Veterinary and Animal Science 29 (2025) 100465

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

The blood biochemical parameters intervals and dynamics in modern broiler chickens

Dana Zálešáková, Jakub Novotný, Michal Řiháček, Lucie Horáková, Eva Mrkvicová, Ondřej Šťastník, Leoš Pavlata *

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

ARTICLE INFO

Keywords:
Blood plasma
Reference value
Poultry
Physiology
Metabolism

ABSTRACT

Blood biochemistry in poultry is an understudied area, requiring the establishment of reference intervals (RIs) to monitor health and metabolism effectively across different life stages. To broaden and complete the spectrum of parameters encountered in animal medicine, we pursued two main objectives: first, to establish the comprehensive spectrum of blood RIs for Ross 308 male chickens, and second, to investigate potential age-specific differences in broilers from the onset of their lives. A total of 228 blood samples from 35-day-old broilers were analysed to determine RIs for key metabolism indicators, including ALT (0.0–0.4 μkat/l), AST (1.4–5.7 μkat/l), GMT (0.2–0.5 μkat/l), ALP (13.3–281.9 μkat/l), LD (14.2–112.6 μkat/l), CK (94.9–925.6 μkat/l), TBili (1.8–7.3 μmol/l), Urea (0.7–2.7 mmol/l), Creat (16.7–37.7 μmol/l), Uric Acid (140.0–594.6 μmol/l), Total protein (24.7–37.1 g/l), Albumin (13.0–21.4 g/l), Globulin (10.3–19.0 g/l), Glucose (5.8–15.1 mmol/l), Cholesterol (2.3–4.1 mmol/l), TG (0.2–1.2 mmol/l). Furthermore, we monitored mineral, nitrogen, fat, and energy metabolism parameters weekly from day 1 to day 35 of chick age to assess biochemical dynamics. Significant age-related variations were found in most parameters, particularly during the first week after hatching ($P < 0.05$). The only stable indicators throughout the observation were creatinine and potassium ($P > 0.05$). These findings contribute to a deeper understanding of broiler physiology, enhance the precision of blood testing interpretations, and offer the potential for the early detection of metabolic disorders or diseases.

1. Introduction

Blood biochemical parameters are important indicators of animals' health status, nutritional, and physiological conditions (Alagawany et al., 2014; Kareem et al., 2024). It can be expected that due to the rising amount of human population, the demand for animal protein contained in chicken meat will increase (USDA, 2024). However, the rapid development and expansion of poultry production are associated with increasing incidences of avian diseases (Talebi et al., 2005). Monitoring changes in blood parameters can facilitate early diagnosis and help mitigate these issues (Arzour-Lakehal and Boudebza, 2021). Blood biochemistry can identify early metabolic disorders, including hypoglycemia, hypernatremia, and hyperkalemia (Klasing and Korver, 2020), as well as other metabolic conditions that may result in calcium deficiency, cardiomyopathies, and starvation. (Livingston et al., 2020; Adams et al., 2022; Kareem et al., 2024). Additionally, it plays a vital role in assessing stress levels (Li et al., 2020; Nwaigwe et al., 2020; Alghirani et al., 2023), which is closely linked to animal welfare. Consequently, veterinarians and nutritionists can gather essential diagnostic data without reducing the overall population of birds in a flock. Nevertheless, in the health assessment of commercial poultry flocks, clinical biochemistry is infrequently used (Martin et al., 2010). This is primarily due to the lack of accurate blood reference intervals (RIs) (Silva et al., 2007; Tang et al., 2013; Board et al., 2018; Arzour-Lakehal and Boudebza, 2021), variability in the methods used to analyse samples (Harr, 2006), and significant differences between breeds, strains, husbandry conditions, and age (Alonso-Alvarez, 2005; Livingston et al., 2020; Jiang et al., 2023).

Several studies have reported RIs mainly in long-lived poultry, including the dynamics in growing turkey (Szabó et al., 2005; Adams et al., 2022), pheasants (Dzikamunhenga et al., 2017), and the blood profiles of commercial layers (Schaal et al., 2016; Sauer et al., 2019; Ding et al., 2021), backyard hens (Board et al., 2018), and broiler breeders (Martin et al., 2010). Additionally, there are several studies

* Corresponding author.
E-mail address: leos.pavlata@mendelu.cz (L. Pavlata).

https://doi.org/10.1016/j.vas.2025.100465

Available online 8 June 2025

regarding the blood biochemistry of broilers (Bowes et al., 1989; Meluzzi et al., 1992; Piotrowska et al., 2011; Arzour-Lakehal and Boudebza, 2021; Ruiz-Jimenez et al., 2022; Ogbuewu et al., 2023; Kareem et al., 2024) and backyard chickens (Kaiser et al., 2022). However, many of these studies are outdated (Bowes et al., 1989; Meluzzi et al., 1992), involve fewer animals (Piotrowska et al., 2011), have different observed parameters, undifferentiated sex of animals, use various analysis methods, or involve different hybrid combinations of broilers compared to our study (Arzour-Lakehal and Boudebza, 2021; Kaiser et al., 2022; Ruiz-Jimenez et al., 2022).

The objectives of this study were: a) to compile comprehensive blood RIs for Ross 308 male chickens based on photometric measurements, and b) to determine whether age-specific differences are present in broilers. This study differs from previous studies in several aspects; a) it focuses on male chicken broilers, b) it includes blood dynamics from the first day of life, measured weekly, and c) as far as we know, it covers a broader spectrum of monitored parameters (especially liver enzymes), and a larger sample size compared to previous studies. Therefore, our work may enhance the understanding of blood dynamics and biochemistry in modern commercial broilers, complementing existing studies.

2. Materials and methods

RIs: This study involved several experiments to establish the RIs for Ross 308 broilers. Samples were collected from selected healthy broilers on the 35th day of age, originating from control groups of feeding experiments conducted in the years 2019–2022. In 11 experimental studies, a total of 2,080 broilers were included in control groups. Average chickens from each experiment (15 – 25 birds) were chosen to collect blood samples (a total of 228 blood samples, 1 from each individual).

Animal procedures were reviewed and approved by the Animal Care Committee of Mendel University in Brno and by the Ministry of Education, Youth, and Sports (MSMT-14021/2022-5).

Blood dynamics: Blood dynamics were monitored in Ross 308 chickens during a trial involving a total of 56 animals. Blood samples were collected weekly, starting from the first day of life. 8 samples were taken from different individuals each week over a period of six weeks, resulting in a total of 48 samples for the entire experiment.

Birds and housing: Male broilers of the Ross 308 hybrid combination were included in the experiment from the first day of life. All animals were housed in accredited facilities at Mendel University in Brno, ensuring that the environmental conditions were consistent throughout all experiments. Their health and availability of feed and water were checked daily. In all cases, the temperature, humidity, and lighting conditions adhered to the breeding guidelines provided by the Aviagen company (2018).

Diet: All chickens were fed a non-pelleted feed mixture ad libitum. From the 1st day to the 10th day of life, they received the starter feed mixture, and from the 11th to the 35th day of life, they were fed the grower diet.

The composition of the feed mixtures used in the blood dynamics trial is outlined in Table 1.

The nutritional composition of the feed mixtures was formulated according to the recommended nutritional requirements of the chosen hybrid combination in all trials.

Blood samples: Blood was collected from the jugular vein using a closed vacuum sampling system, which included 4-ml vacuum collection tubes flushed with lithium and heparin and 22-gauge needles with holders also treated with heparin (Vacutest Kima, Italy). The volume of blood collected ranged from 0.5 to 3 ml, depending on the age of the chickens. After sampling, the tubes were gently mixed and placed in a centrifuge within 30 minutes, where blood elements and plasma were separated. Centrifugation was carried out for 10 minutes at 3,000 rpm. All samples were then refrigerated and stored frozen at -20°C until biochemical determination was conducted.

Table 1
Composition and nutrient content of starter (S) and grower (G) diets.

Components and chemical composition	S	G
Wheat (g)	195	224.7
Maze (g)	290	330
Soybean meal (g)	430	323.3
Limestone milled (g)	6	4
Rapeseed oil (g)	41	45
Monocalcium phosphate (g)	8	7.3
L-Lysine (g)	-	0.3
Wheat gluten(g)	-	35
Vitamin-mineral premix:	30	30
L-lysine (g)	2.34	2.58
DL-Methionine (g)	2.4	2.52
Threonine (g)	0.99	1.47
Calcium (g)	5.25	5.04
Phosphorus (g)	1.95	1.65
Sodium (g)	1.44	1.38
Copper (mg)	15	15
Iron (mg)	84	75
Zinc (mg)	99	99
Manganese (mg)	99	99
Iodine (mg)	0.99	0.9
Selenium (mg)	0.18	0.36
Retinol (IU)	13,500	9,900
Calciferol (IU)	5,001	5,001
Tocopherol (mg)	45	45
Phylloquinone (mg)	1.5	1.5
Thiamine (mg)	4.2	4.2
Riboflavin (mg)	8.4	8.4
Pyridoxin (mg)	6	6
Cobalamin (μg)	30	28.8
Biotin (mg)	0.21	0.18
Niacinamide (mg)	36	36
Folic acid (mg)	1.8	1.71
Calcium pantothenate (mg)	13.5	13.35
Choline chloride (mg)	180	180
ME_N (MJ)[1]	12.55	12.97
Dry matter (g)	860	860
Crude protein (g)	222.75	212.12
Ether extract (g)	57.77	69.79
Crude fibre (g)	26.48	24.91
Ash (g)	65.22	52.39

[1] Apparent metabolise energy, calculated value

To monitor blood dynamics, eight animals from a group were selected each week. Blood sampling repeated once a week: from the 1st day of life until the 35th day. Each sampled chicken chosen for blood dynamics monitoring was marked with a coloured ring on the runner to prevent repeated sampling. This procedure was not applied to 1-day-old chicks that were slaughtered during collection.

Biochemical analyses: Standardised biochemical methods using commercial Erba Lachema kits (Czech Republic) were used, and analyses were performed on an Ellipse automated biochemical analyser (AMS, Italy). The following parameters were evaluated: enzyme activity AST – aspartate aminotransferase (AST/GOT kit; IFCC method); ALT – alanine aminotransferase (ALT/GPT kit; IFCC method); GMT – gamma-glutamyl transferase (γ-GT kit; IFCC method); ALP – alkaline phosphatase (ALP – AMP kit; AMP Buffer method); LD – lactate dehydrogenase (LDH kit; IFCC method), and creatine kinase (CK kit; IFCC-NAC method). As other markers of liver metabolism, nitrogen and fat metabolism were determined concentrations of total bilirubin – TBili (DPD method), urea (UREA/BUN – UV kit; Glutamate DH method), creatinine (Jaffé method), uric acid (Uricase-Peroxidase method), total protein (Biuret method), albumin (Bromocresol Green method), glucose (Oxidase method), cholesterol (Oxidase-Peroxidase method), and TG – triglycerides (Glycerol P Oxidase-Peroxidase method) were determined. The content of globulins (total protein minus albumin) was calculated. Furthermore, the mineral metabolism parameters were monitored in the

blood dynamics trial. The values of the following minerals were determined using Erba Lachema kits: calcium, phosphorus, magnesium, sodium, potassium, chlorine, zinc, copper, and iron. The analytical measurement error of individual parameters (intra- and inter-assay) is shown in Table 2.

Statistical analyses: Statistical analyses were performed using Tibco Statistica 14, Microsoft Excel, and a set of macroinstructions for Excel, the Reference Value Advisor V 2.1 (Geffré et al., 2011). Graphical representations of the data (boxplots and histograms) were generated using GraphPad Prism 9.

Establishing RIs corresponds to the recommendation published by the International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI, 2008). Tukey and the Dixon-Reed tests were used to identify outliers, which were then removed. For each analyte, descriptive statistics were generated that included mean, median, standard deviation, coefficient of variation, skewness, kurtosis, minimum, and maximum values. Subsequently, the 2.5^{th} and 97.5^{th} fractile were formulated as the lower and upper reference limits, and 95% confidence intervals (CI) were formulated for each parameter. Due to the high number of cases ($n > 120$), a non-parametric method was used for this determination.

In the evaluation of blood dynamics, mean values and standard errors were generated for each indicator. The normality test was performed with the Shapiro-Wilk W test. Based on the results of normality, significant differences ($P < 0.05$) in the changes of individual parameters monitored for the entire period of the experiment (among all sampling) were formulated. Statistically significant differences between values with a non-parametric distribution were calculated by Kruskal-Wallis ANOVA, and values with a parametric distribution by the Scheffé test. Additionally, linear Pearson's correlation was applied to establish associations between age and the values of biochemical analytes.

3. Results

The reference intervals (RI) for blood plasma parameters in broiler chickens are presented in Table 3. Aside from reference intervals, we also assessed confidence intervals (95% CI for lower and upper limits) and calculated the minimum and maximum values, as well as the mean, median, and standard deviation (SD) of the chosen parameters. According to coefficient of variation, some of indicators were more variable (ALT – 70.43 %, ALP – 78.81 %, CK – 58.23 %), other were less variable (AST – 24.86 %, GMT – 26.84 %, Creatinine – 19.81 %, Total protein – 9.66 %, Cholesterol – 13.80 %, Glucose – 24.11 %).

