4 h of fasting for ALT activity follow the patterns reported by Veiga et al. (1978), who observed an increase in AST and ALT activities as fasting time increased due to the greater hepatic influx of gluconeogenic substrates. However, these authors found that AST activity correlated more strongly with hepatic gluconeogenesis than ALT activity.

In the present study, there was a linear increase in GGT activity due to accelerated protein catabolism and increased blood transport of amino acids as this enzyme acts in the extracellular environment by performing catalysis and converting glutathione to glutamate or cysteine (Yu & Long, 2016). In addition, our results indicate that during fasting, there is a deprivation of the amino acid supply, which plays an important role in several biochemical pathways; hence an activation of GGT was observed.

As previously reported, the AST activity in the present study did not differ among the freezing times, which was expected. This is in agreement with the findings of Kaneko et al. (2008), who reported that the activity of this enzyme does not change upon freezing and usually remains stable. In addition, the same stability response of AST according to storage time was observed in studies with rats (Cray et al., 2009), dogs (Thoresen et al., 1995), sheep (Oliveira et al., 2011), and humans (Cuhadar et al., 2013).

Serum ALT activity showed a maximum point at 30 days of storage, and the concentration of this enzyme in plasma showed a fast decrease, with a minimum point at 90 days. This corroborates the study on humans and pigs conducted by Kaneko et al. (2008), who evidenced a decrease in ALT concentrations when the samples were subjected to freezing. A reduced serum ALT activity concentration indicates unstable changes in enzyme isoforms (Cray et al., 2009).

The increase in AST and ALT activities in broiler chickens subjected to higher light intensity may also be associated with the level of intense stress, causing liver damage. In addition, Hosseini-Vashan et al. (2016) found increased concentrations of these liver enzymes in broiler chickens under heat stress. The response to AST activity follows the finding by Fidan et al. (2017), who observed increased concentrations of this enzyme in broiler chickens subjected to higher light intensity.

When analyzed together, the concentrations of glucose, cholesterol, and AST, ALT, and GGT activity were higher in the serum fraction. This result, according to Alper (1974), is attributed to low-molecular-weight anticoagulants (e.g. sodium fluoride) that exert an osmotic effect, transferring a large amount of water from the erythrocytes to the plasma. Also, according to Grande et al. (1964), this leads to the dilution of plasma constituents, resulting in lower concentrations of metabolites from plasma samples compared to serum samples. Another factor to consider is that plasma samples in sodium fluoride have higher hemolysis rates than serum samples (Al-Kharusi et al., 2014; Bonetti et al., 2016; Fernandez et al., 2013). Such a result, according to Sabater and Forbes (2015), causes changes in the determined concentrations of blood biochemical analytes.

Collectively, as far as we know, little or no attention has been addressed to the effects of the variables investigated in this study (e.g. light intensity, blood fraction, fasting and storage time) on blood components in broilers. Du et al. (2023), who examined serum blood metabolites after fasting for 16 h in broilers at 49 d of age on restricted feeding found lower values than our findings for total cholesterol (67.14 vs. 135 mg dL^{-1}), triglycerides (7.92 vs. 38 mg dL^{-1}), and glucose (151 vs. 227 mg dL^{-1}) and ALT (2.06 vs. 7.25 IU L^{-1}) compared to the 12-h fasting in our study. However, the values for AST were higher (587 vs. 326 IU L^{-1}), whereas the GGT values were close (24.78 vs. 29.59 IU L^{-1}) to those of the present study. Corroborating the results of the present study, Hagan et al. (2022) reported normal serum reference values of 197 to 299 mg dL^{-1} for glucose, 129 to 297 mg dL^{-1} for cholesterol, and average values of 96.15 mg dL^{-1} for the triglyceride concentrations in 56-d-old broiler chickens (Cobb and Ross) fasted for 12 h.

Conclusions

Samples for AST analysis can be stored up to 120 days, whereas the cholesterol and serum triglyceride levels as well as the GGT activity increase with storage time. Triglyceride analyses can be performed on serum or plasma, but serum analysis of glucose, cholesterol, triglyceride, ALT, and GGT should be used, with a 6-h fast. In addition, it is recommended to specify the light intensity to which the broiler chickens are subjected.

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