Table 2
Intra- and inter-assay of monitored indicators.

	CV% – intra assay	CV% – inter assay
AST	0.77	0.69
ALT	2.62	3.08
GMT	0.89	1.61
ALP	1.07	2.34
LD	1.60	2.09
CK	0.70	1.01
TBili	1.38	3.52
Urea	1.07	1.37
Creatinine	1.26	2.05
Uric acid	2.86	2.58
Total protein	0.55	0.73
Glucose	0.67	0.85
Cholesterol	1.26	1.65
Triglycerides	0.52	1.54
Ca	1.20	2.01
P	0.94	1.13
Mg	3.66	2.05
Na	0.31	0.70
K	0.36	0.57
Cl	0.31	0.21
Zn	0.91	3.13
Cu	1.06	1.74
Fe	0.79	1.02

CV – coefficient of variation

Table 4 presents mean values for various blood parameters, including both macroelements and microelements, and their age-related variations and correlation (r values). Except for two parameters (creatinine and potassium), all other monitored variables exhibited statistically significant changes over time ($P < 0.05$). The most substantial alterations in most parameters occurred following the first day of the chicks' lives. For a clearer overview, the distribution of individual parameters for each day is illustrated in Fig. 1–25. Fig. 26–50 presents aggregated values of the respective parameters, including general means. Fig. 51–75 presents histograms offering a more detailed overview of the frequency distributions and variability of the measured parameters.

Liver enzyme activity, such as AST, GMT, and ALP, as well as triacylglycerol levels, significantly increased after the 1^{st} day of age, followed by a subsequent decline. In the case of ALT, this trend was reversed. LD enzyme activity, total bilirubin, and globulin values remained relatively stable throughout the broilers' lifespan, with only occasional individual increases.

In terms of nutrient metabolism, levels of uric acid, urea, and cholesterol decreased significantly after the 1^{st} day of age and remained relatively stable until the end of the fattening period. Conversely, total protein and albumin values showed a significant increase after the 1^{st} day of age and remained stable throughout the chickens' lives. Glucose levels followed a similar pattern. Triacylglycerol levels exhibited a significant increase from the beginning of life, followed by a gradual decrease.

Regarding mineral metabolism, significant changes were observed in the concentration of individual elements in blood plasma, particularly following the 1^{st} day of age. Calcium and zinc concentrations showed a significant increase, then decreased and subsequently rose again towards the end of the fattening period. In comparison, phosphorus levels remained relatively stable after the 1^{st} day, with no further significant changes. Copper and iron levels rose slightly after birth and stayed stable throughout the fattening period. Sodium levels reached their lowest point on the seventh day of life and remained relatively stable thereafter. Conversely, magnesium level increased at the start of fattening, decreased after the 7^{th} day, and remained unchanged thereafter. Chlorides, in contrast to most other mineral substances, decreased after the 1^{st} day of age and remained stable until the end of the fattening period.

As mentioned, within the framework of the dynamics, significant changes in almost all parameters occurred during the life of the chickens. The correlation related to the age of the chickens was demonstrated for most monitored parameters, the strongest for nitrogen metabolism parameters: urea ($r = 0.63$), uric acid ($r = 0.59$), total protein ($r = 0.64$), and albumin ($r = 0.66$). A strong positive correlation was also observed for cholesterol parameters ($r = 0.60$) and triacylglycerols ($r = 0.52$).

4. Discussion

4.1. Reference intervals (RIs)

In this study, our objective was to establish RIs for broiler chickens, specifically focusing on the Ross 308 hybrid combination, which is widely bred in the Czech Republic and globally. RIs play a crucial role in monitoring the health and well-being of broiler chickens and optimising their production. To improve clarity, we have compared the results of our study with older and more recent broiler chicken studies, as presented in Table 5. Furthermore, we added the values of the control groups from individual recent trials with modern broilers for an adequate comparison, which is shown in Table 6. The selected values were adjusted to align with the sampling at approximately the same age for all the listed hybrid lines. In instances where parameters were

Table 3
Reference intervals (RI) of plasma biochemistry in Ross 308 broilers.

Analyte	n	Mean	Median	SD	CV	Skewness	Kurtosis	Min	Max	Lower limit of RI (95% CI for lower limit)	Upper limit of RI (95% CI for upper interval)
ALT (μkat/l)	221	0.2	0.1	0.1	70.4	0.98	-0.00	0.02	0.49	**0.0** (0.03–0.05)	**0.4** (0.41–0.49)
AST (μkat/l)	208	3.7	3.8	0.9	24.9	-0.50	0.94	0.67	5.92	**1.4** (0.97–1.70)	**5.7** (5.43–5.92)
GMT (μkat/l)	224	0.3	0.3	0.1	26.8	0.39	-0.38	0.15	0.51	**0.2** (0.15–0.19)	**0.5** (0.45–0.51)
ALP (μkat/l)	227	98.7	78.1	77.8	78.8	0.93	0.03	5.27	337.17	**13.3** (5.27–18.64)	**281.9** (267.12–328.10)
LD (μkat/l)	224	58.5	57.9	25.5	43.7	0.23	-0.35	10.89	124.95	**14.2** (12.33–16.67)	**112.6** (105.18–123.30)
CK (μkat/l)	216	387.2	330.1	225.5	58.2	0.86	0.02	52.3	972.7	**94.9** (58.70–112.10)	**925.6** (901.49–972.74)
TBili (μmol/l)	208	4.7	4.7	1.3	27.0	-0.17	0.00	1.4	7.6	**1.8** (1.50–2.30)	**7.3** (6.80–7.60)
Urea (mmol/l)	214	1.5	1.5	0.5	33.7	0.45	0.03	0.6	3.09	**0.7** (0.61–0.76)	**2.7** (2.51–3.09)
Creatinine (μmol/l)	226	28.0	28.6	5.5	19.8	-0.22	-0.17	13.6	43	**16.7** (13.80–18.40)	**37.7** (36.50–42.10)
Uric acid (μmol/l)	213	313.6	298.4	113.2	36.1	0.57	-0.09	108	618.5	**140.0** (128.90–152.20)	**594.6** (512.90–610.80)
Total protein (g/l)	220	30.7	30.7	3.0	9.7	0.13	-0.23	23.1	38.1	**24.7** (24.40–26.30)	**37.1** (35.55–37.60)
Albumin (g/l)	227	16.7	16.8	2.3	13.5	-0.01	-0.44	10.3	21.9	**13.0** (10.42–13.36)	**21.4** (20.34–21.86)
Globulin (g/l)	222	14.1	14.0	2.2	15.8	0.40	-0.44	9.8	20.0	**10.3** (9.90–11.05)	**19.0** (18.30–19.10)
Glucose (mmol/l)	226	10.7	10.7	2.6	24.1	-0.10	-0.81	4.19	16.58	**5.8** (5.00–6.57)	**15.1** (14.50–15.32)
Cholesterol (mmol/l)	217	3.2	3.1	0.4	13.8	0.39	-0.14	2.24	4.35	**2.3** (2.27–2.54)	**4.1** (3.99–4.23)
TG (mmol/l)	226	0.7	0.7	0.3	39.0	0.19	-0.46	0.11	1.42	**0.2** (0.16–0.28)	**1.2** (1.15–1.29)

Table 4
Plasma biochemistry blood dynamics and age correlation (*r*) of Ross 308 broilers from the 1st to the 35th day of life.

	n	1st day	7th day	14th day	21st day	28th day	35th day	SEM	*P*	*r*
ALT (μkat/l)	47	0.43^{a}	0.14^{c}	0.15^{c}	0.07^{c}	0.16^{c}	0.31^{b}	0.02	0.00	0.14
AST (μkat/l)	46	2.64^{c}	3.15^{bc}	6.04^{a}	4.61^{ab}	3.40^{bc}	3.62^{bc}	0.23	0.00	0.15
GMT (μkat/l)	47	0.12^{c}	0.16^{bc}	0.21^{b}	0.38^{a}	0.24^{b}	0.23^{b}	0.01	0.00	0.44*
ALP (μkat/l)	46	31.46^{c}	354.56^{a}	258.46^{b}	256.12^{b}	108.13^{c}	92.33^{c}	18.43	0.00	0.17
LD (μkat/l)	47	12.61^{b}	14.30^{b}	14.13^{b}	16.42^{b}	40.65^{a}	15.37^{b}	1.61	0.00	0.42*
CK (μkat/l)	44	31.60^{d}	136.43^{ab}	85.56^{bd}	71.14^{cd}	124.66^{bc}	185.16^{a}	8.99	0.00	0.47*
TBili (μmol/l)	47	4.08^{ab}	2.25^{b}	2.01^{b}	1.71^{b}	3.45^{b}	6.71^{a}	0.35	0.00	0.37
Urea (mmol/l)	48	5.99^{a}	1.79^{b}	1.03^{bd}	0.80^{cd}	0.67^{d}	1.62^{bc}	0.28	0.00	0.63*
Creatinine (μmol/l)	47	33.53	28.84	33.53	29.10	32.19	30.80	0.54	0.21	0.09
Uric acid (μmol/l)	48	896.94^{a}	651.36^{ab}	362.20^{bc}	524.30^{ac}	377.99^{bc}	226.75^{c}	48.19	0.00	0.56*
Total protein (g/l)	48	22.18^{c}	27.69^{ab}	25.51^{bc}	31.56^{a}	31.24^{a}	30.95^{a}	0.67	0.00	0.64**
Albumin (g/l)	45	5.59^{c}	15.26^{a}	13.19^{b}	13.72^{ab}	14.92^{a}	15.19^{a}	0.54	0.00	0.66**
Globulin (g/l)	46	16.58^{a}	13.14^{ab}	11.11^{b}	16.00^{a}	16.32^{a}	15.72^{a}	0.47	0.00	0.14
Glucose (mmol/l)	47	11.84^{b}	14.89^{a}	14.79^{a}	14.75^{a}	13.94^{a}	14.27^{a}	0.21	0.00	0.29
Cholesterol (mmol/l)	47	8.34^{a}	3.16^{c}	2.86^{c}	4.60^{b}	3.40^{c}	2.82^{c}	0.30	0.00	0.60**
TG (mmol/l)	46	0.65^{b}	0.93^{a}	0.51^{b}	0.51^{b}	0.42^{b}	0.49^{b}	0.03	0.00	0.52*
Ca (mmol/l)	47	2.08^{bc}	2.66^{a}	2.08^{c}	2.17^{bc}	2.46^{ab}	2.67^{a}	0.05	0.00	0.33
P (mmol/l)	46	1.15^{b}	2.31^{a}	2.50^{a}	1.87^{ab}	2.35^{a}	2.13^{a}	0.09	0.00	0.33
Mg (mmol/l)	46	0.74^{b}	1.20^{a}	0.73^{b}	0.97^{ab}	1.03^{ab}	0.95^{ab}	0.04	0.00	0.08
Na (mmol/l)	46	135.87^{bc}	133.04^{c}	142.79^{ab}	146.73^{a}	147.53^{a}	139.38^{bc}	1.04	0.00	0.26
K (mmol/l)	48	4.45	4.94	4.91	5.10	5.21	4.50	0.10	0.11	0.09
Cl (mmol/l)	45	117.70^{a}	106.90^{c}	108.85^{bc}	109.99^{bc}	109.94^{bc}	111.77^{b}	0.63	0.00	0.33
Zn (umol/l)	48	16.56^{c}	24.88^{a}	24.45^{a}	18.12^{bc}	16.01^{c}	21.31^{ab}	0.67	0.00	0.11
Cu (umol/l)	47	3.80^{c}	5.18^{bc}	6.24^{ab}	7.69^{a}	4.66^{bc}	5.65^{ac}	0.28	0.00	0.18
Fe (umol/l)	48	9.91^{b}	16.43^{ab}	11.86^{b}	18.80^{ab}	26.20^{a}	14.76^{b}	1.23	0.01	0.35

ALT – alanine aminotransferase; AST – aspartate aminotransferase; GMT – gamma-glutamyl transferase; ALP – alkaline phosphatase; LD – lactate dehydrogenase; CK – creatine kinase; TBili – total bilirubin; TG – triglycerides; SEM – standard error of mean; n – the number of samples without outliers; different letters in a row indicates a statistically significant difference $P < 0.05$; */** indicates medium * or strong ** Pearson's correlation according to Evans (1996)

expressed in different units, we meticulously recalculated and standardised the results.

Our findings revealed several important insights into RIs for broiler chickens. AST and creatinine showed the least variation across studies. AST had a narrow reference range, especially in the ISA 15 hybrid line (Arzour-Lakehal et al., 2021), and varied slightly compared to other studies. Meluzzi et al. (1992) reported a lower AST lower limit, while Ruiz-Jimenez et al. (2022) found a higher upper limit compared to our study. ALP and CK activities also showed slight differences among studies.

For uric acid, only the upper reference limit for ISA 15 and Ross 708 hybrids was provided in studies by Arzour-Lakehal et al. (2021) and Ruiz-Jimenez et al (2022). Our study presented the narrowest RIs for total protein. The lower limit for ISA 15 was found to be lowest in Arzour-Lakehal et al. (2021) and highest in Ruiz-Jimenez et al. (2022). Meluzzi et al. (1992) reported the highest upper limit for albumin. Creatinine RIs from Arzour-Lakehal et al. (2021) were consistent but narrower than our findings. Plasma glucose values reported in other studies ranged from 2.8 to 17.96 mmol/L, which is broader than our range. Cholesterol levels ranged from 0.52 to 4.97 mmol/l in other studies, again wider than ours. Additionally, TG levels also had a wider range in some trials.

It is important to emphasize that, based on the experiences of the authors of both current and earlier studies, evaluating blood parameters in broiler chickens requires careful consideration of several factors. These factors include the age of the chickens at the time of sampling, the

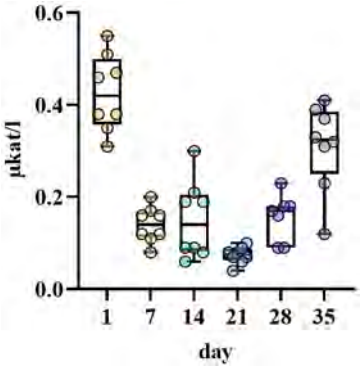

Fig. 1. ALT dynamics from the 1st to the 35th day of life.

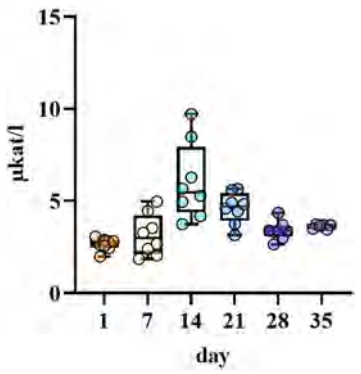

Fig. 2. AST dynamics from the 1st to the 35th day of life.

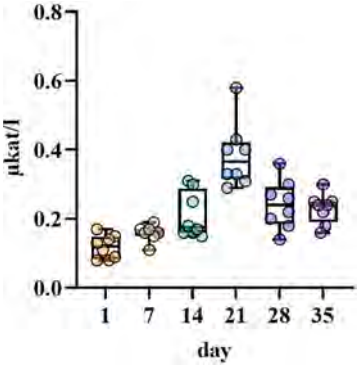

Fig. 3. GMT dynamics from the 1st to the 35th day of life.

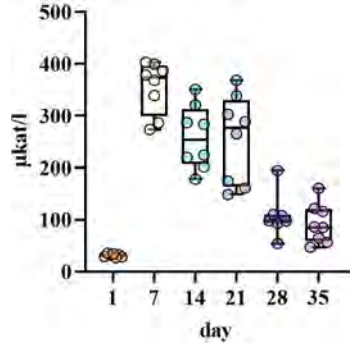

Fig. 4. ALP dynamics from the 1st to the 35th day of life.

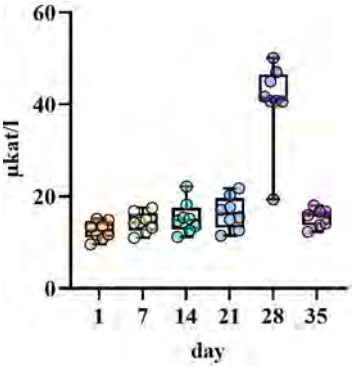

Fig. 5. LD dynamics from the 1st to the 35th day of life.

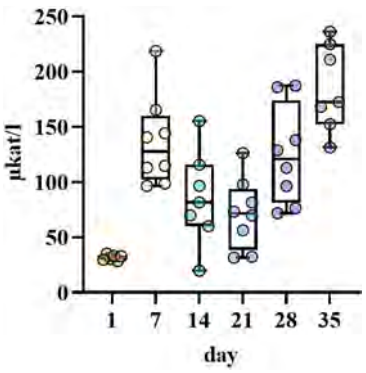

Fig. 6. CK dynamics from the 1st to the 35th day of life.

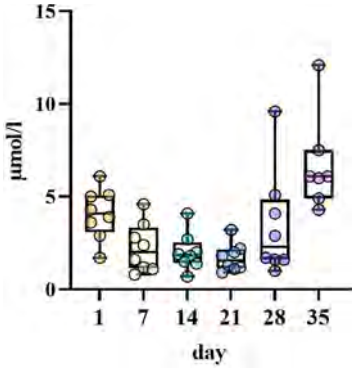

Fig. 7. TBili dynamics from the 1st to the 35th day of life.

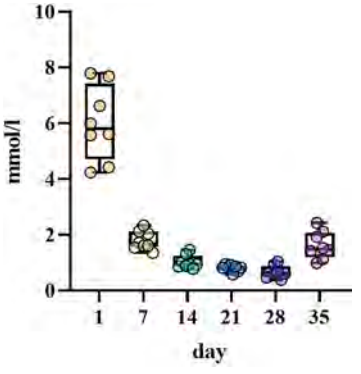

Fig. 8. Urea dynamics from the 1st to the 35th day of life.

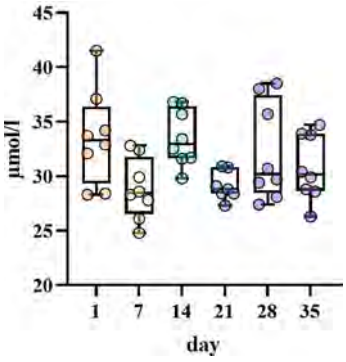

Fig. 9. Creatinine dynamics from the 1st to the 35th day of life.

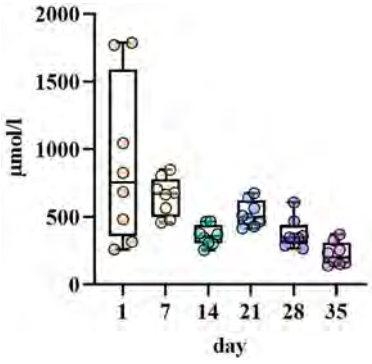

Fig. 10. Uric acid dynamics from the 1st to the 35th day of life.

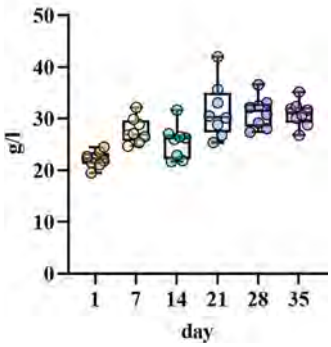

Fig. 11. Total protein dynamics from the 1st to the 35th day of life.

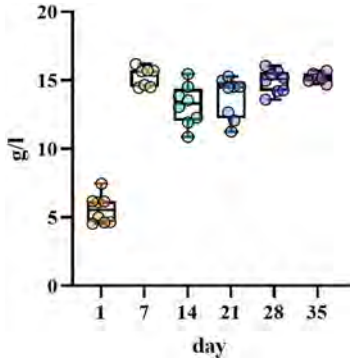

Fig. 12. Albumin dynamics from the 1st to the 35th day of life.

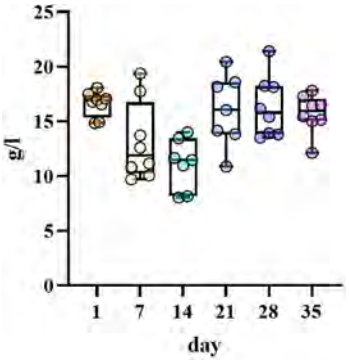

Fig. 13. Globulin dynamics from the 1st to the 35th day of life.

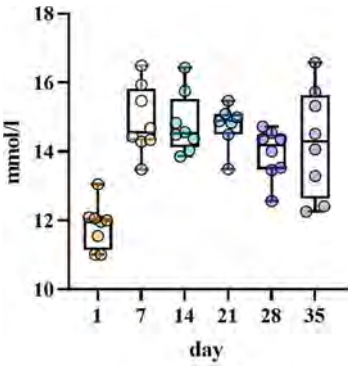

Fig. 14. Glucose dynamics from the 1st to the 35th day of life.

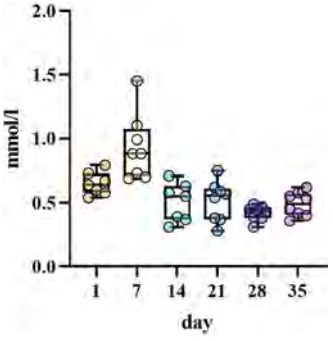

Fig. 15. Cholesterol dynamics from the 1st to the 35th day of life.

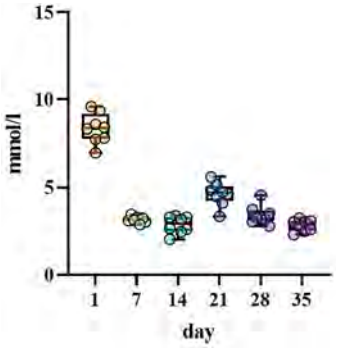

Fig. 16. Triglycerides dynamics from the 1st to the 35th day of life.

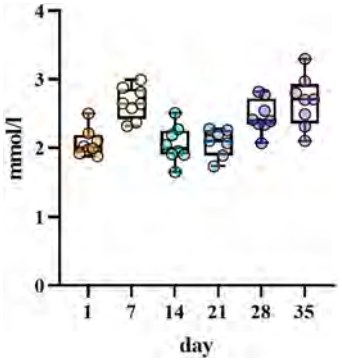

Fig. 17. Calcium dynamics from the 1st to the 35th day of life.

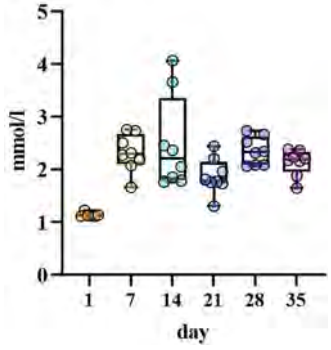

Fig. 18. Phosphorus dynamics from the 1st to the 35th day of life.

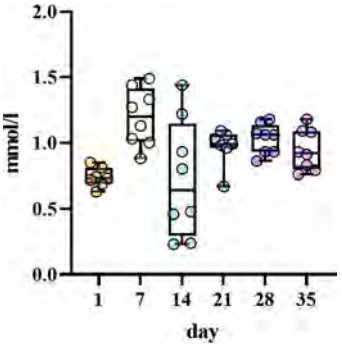

Fig. 19. Magnesium dynamics from the 1st to the 35th day of life.

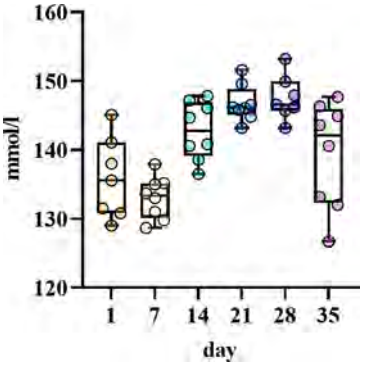

Fig. 20. Sodium dynamics from the 1st to the 35th day of life.

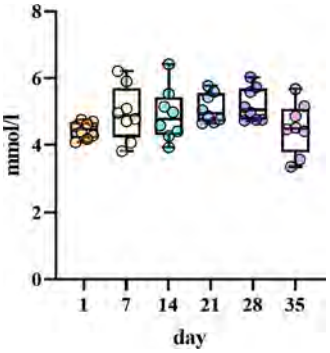

Fig. 21. Potassium dynamics from the 1st to the 35th day of life.

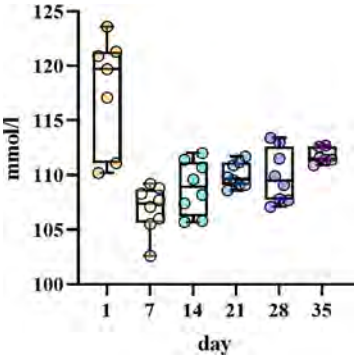

Fig. 22. Chlorine dynamics from the 1st to the 35th day of life.

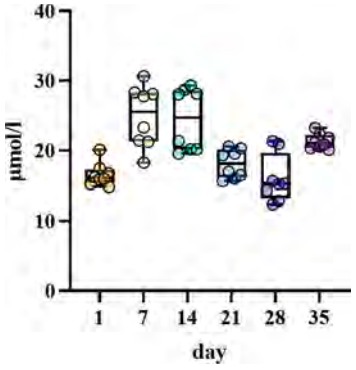

Fig. 23. Zinc dynamics from the 1st to the 35th day of life.

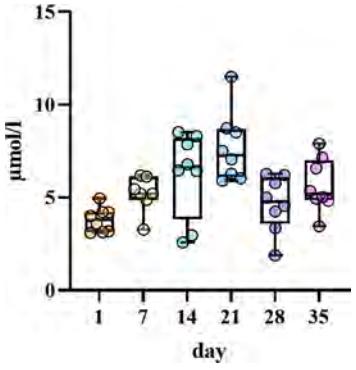

Fig. 24. Copper dynamics from the 1st to the 35th day of life.

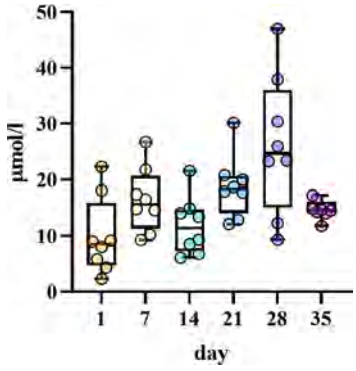

Fig. 25. Iron dynamics from the 1st to the 35th day of life.

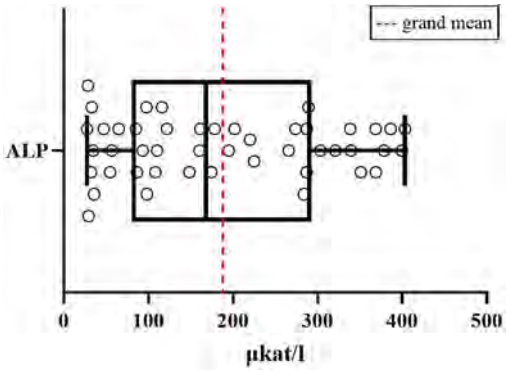

Fig. 29. Summary of ALP dynamics (days 1–35).

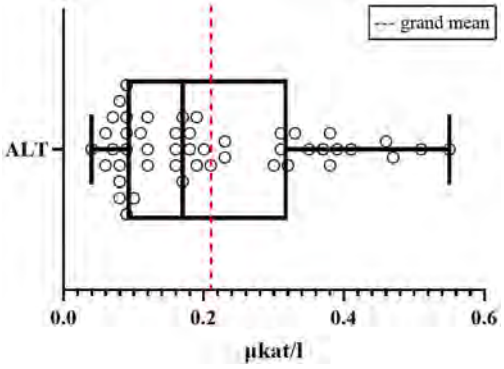

Fig. 26. Summary of ALT dynamics (days 1–35).

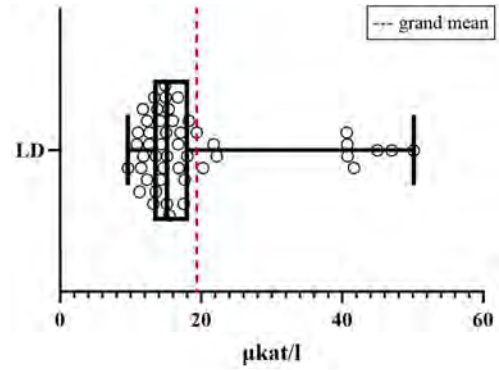

Fig. 30. Summary of LD dynamics (days 1–35).

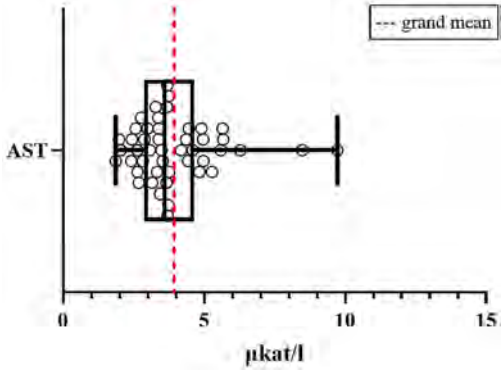

Fig. 27. Summary of AST dynamics (days 1–35).

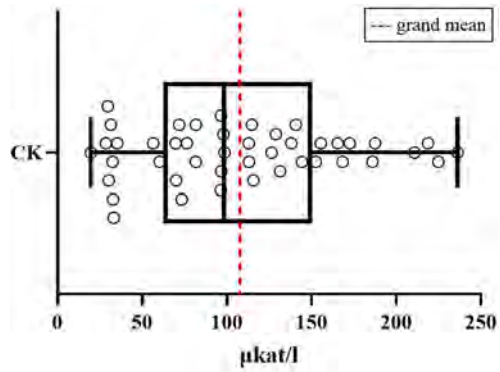

Fig. 31. Summary of CK dynamics (days 1–35).

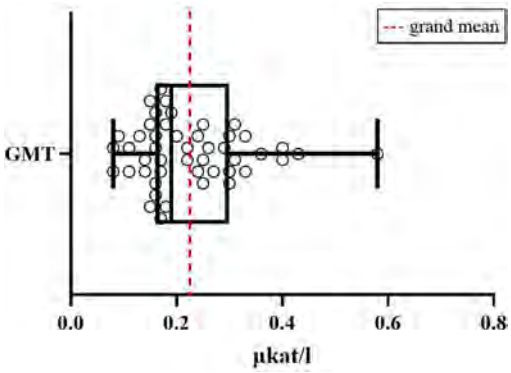

Fig. 28. Summary of GMT dynamics (days 1–35).

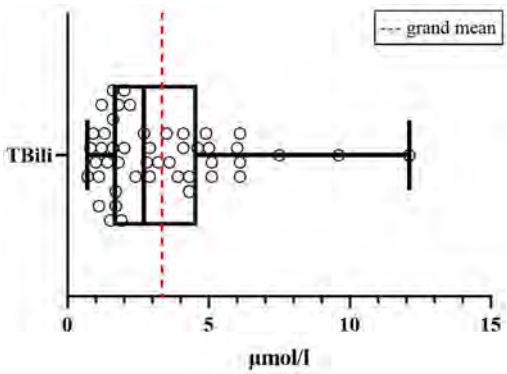

Fig. 32. Summary of TBili dynamics (days 1–35).

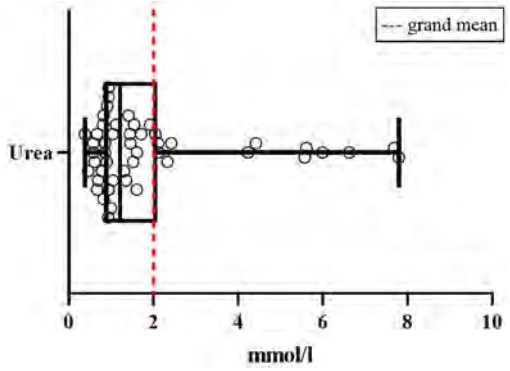

Fig. 33. Summary of urea dynamics (days 1–35).

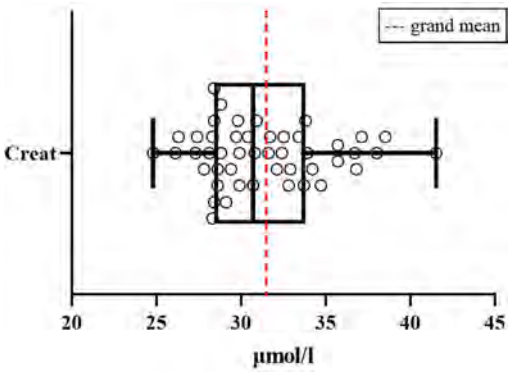

Fig. 34. Summary of creatinine dynamics (days 1–35).

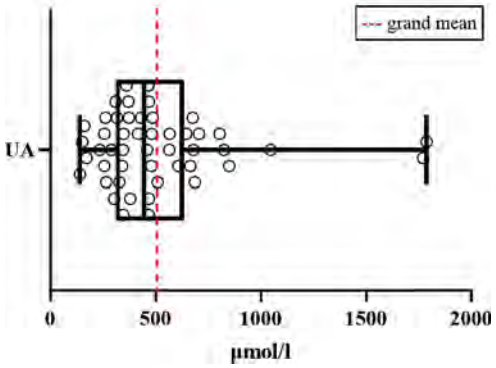

Fig. 35. Summary of uric acid dynamics (days 1–35).

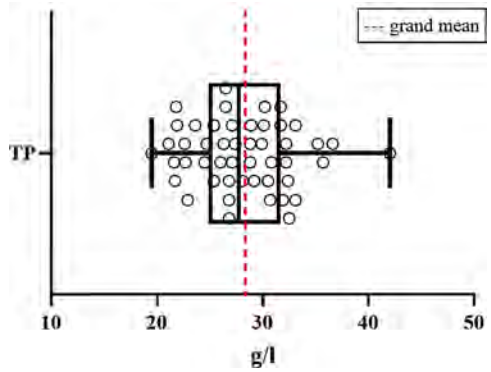

Fig. 36. Summary of total protein dynamics (days 1–35).

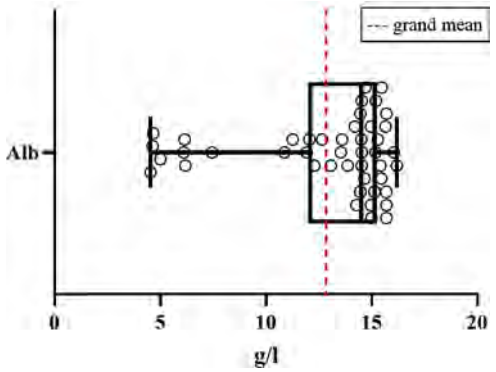

Fig. 37. Summary of albumin dynamics (days 1–35).

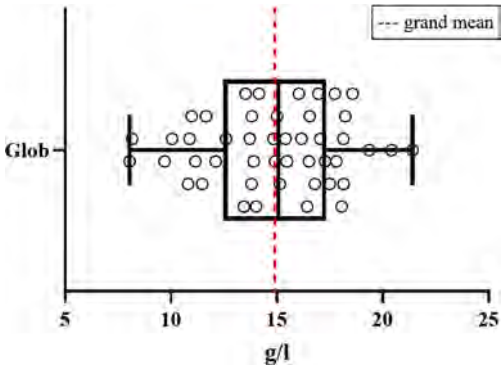

Fig. 38. Summary of globulin dynamics (days 1–35).

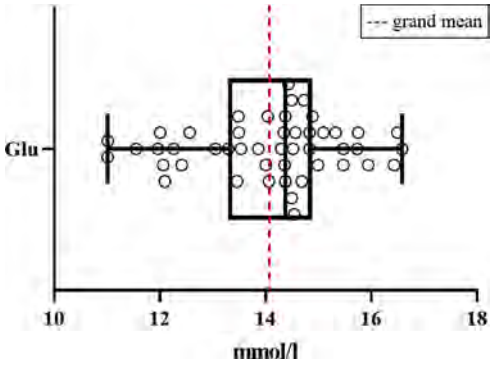

Fig. 39. Summary of glucose dynamics (days 1–35).

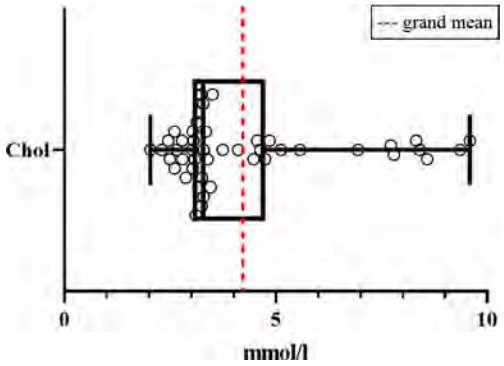

Fig. 40. Summary of cholesterol dynamics (days 1–35).

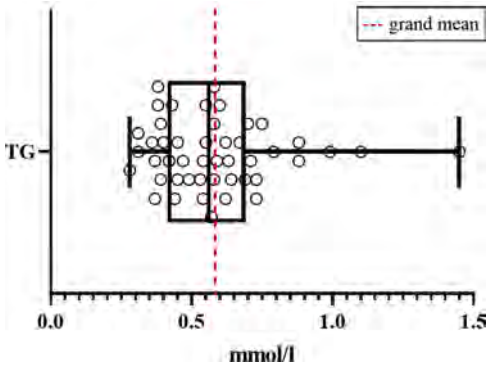

Fig. 41. Summary of triglycerides dynamics (days 1–35).

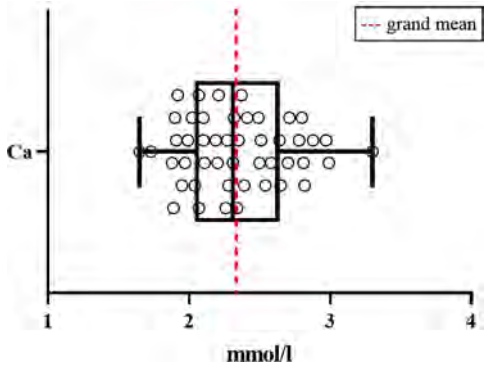

Fig. 42. Summary of calcium dynamics (days 1–35).

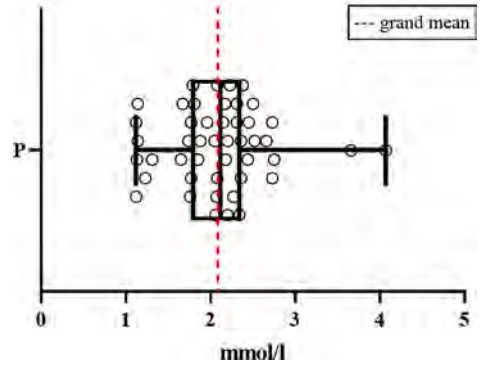

Fig. 43. Summary of phosphorus dynamics (days 1–35).

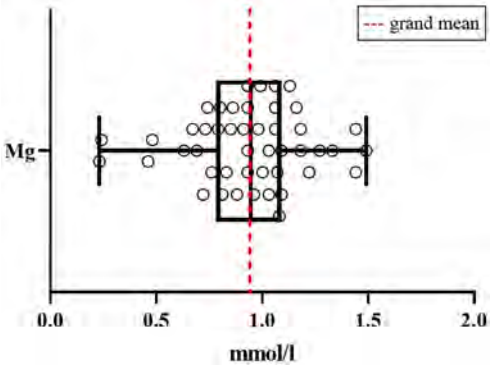

Fig. 44. Summary of magnesium dynamics (days 1–35).

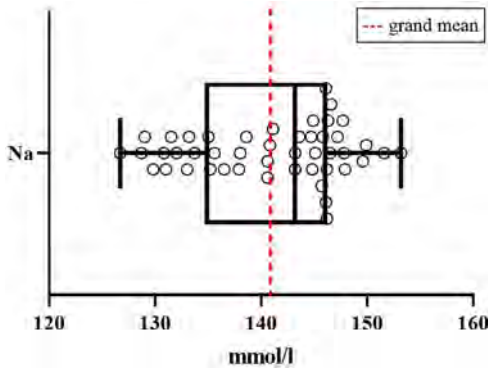

Fig. 45. Summary of sodium dynamics (days 1–35).

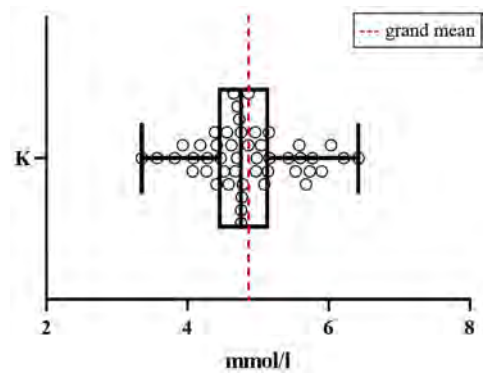

Fig. 46. Summary of potassium dynamics (days 1–35).

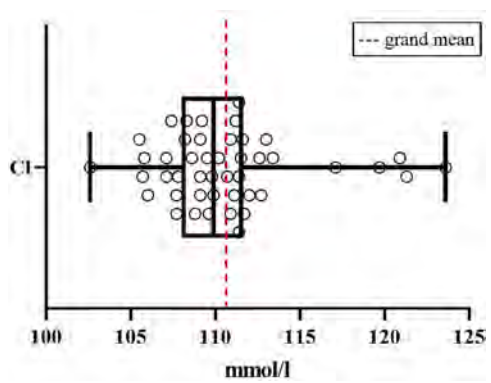

Fig. 47. Summary of chlorine dynamics (days 1–35).

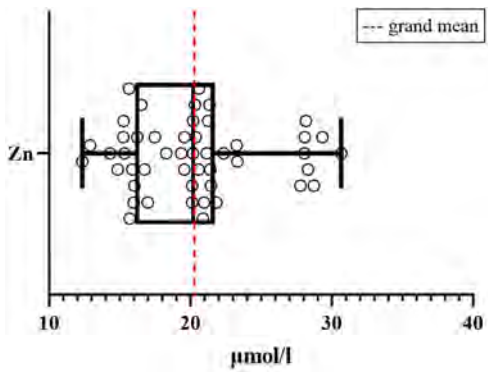

Fig. 48. Summary of zinc dynamics (days 1–35).

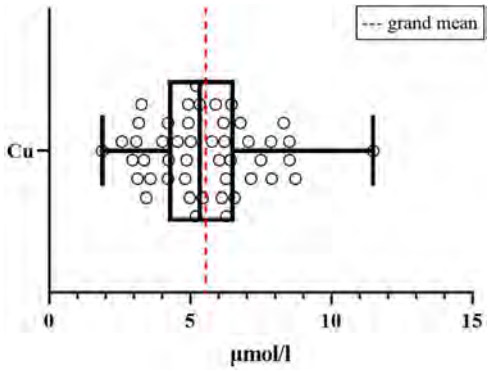

Fig. 49. Summary of copper dynamics (days 1–35).

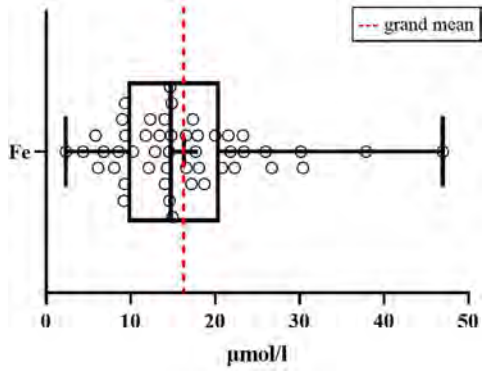

Fig. 50. Summary of iron dynamics (days 1–35).

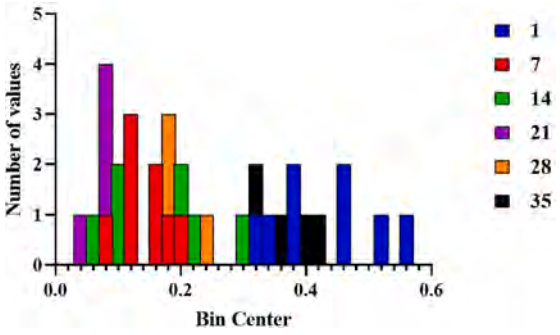

Fig. 51. Frequency distribution of ALT (days 1–35).

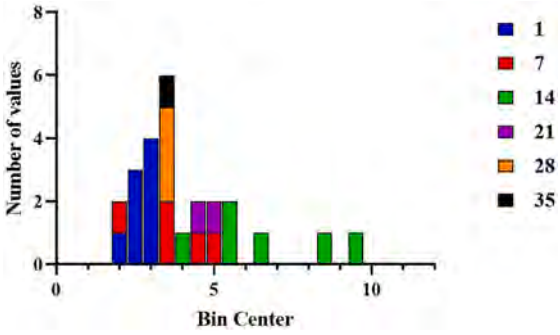

Fig. 52. Frequency distribution of AST (days 1–35).

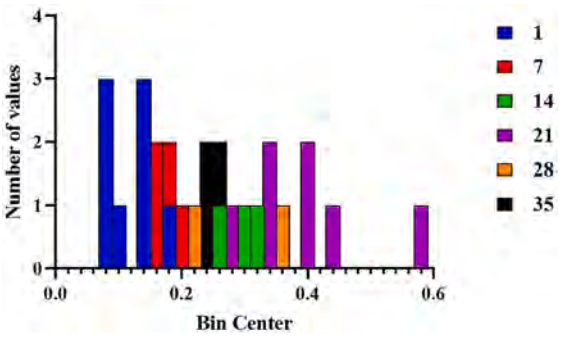

Fig. 53. Frequency distribution of GMT (days 1–35).

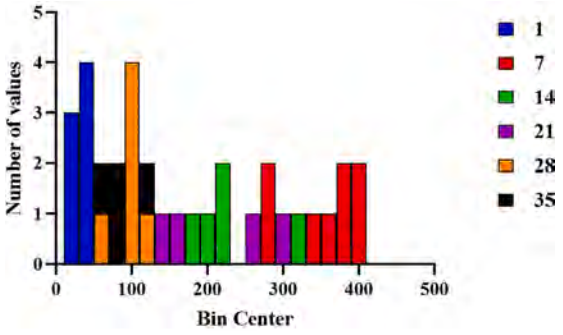

Fig. 54. Frequency distribution of ALP (days 1–35).

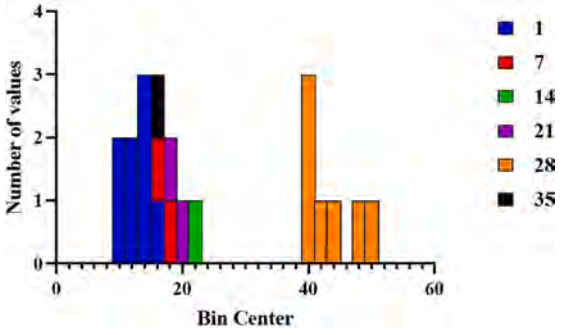

Fig. 55. Frequency distribution of LD (days 1–35).

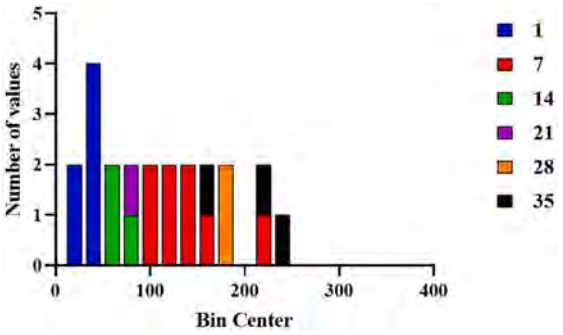

Fig. 56. Frequency distribution of CK (days 1–35).

choice of hybrid line, feeding practices, housing methods, and other relevant parameters (Bowes et al., 1989; Board et al., 2018; Arzour--Lakehal, 2021). As a result, even minor nuances in the evaluation may be influenced by these factors. All the authors mentioned utilized

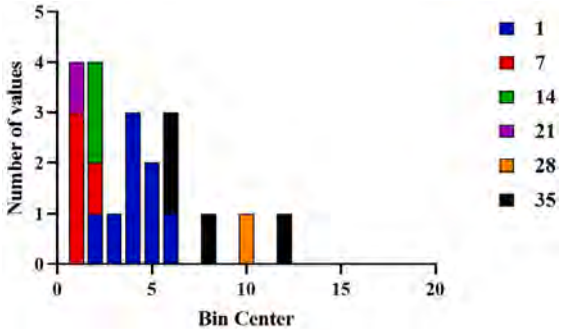

Fig. 57. Frequency distribution of TBili (days 1–35).

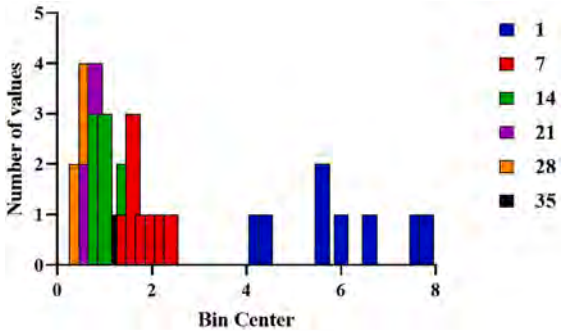

Fig. 58. Frequency distribution of urea (days 1–35).

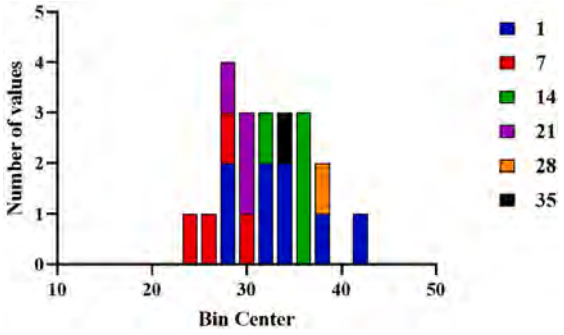

Fig. 59. Frequency distribution of creatinine (days 1–35).

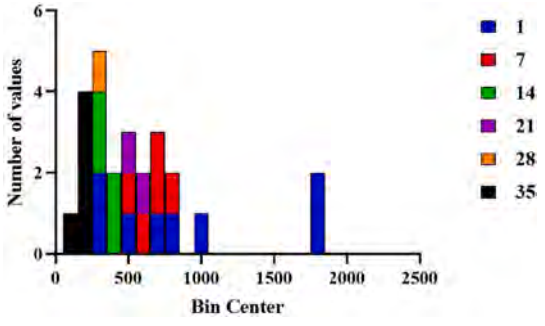

Fig. 60. Frequency distribution of uric acid (days 1–35).

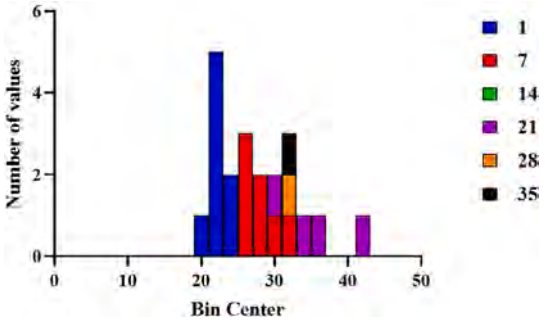

Fig. 61. Frequency distribution of total protein (days 1–35).

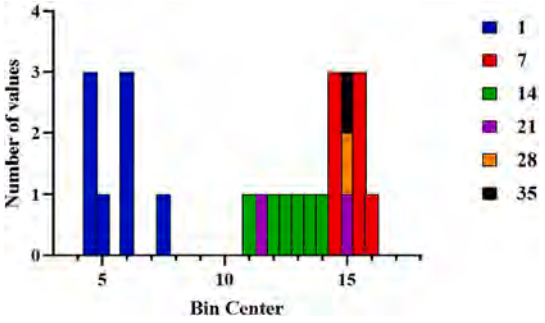

Fig. 62. Frequency distribution of albumin (days 1–35).

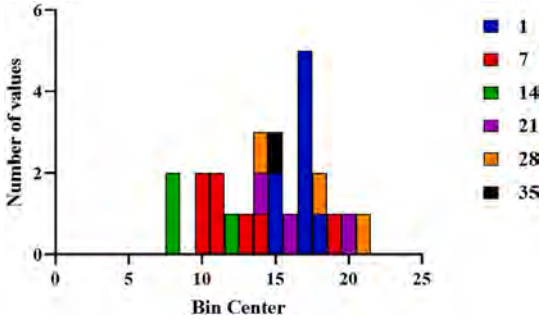

Fig. 63. Frequency distribution of globulin (days 1–35).

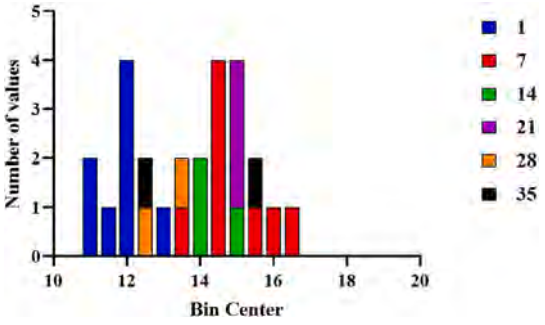

Fig. 64. Frequency distribution of glucose (days 1–35).

control groups of animals that were fed according to the nutritional requirements of their respective hybrids and were housed under conditions that adhered to established technological guidelines. Thus, any potential pathophysiological changes should be ruled out.

Enzyme activity is often relatively neglected in chickens. Recent studies, as outlined in Table 6, measured enzyme activity while examining various factors affecting the health and productivity of broilers. These factors included the impact of different feed mixture structures (Novotný et al., 2023), the effect of crude protein deficiency on the blood profile of chickens (Mousa et al., 2023), the addition of natural antioxidants to the feed mixture (Al-Azzami and Mohammed, 2023), the

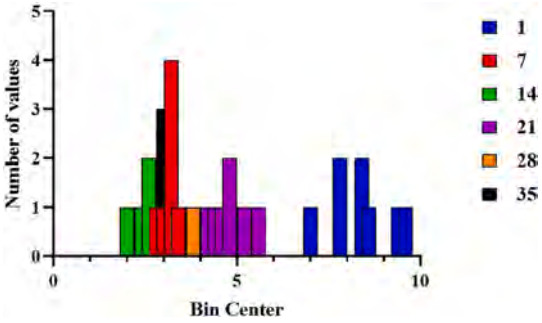

Fig. 65. Frequency distribution of cholesterol (days 1–35).

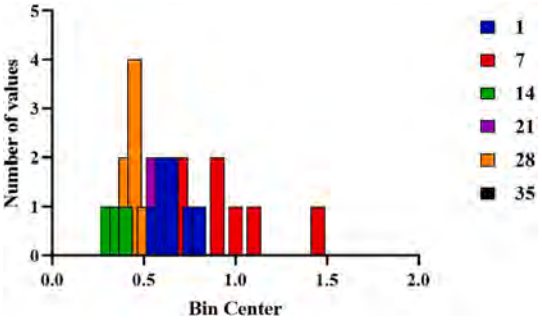

Fig. 66. Frequency distribution of triglycerides (days 1–35).

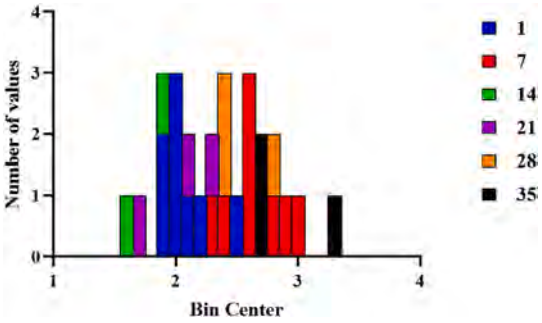

Fig. 67. Frequency distribution of calcium (days 1–35).

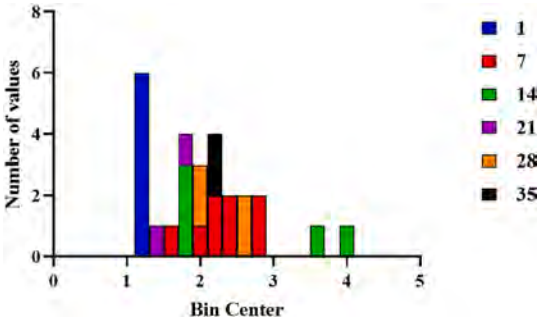

Fig. 68. Frequency distribution of phosphorus (days 1–35).

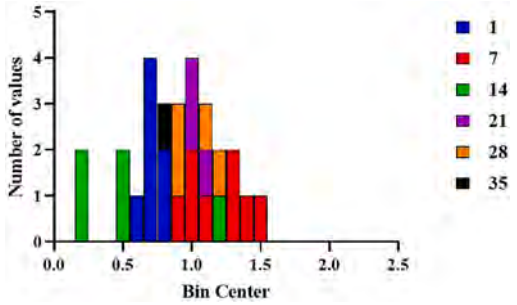

Fig. 69. Frequency distribution of magnesium (days 1–35).

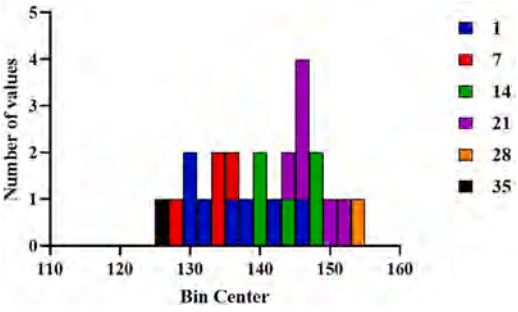

Fig. 70. Frequency distribution of sodium (days 1–35).

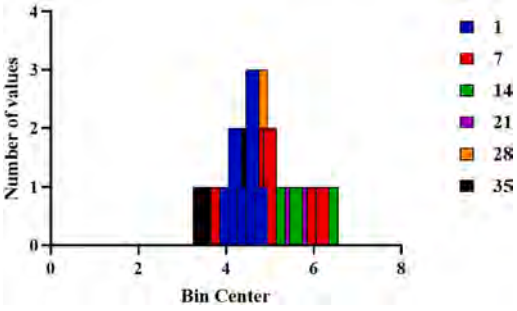

Fig. 71. Frequency distribution of potassium (days 1–35).

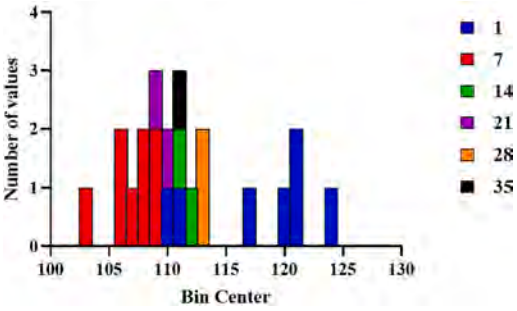

Fig. 72. Frequency distribution of chlorine (days 1–35).

addition of probiotics (Oladokun and Adewole, 2023), as well as the combination of probiotics and antibiotics in chickens challenged with *Salmonella* (Cirilo et al., 2023). Only the AST values, which were lower in Azzami and Mohammed (2023) and slightly higher in Cirilo et al. (2023), did not correspond to our reference range. However, it is important to note that several aspects need to be considered when monitoring RIs for liver enzymes. AST is an enzyme specific to the liver and muscles, and elevated levels can indicate potential damage to hepatocytes or muscle fibres (Tully et al., 2009). ALP activity is typically higher in younger chicks and, like GMT, is associated with cell membrane structures. Increased activity of both enzymes can also be considered an indicator of tissue cell integrity disruption (Melillo, 2007). Therefore, it can be concluded that even if the lower reference interval or a single value of liver enzyme activity in some studies does not fall within the range observed in our study, it may not necessarily

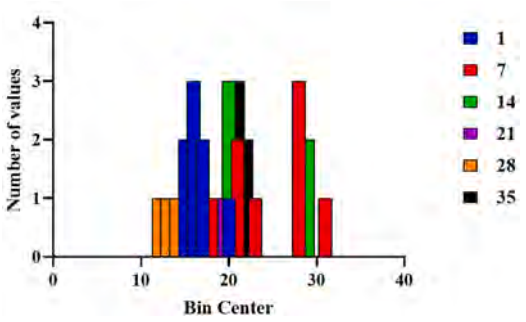

Fig. 73. Frequency distribution of zinc (days 1–35).

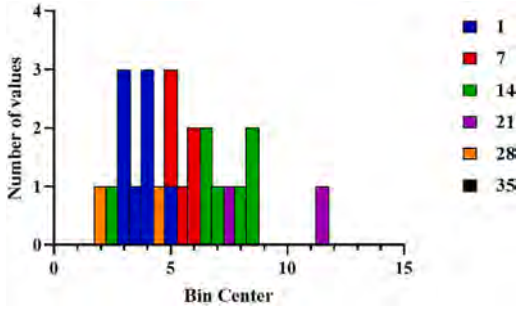

Fig. 74. Frequency distribution of copper (days 1–35).

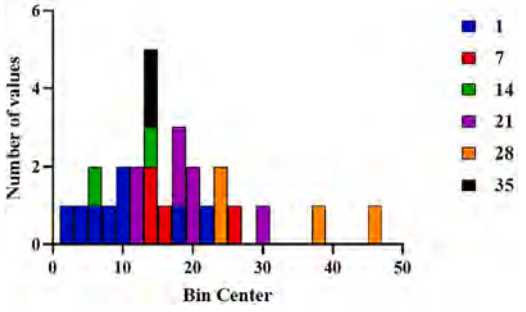

Fig. 75. Frequency distribution of iron (days 1–35).

represent a significant problem in potential diagnostics. However, this may not be the case for enzymes whose activity significantly exceeds the upper RI. Exceeding may indicate significant problems, as mentioned above.

The levels of uric acid can vary significantly among different animal species (Coles et al., 2007). As a final product of nitrogen metabolism, uric acid serves as an important indicator in the blood plasma of birds (Juráni et al., 2004). This explains why some authors overlook RIs for urea, which holds much less diagnostic significance in avian medicine (Ritchie et al., 1994). Outside of our established reference range, a lower urea value was only reported in the experiment by Oladokun and Adewole (2023); however, the uric acid was not reduced, leading to the conclusion that this was only a slight deviation. Significant changes in uric acid can primarily indicate kidney disease or disorders (in which it is excreted), as well as dehydration (Coles et al., 2007). In terms of total protein, very high concentrations in the blood can indicate acute or chronic inflammation (Thrall et al., 2012). In recent studies, El-Kasrawy et al. (2023) investigated the effect of Ginkgo biloba oil supplementation on blood parameters of chickens, revealing significantly elevated globulin levels. This increase may indicate inflammation linked to a rise in immunoglobulins (Allison, 2012), but further analysis would be necessary to confirm this. The level of glucose in the body is regulated by homeostasis through hepatic glycogenolysis. A high glucose level is often detected during periods of increased stress, while a lower level results from prolonged starvation or other disorders (Thrall et al., 2012). In a study conducted by Oladokun and Adewole (2023), the glucose value recorded was only slightly above our RI. In contrast, most studies examining cholesterol and triacylglycerol levels in the blood plasma of chickens have found values higher than those recommended by our RI or those from other authors. According to Thrall et al. (2012), elevated cholesterol and TG levels can indicate overfeeding, steatosis, or other liver damage, whereas lower levels may suggest starvation. Therefore, in the studies mentioned, both the composition of the feed mixture and the timing of blood sampling after the last feeding are crucial factors to consider.

Significant differences in blood parameters can be observed even among chickens of the same utility type and similar age. These variations may be due to differences in population characteristics, including the use of various chicken genotypes, diets, and housing conditions, as noted in studies by Weiss and Wardrop (2010) and Board et al. (2018). Nevertheless, most reference values are relatively consistent and can serve as effective tools for understanding the biochemistry of blood parameters in modern broiler chickens.

Table 5
A comparison of reference blood intervals (RIs) for blood biochemical values of broilers from available studies reported in previous years.

Author	**Current study**	Meluzzi et al., 1992	Arzour-Lakehal and Boudebza, 2021	Arzour-Lakehal and Boudebza, 2021	Ruiz-Jimenez et al., 2022
Hybrid	**Ross 308**	Arbor Acres and Hybro	ISA 15	Arbor Acres Plus	Ross 708
n	**228**	800	60	60	45
Analytes	Existing reference intervals				
ALT (μkat/l)	**0.0–0.4**				
AST (μkat/l)	**1.4–5.7**	1.16–3.65	3.27–4.89	2.50–4.79	2.64–18.42
GMT (μkat/l)	**0.2–0.5**				
ALP (μkat/l)	**13.3–281.9**	9.47–147.21			
LD (μkat/l)	**14.2–112.6**				
CK (μkat/l)	**94.9–925.6**				4.13->233.38
TBili (μmol/l)	**1.8–7.3**				
Urea (mmol/l)	**0.7–2.7**				
Creat (μmol/l)	**16.7–37.7**		17.68–26.53	17.68–32.72	
Uric acid (μmol/l)	**140.0–594.6**		98.16–312.91	137.42–666.86	100.53–513.38
Total protein (g/l)	**24.7–37.1**	25.8–52.2	10.5–36.8	21.6–47.5	26.2–86.3
Albumin (g/l)	**13.0–21.4**	11.0–27.4			18.9–25.4
Globulin (g/l)	**10.3–19.0**				
Glucose (mmol/l)	**5.8–15.1**		2.8–15	3.9–17.8	10.82–17.96
Cholesterol (mmol/l)	**2.3–4.1**	2.30–4.97	0.52–3.62	0.52–3.88	
TG (mmol/l)	**0.2–1.2**	0.52–1.94	0.34–1.47	0.26–1.47	

ALT – alanine aminotransferase; AST – aspartate aminotransferase; GMT – gamma-glutamyl transferase; ALP – alkaline phosphatase; LD – lactate dehydrogenase; CK – creatine kinase; TBili – total bilirubin; TG – triglycerides; n – the number of samples before subtracting outliers

Table 6
Selection of blood biochemical profiles of broilers from the control groups of the present studies.

Author	Alhayaly et al., 2024	El-Kasrawy et al., 2023	Novotný et al., 2023	Hassan et al., 2023	Al-Azzami and Mohammed, 2023	Oladokun and Adewole, 2023	Mousa et al., 2023	Cirilo et al., 2023
Hybrid	Ross 308	Cobb 500	Ross 308	Hubbard strain	Ross 308	Cobb 500	Arbor Acres Plus	Cobb 500
n	9	9	10	6	6	9	5	16
ALT (μkat/l)			0.09		0.43	0.04	0.13	0.15
AST (μkat/l)			3.81		0.81	2.77	4.47	5.91
GMT (μkat/l)			0.22			0.20		0.29
ALP (μkat/l)			206.27					
LD (μkat/l)			57.67					
CK (μkat/l)						110		
TBili (μmol/l)			4.41				0.33	
Urea (mmol/l)			1.84			0.31	0.86	
Creat (μmol/l)								
Uric acid (μmol/l)			298.26			364		410.18
Total protein (g/l)	36.4	59.5	29.27	27.8	39.6	28.6	26	29.24
Albumin (g/l)		19.8	17	15.1	26	11.8	13	16.45
Globulin (g/l)		39.7			13.6	16.7	13	
Glucose (mmol/l)	12.69			12.51	13.1	15.5		
Cholesterol (mmol/l)	5.94	5.57		3.58	8.17	3.49	7.78	6.69
TG (mmol/l)	4.53	3.03		3.67	8.01		4.17	1.82

ALT – alanine aminotransferase; AST – aspartate aminotransferase; GMT – gamma-glutamyl transferase; ALP – alkaline phosphatase; LD – lactate dehydrogenase; CK – creatine kinase; TBili – total bilirubin; TG – triglycerides; n – the number of samples

4.2. Blood dynamics

To the best of our knowledge, there are a limited number of studies that have investigated blood parameters of broilers during their early fattening stages. In earlier studies, Bowes et al. (1989) examined differences in selected parameters in broilers at 9, 20, 30, and 42 days of age. Three years later, Meluzzi et al. (1992) studied these parameters at 21 and 45 days of age. Piotrowska et al. (2011) assessed variations at 14, 21, and 42 days of age in Ross 308 broilers. Similar to our study, Café et al. (2012) observed differences at weekly intervals from the 14th day to the 42nd day of the chickens' age. More recently, Ruiz-Jimenez et al. (2022) examined blood parameters at 7, 21, and 35 days of age.

According to our findings, the most significant changes in blood parameters occur during the 1st week of a chicken's life. While we cannot support these results in any of the publications found, it can be inferred that there are substantial metabolic changes processes during the early stages of broilers' lives. During this period, chicks begin consuming food, adapt to their new environmental conditions, and experience dynamic growth and development of the organism, accompanied by the acquisition of muscle mass. These changes are likely to be reflected in alterations in blood parameters, as confirmed by our study. Additionally, it is important to consider the nutrient consumption of the yolk sac during the chick's embryonic period, as this is closely related to the feeding of the broiler breeder. This can impact the quality of the chicks after hatching (including initial weight, lower mortality, improved immune response, and growth) (Chang et al., 2016) and potentially affect their blood parameters.

Enzymes: The exploration of enzymatic activity during broiler fattening remains limited in the literature. Our study identified a significant correlation between age and GMT, LD, and CK parameters ($P < 0.05$). GMT reached its peak on day 21, similar to findings by Café et al. (2012). LD activity showed a decline after 30 days, consistent with Bowes et al. (1989). CK levels increased from day 21, partially aligning with Ruiz-Jimenez et al. (2022). ALT remained stable, gradually increasing towards the end of the fattening period, as observed by Café et al. (2012). In contrast, AST displayed a different trend, increasing from day 7 to 14 before decreasing, corroborating findings from Meluzzi et al. (1992). ALP activity decreased from day 21, also noted by Meluzzi et al. (1992). Variations in enzymatic activity can be due to differences in hybrid lines, housing, and feed conditions (Weiss and Wardrop, 2010). We propose that the pronounced changes in enzymatic levels could be attributed to factors such as housing, movement, stress, and handling before blood collection. To minimize potential bias, we carefully managed the animals before and during blood sampling to reduce these negative influences.

Indicators of nitrogen metabolism: Key indicators of nitrogen metabolism in birds, including uric acid, total protein, and albumin, as well as urea, showed a positive correlation with the age of the chickens ($P < 0.05$). Our study observed that total protein and albumin levels gradually increased with age, consistent with similar findings in studies by Meluzzi et al. (1992), Piotrowska et al. (2011), and Ruiz-Jimenez et al. (2022). Conversely, uric acid levels decreased gradually from day 1, a trend supported by Ruiz-Jimenez et al. (2022) and partially by Piotrowska et al. (2011), who noted a rise in uric acid towards the end of the fattening period. Creatinine was one of the few parameters that did not show significant changes throughout the chickens' lives, aligning with a result from Bowes et al. (1989) and Rajman et al. (2006). Café et al. (2012) reported an individual increase in creatinine on day 35, while Piotrowska et al. (2011) observed a significant individual decrease at the 21st day of age, potentially related to a similar decrease in uric acid noted in their study. Urea, a less-studied parameter in birds, followed a pattern similar to that of uric acid in our research. These two parameters showed a significantly positive correlation with each other. It's important to note that some older studies reported less pronounced significant changes or no changes in the observed parameters (Bowes et al., 1989; Meluzzi et al., 1991). However, these discrepancies may be attributed to genetic development shifts, metabolic intensification, and differences in the life stages of the birds sampled over the past decades.

Indicators of fat metabolism and glucose: A significant positive correlation was found between age and TG, cholesterol, and glucose ($P < 0.05$). Most of the parameters assessed in our study changed with age, as confirmed by other studies. For example, TG, as examined in Piotrowska et al. (2011) and Café et al. (2012), showed a decline starting from the 14th day, consistent with our observations. These studies also reported that cholesterol levels were lowest in chickens' blood on the 42nd day of age, a trend consistent with our results. While Café et al. (2012) noted an increase in glucose levels starting from day 14, Ruiz-Jimenez et al. (2022) observed an increase from the seventh day of age.

In our study, a significant increase in glucose level was observed from the 1st day of age, which does not contradict previous studies that did not provide this data. During the later stages of fattening, glucose levels remained relatively stable.

It's important to consider that changes in metabolic parameters can occur due to the different stages of digestive system development and possible metabolic shifts during growth. For example, elevated cholesterol levels during the early stages of fattening may arise from its presence in the yolk sac, while triglyceride levels could be affected by the development of the lymphatic system (Anca et al., 2019).

Indicators of mineral substances metabolism: In contrast to previous findings, we observed a significant positive correlation with age ($P < 0.05$) for Ca, P, Cl, and Fe. However, we did not find a high positive correlation with age for Mg, Na, K, Zn, and Cu ($P > 0.05$). In our study, most of the monitored mineral substances showed changes after the 1st day of age, with only minor exceptions, after which their levels remained relatively stable. It is important to note that in available studies, blood sampling was typically conducted at later stages of the chickens' lives, and they largely confirmed the stability of these elements.

Bowes et al. (1989) reported no significant changes in P, Mg, Na, K, and Cl concerning the age of the chickens. Similarly, Meluzzi et al. (1992) found no changes in Ca, and Piotrowska et al. (2011) did not observe changes in P.

For the parameters where changes were observed, Bowes et al. (1989) noted an increase in plasma Ca concentration from day 9, Piotrowska et al. (2011) from day 14, and Ruiz-Jimenez et al. (2022) from the 7th day of age. Our findings partially align with previous studies, as we also observed an increase in calcium levels beginning on the 14th day of age in chickens, except for the 1st day of age. This phenomenon can be attributed to the period of intensive growth and mineralization of bone tissue, which leads to higher calcium intake requirements Meluzzi et al. (1992) and Ruiz-Jimenez et al. (2022) also noted an increase in P levels from the 21st and 7th days of age of the chickens, a trend that was not confirmed in our study. Furthermore, Piotrowska et al. (2011) reported a decrease in Mg and Fe levels starting from day 14, which we did not observe in our experiment.

In our study, Na, as the primary extracellular cation, significantly decreased from the 1st day to the 7th day of the chicks' age. After this initial decrease, Na levels stabilized in the following weeks. This phenomenon was also confirmed by Ruiz-Jimenez et al. (2022), who reported an increase in Na concentration in the blood. However, due to a considerable lack of studies on this topic, it was challenging to objectively evaluate the dynamics of sodium levels, especially concerning microelements. This presents an opportunity for future research on the life cycle of chickens, both in our experiment and in previous studies.

It's worth noting that a significant portion of the microelements detected in the blood plasma of poultry have not been extensively studied in previous experiments. Due to their indispensable role in the organism, this represents a fundamental area where future research could be focused.

5. Conclusion

In conclusion, our study provides a comprehensive range of reference blood intervals that cover enzymes and parameters related to nitrogen and lipid metabolism in broiler chickens, thereby complementing and expanding existing research. In addition, within the framework of blood dynamics, mineral metabolism parameters are also included. Our findings indicate that many of these parameters undergo significant changes during the fattening period, and these variations should be considered when sampling at different stages of the chickens' lives. However, due to the lack of studies, it was impossible to objectively evaluate the dynamics of microelements, which could be one of the directions of future research.

The results of our study can significantly enhance our understanding of broiler physiology and the potential use of blood tests to monitor nutrition, metabolism, and animal welfare. This could lead to more precise blood test assessments and facilitate early detection of metabolic disorders or diseases.

CRediT authorship contribution statement

Dana Zálešáková: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jakub Novotný:** Methodology, Formal analysis. **Michal Řiháček:** Methodology, Formal analysis. **Lucie Horáková:** Methodology, Formal analysis. **Eva Mrkvicová:** Resources. **Ondřej Šťastník:** Validation, Supervision, Resources. **Leoš Pavlata:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was supported by the grant no. AF-IGA-2021-IP064 from the Internal Agency of Mendel University. We are grateful to Emir Dzomba, Prof. Dr. from the University of Sarajevo, for sharing his insights for the manuscript.

References

Adams, D., Gruber, E., Sather, H., Correa, M., & Crespo, R. (2022). Evaluation of growing turkey blood biochemistry panel measured using the vetscan VS2. *Poultry, 1*(2), 138–146. https://doi.org/10.3390/poultry1020012

Alagawany, M., El-Hack, M. E. A., Laudadio, V., & Tufarelli, V. (2014). Effect of low-protein diets with crystalline amino acid supplementation on egg production, blood parameters and nitrogen balance in laying Japanese quails. *Avian Biology Research, 7*(4), 235–243. https://doi.org/10.3184/175815514X141529451666

Al-Azzami, A. A., & Mohammed, T. T. (2023). Effect of adding dry leaves of lemongrass (cymbopogon citratus) to the diet on some biochemical tests of blood in broiler (Ross 308). In *, 1252. IOP Conference Series: Earth and Environmental Science*. IOP Publishing, Article 012125. https://doi.org/10.1088/1755-1315/1252/1/012125.

Alghirani, M. M., Chung, E. L. T., Kassim, N. A., Ong, Y. L., Jesse, F. F. A., Sazili, A. Q., & Loh, T. C. (2023). Blood biochemistry and stress biomarkers of broiler chickens supplemented with different levels of Yucca schidigera saponins reared under tropical conditions. *Veterinary Integrative Sciences, 21*(1), 1–15. https://doi.org/10.12982/VIS.2023.001

Alhayaly, H. N., Saadi, A. M., & Younis, D. T. (2024). Use of Linseed oil and animal tallow in nutrition and its effect on blood Characteristics and meat composition in Broilers. *Egyptian Journal of Veterinary Science, 55*(2), 435–442. https://doi.org/10.21608/EJVS.2023.234921.1604

Allison, Robin W. (2012). Laboratory evaluation of plasma and serum proteins. In M. A. Thrall, G. Weiser, W. R. Allison, & W. T. Campbell (Eds.), *Veterinary hematology and clinical chemistry* (pp. 460–475). Oxford, UK: Blackwell Publishing.

Alonso-Alvarez, C. (2005). Age-dependent changes in plasma biochemistry of yellow-legged gulls (Larus cachinnans). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140*(4), 512–518. https://doi.org/10.1016/j.cbpb.2005.03.001

Anca, G., Hăbeanu, M., Lefter, N. A., & Ropotă, M. (2019). Performance parameters, plasma lipid status, and lymphoid tissue fatty acid profile of broiler chicks fed camelina cake. *Brazilian Journal of Poultry Science, 21*(4). https://doi.org/10.1590/1806-9061-2019-1053

Arzour-Lakehal, N., & Boudebza, A. (2021). Biochemical reference intervals in broiler chickens according to age and strain. *Agricultural Science and Technology, 13*(4), 357–364. https://doi.org/10.15547/ast.2021.04.058

Aviagen. (2018). Ross broiler management pocket guide. Available at https://aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Pocket-Guide-2020-EN.pdf Accessed November 23, 2024.

Board, M. M., Crespo, R., Shah, D. H., & Faux, C. M. (2018). Biochemical reference intervals for backyard hens. *Journal of Avian Medicine and Surgery, 32*, 301–306. https://doi.org/10.1647/2017-310

Bowes, V. A., Julian, R. J., & Stirtzinger, T. (1989). Comparison of serum biochemical profiles of male broilers with female broilers and white Leghorn chickens. *Canadian Journal of Veterinary Research, 53*, 7–11.

Café, M. B., Rinaldi, F. P., Morais, H. R., Nascimento, M. R. B. M., Mundim, A. V., & Marchini, C. F. P. (2012). Biochemical blood parameters of broilers at different ages under thermoneutral environment. *World's Poultry Science Journal, 5*(9), 143–146.

Cirilo, E. H., Junior, N. R., Andrade, T. S., Souza, C., Kaufmann, C., Kohler, T. L., Datsch, L. I., Vieira, B. S., Vargas Junior, J. G., Carvalho, P. L. O., Eyng, C., & Nunes, R. V. (2023). Effects of probiotics on blood metabolites, enterocytes, growth, and carcass characteristics of broilers challenged with Salmonella Serovar Heidelberg. *Livestock Science, 270*, Article 105188. https://doi.org/10.1016/j.livsci.2023.105188

CLSI. (2008). Defining, Establishing, & Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed. Wayne, PA.

Coles, B. H., Krautwald-Junghanns, M., Orosz, S. E., & Tully, T. N., Jr (2007). *Essentials of Avian Medicine and Surgery* (3rd ed.). Oxford, UK: Blackwell Publishing.

Ding, H., Yue, Q., Chang, L., Xi, J., Li, F., Wang, D., & Zhou, R. (2021). Whole blood gas and biochemical reference intervals for Lohmann silver layers. *Poultry science, 100* (9), Article 101368. https://doi.org/10.1016/j.psj.2021.101368

Dzikamunhenga, R. S., Griffith, R. W., Hostetter, S., Fisher, P., & Larson, W. (2017). Hematology and serum biochemistry reference intervals for six-week-old, farm-reared chinese ring-necked pheasants (phasianus colchicus) from minnesota. *Avian Diseases, 61*(2), 211–213. https://doi.org/10.1637/11588-011017-Reg.1

El-Kasrawy, N. I., Majrashi, K. A., El-Naggar, K., Abd Elreheim, A. M., Essa, B. H., Mahmoud, S. F., Ibrahim, S. I., Raafat, M., Abd El-Hack, M. E., & Aboghanima, M. M. (2023). Impacts of supplemental Ginkgo biloba oil on broilers' growth, blood indices, intestinal and hepatic morphology and expression of growth-related genes. *Poultry Science, 102*(4), Article 102520. https://doi.org/10.1016/j.livsci.2023.105188

Evans, J. D. (1996). *Straightforward statistics for the behavioral sciences*. Thomson Brooks/Cole Publishing Co.

Geffré, A., Concordet, D., Braun, J. P., & Trumel, C. (2011). Reference Value Advisor: A new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Veterinary Clinical Pathology, 40*(1), 107–112. https://doi.org/10.1111/j.1939-165X.2011.00287.x

Harr, K. E. (2006). Diagnostic value of biochemistry. In G. J. Harrison, & T. L. Lightfoot (Eds.), *Clinical avian medicine* (pp. 611–630). Palm Beach, Florida, USA: Spix Publishing Inc.

Hassan, M. I., Khalifah, A. M., El Sabry, M. I., Mohamed, A. E., & Hassan, S. S. (2023). Performance traits and selected blood constituents of broiler chicks as influenced by early access to feed post-hatch. *Animal Biotechnology, 34*(7), 2855–2862. https://doi.org/10.1080/10495398.2022.2124164

Chang, A., Halley, J., & Silva, M. (2016). Can feeding the broiler breeder improve chick quality and offspring performance? *Animal Production Science, 56*(8), 1254–1262. https://doi.org/10.1071/AN15381

Jiang, X., Zhang, B., Lan, F., Zhong, C., Jin, J., Li, X., Zhou, Q., Li, J., Yang, N., Wen, C., & Sun, C. (2023). Host genetics and gut microbiota jointly regulate blood biochemical indicators in chickens. *Applied Microbiology and Biotechnology, 107*, 7601–7620. https://doi.org/10.1007/s00253-023-12814-8

Juráni, M., Výboh, P., Zeman, M., Lamošová, D., Košťál, L. U., & Blažíček, P. (2004). Post-hatching dynamics of plasma biochemistry in free-living European starlings (Sturnus vulgaris). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 138*(1), 89–95. https://doi.org/10.1016/j.cbpb.2004.03.005

Kaiser, J. C., Reider, H., Pabilonia, K. L., & Moore, A. R. (2022). Establishment of biochemical reference values for backyard chickens in Colorado (Gallus gallus domesticus). *Veterinary Clinical Pathology, 51*(4), 577–584. https://doi.org/10.1111/vcp.13136

Kareem, D. U., Amos, A. T., Idowu, O. P. A., Bonagurio, L. P., & Idowu, O. M. O. (2024). Blood profile as a health indicator in broiler chickens fed diets of different particle sizes supplemented with multienzyme. *Agricultura Tropica et Subtropica, 57*(1), 45–59. https://doi.org/10.2478/ats-2024-0005

Klasing, K. C, & Korver, D. R. (2020). In D. E. Swayne, M. Boulianne, C. M. Logue, L. R. McDougald, V. Nair, & D. L. Suarez (Eds.), *Nutritional diseases. Pages 1257-1285 in Diseases of Poultry* (14th ed.). Hoboken, New Jersey, USA: Wiley Blackwell.

Li, D., Tong, Q., Shi, Z., Li, H., Wang, Y., Li, B., Yan, B.,G., Chen, H., & Zheng, W. (2020). Effects of chronic heat stress and ammonia concentration on blood parameters of laying hens. *Poultry Science, 99*(8), 3784–3792. https://doi.org/10.1016/j.psj.2020.03.060

Livingston, M. L., Cowieson, A. J., Crespo, R., Hoang, V., Nogal, B., Browning, M., & Livingston, K. A. (2020). Effect of broiler genetics, age, and gender on performance and blood chemistry. *Heliyon, 6*(7). https://doi.org/10.1016/j.heliyon.2020.e04400

Martin, M. P., Wineland, M., & Barnes, H. J. (2010). Selected blood chemistry and gas reference ranges for broiler breeders using the i-STAT® handheld clinical analyzer. *Avian Diseases, 54*(3), 1016–1020. https://doi.org/10.1637/9471-922310-DIGEST.1

Melillo, A. (2007). Rabbit clinical pathology. *Journal of Exotic Pet Medicine, 16*(3), 135–145. https://doi.org/10.1053/j.jepm.2007.06.002

Meluzzi, A., Primiceri, G., Giordani, R., & Fabris, G. (1992). Determination of blood constituents reference values in broilers. *Poultry Science, 71*(2), 337–345. https://doi.org/10.3382/ps.0710337

Mousa, M. A., Asman, A. S., Ali, R. M., Sayed, R. K., Majrashi, K. A., Fakiha, K. G., Alhotan, R. A., & Selim, S. (2023). Impacts of dietary lysine and crude protein on performance, hepatic and renal functions, biochemical parameters, and histomorphology of small intestine, liver, and kidney in broiler chickens. *Veterinary Science, 10*(2), 98. https://doi.org/10.3390/vetsci10020098

Novotný, J., Horáková, L., Řiháček, M., Zálešáková, D., Šťastník, O., Mrkvicová, E., Kumbár, V., & Pavlata, L. (2023). Effect of different feed particle size on gastrointestinal tract morphology, ileal digesta viscosity, and blood biochemical parameters as markers of health status in broiler chickens. *Animals, 13*, 2532. https://doi.org/10.3390/ani13152532

Nwaigwe, C. U., Ihedioha, J. I., Shoyinka, S. V., & Nwaigwe, C. O. (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. *Veterinary World, 13*(10), 2294. https://doi.org/10.14202/vetworld.2020.2294-2300

Ogbuewu, I. P., Mabelebele, M., & Mbajiorgu, C. A. (2023). Meta-analysis of blood indices and production physiology of broiler chickens on dietary fermented cassava intervention. *Tropical Animal Health and Production, 55*(6), 368. https://doi.org/10.1007/s11250-023-03783-1

Oladokun, S., & Adewole, D. (2023). The effect of Bacillus subtilis and its delivery route on hatch and growth performance, blood biochemistry, immune status, gut morphology, and microbiota of broiler chickens. *Poultry science, 102*(4), Article 102473. https://doi.org/10.1016/j.psj.2022.102473

Piotrowska, A., Burlikowska, K., & Szymeczko, R. (2011). Changes in blood chemistry in broiler chickens during the fattening period. *Folia Biologica (Krakow), 59*(3-4), 183–187. https://doi.org/10.3409/fb59_3-4.183-187

Rajman, M., Juráni, M., Lamošová, D., Máčajová, M., Sedlačková, M., Košťál, Ľ., Ježová, D., & Výboh, P. (2006). The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 145*(3), 363–371. https://doi.org/10.1016/j.cbpa.2006.07.004

Ritchie, B. W., Harrison, G. J., & Harrison, L. R. (1994). *Avian medicine: principles and application*. Lake Worth, Florida, USA: Wingers Publishing Inc.

Ruiz-Jimenez, F., Gruber, E., Correa, M., & Crespo, R. (2022). Establishment of age-specific whole blood biochemistry and gas reference intervals in broiler chickens using the i-STAT and the vetscan vs2 portable analyzers. *Avian Diseases, 66*(1), 95–100. https://doi.org/10.1637/21-00011

Sauer, Z. C., Taylor, K., Wolc, A., Viall, A., O'Sullivan, N., Fulton, J. E., Rubinoff, I., Schaal, T., & Sato, Y. (2019). Establishment of Hy-Line commercial laying hen whole blood gas and biochemistry reference intervals utilizing portable iSTAT clinical analyzer. *Poultry Science, 98*(6), 2354–2359. https://doi.org/10.3382/ps/pey600

Schaal, T. P, Arango, J., Wolc, A., Brady, J. V., Fulton, J. E., Rubinoff, I., Ehr, I. J., Persia, M. E., & O'Sullivan, N. P. (2016). Commercial Hy-Line W-36 pullet and laying hen venous blood gas and chemistry profiles utilizing the portable i-STATt1 analyzer. *Poultry Science, 95*(2), 466–471. https://doi.org/10.3382/ps/pev350

Silva, P. R. L., Freitas, O. C., Laurentiz, A. C., Junqueira, O. M., & Fagliari, J. J. (2007). Blood serum components and serum protein test of Hybro-PG broilers of different ages. *Brazilian Journal of Poultry Science, 9*(4), 229–232. https://doi.org/10.1590/S1516-635X2007000400004

Szabó, A., Mézes, M., Horn, P., Sütő, Z., Bázár, G. Y., & Romvári, R. (2005). Developmental dynamics of some blood biochemical parameters in the growing turkey (Meleagris gallopavo). *Acta veterinaria Hungarica, 53*(4), 397–409. https://doi.org/10.1556/avet.53.2005.4.1

Talebi, A., Asri-Rezaei, S., Rozeh-Chai, R., & Sahraei, R. (2005). Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian). *International Journal of Poultry Science, 4*(8), 573–579. https://doi.org/10.3923/ijps.2005.573.579

Tang, F., Messinger, S., & Cray, C. (2013). Use of an indirect sampling method to produce reference intervals for hematologic and biochemical analyses in Psittaciform species. *Journal of Avian Medicine and Surgery, 27*(3), 194–203. https://doi.org/10.1647/1082-6742-27.3.194

Thrall, M. A., Weiser, G., Allison, W. R., & Campbell, W. T. (2012). *Veterinary hematology and clinical chemistry* (2nd ed.). Oxford, UK: Blackwell Publishing.

Tully, T. N., Dorrestein, G. M., & Jones, A. K. (2009). *Handbook of Avian Medicine* (2nd ed.). New York, USA: Elsevier/Saunders.

USDA. (2024). *Livestock and poultry: World markets and trade*. United States Department of Agriculture, Foreign Agricultural Service. Available at https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf. Accesed November 23, 2024.

Weiss, D. J., & Wardrop, K. J. (2010). *Schalm's veterinary hematology* (6th edition). Baltimore, Maryland, USA: Lippincott William and Wilkins.