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Biochemical reference intervals in broiler chickens according to age and strain

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Abstract. *The objective of the research was to study the physiological pattern of biochemical variables and to obtain reference intervals for young (less than 1 month) and adult (more than 1 month) broiler chickens of 2 strains. From the jugular vein, blood for analysis was collected, separated, and then immediately analyzed. The influence of age was significant for the majority of the investigated variables in the 2 groups. In Isa15 strain, a significant age-related decrease in plasma uric acid, glucose, cholesterol, triglycerides, phosphorus, potassium and iron was established. Also, a significant age-related increase in plasma calcium and ASAT was obtained in the same broiler strain. In Arbor Acres Plus strain, a significant age-related decrease in plasma glucose, cholesterol, triglycerides and phosphorus was observed. A significant age-related increase in plasma total protein, calcium and ASAT has also been reported in this broiler strain. A significant difference between the two strains was reported for plasma uric acid, glucose, cholesterol, phosphorus, magnesium and iron (p<0.05). For most estimated parameters in the 2 strains, calculation of separate reference intervals for young and adult animals was appropriate. Except for potassium in young Isa15 strain, and phosphorus in young Arbor Acres Plus strain, all variables did not follow a normal distribution. Reference intervals are presented for both ages using non-parametric or robust method. 90% confidence intervals for both groups were calculated non-parametrically, or by the bootstraping method. The established reference intervals will be a useful guide for interpreting plasma biochemical variables in different strains of broiler chickens raised in Algeria, and reared in a mild Mediterranean climate.*

Keywords: blood analysis, domestic chicken, meat type, plasma, reference value

Introduction

Reference values are generally utilized as a veterinary diagnostic tool. Specific reference ranges are therefore needed for each animal species for a correct interpretation of laboratory tests (Miranda et al., 2008). The analysis of blood variables can provide useful information on the animal’s general condition, and can be used to determine the health status or a disaese’s diagnosis or differential diagnosis (Bowes et al., 1989; Omer et al., 2006; Al-Busadah, 2007). However, avian clinical pathology is a field with real scarcity in reference intervals (Tang et al., 2013; Board et al., 2018), and compared to their use in large animals, laboratory ranges have been measured much less frequently in avian medicine.

The International Federation of Clinical Chemistry (IFCC), the Clinical Laboratory and Standards Institute (CLSI, 2008), and the American Society for Veterinary Clinical Pathology (ASVCP) based on those in human clinical pathology (Friedrichs et al., 2012), carried out a review of the recommendations for the establishment of reference intervals, and common guidelines have been selected. More previous rules are reaffirmed as the determination of reference intervals using the non-parametric approach with a minimum of 120 “reference individuals”. However, given the difficulties encountered in creating these references (time, cost, sample size), alternative statistical methods such as Robust method that would be suitable for smaller reference samples are now allowed to be used. Blood chemistry of broiler chickens is a scientific field that is still little exploited in Algeria, and studies are needed to define reference values in different strains reared in our country. Therefore, the aim of this paper was to establish reference intervals for selected biochemical indices for 2 age classes of broiler chickens (young and adult) in Isa15 and Arbor Acres Plus strain using Reference Value Advisor version 2.1, and to compare reference limits of the two strains.

Material and methods

Ethical statement for the study

This research involving animals adheres to all legal and ethical requirements and other applicable guidelines. The research protocol has been reviewed by the scientific committee of the

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Institute of Veterinary Sciences of Constantine - Algeria. The guiding principle of this committee is the 3Rs (reduction, replacement and refinement). The scientific committee has ensured that the animals were housed in appropriate facilities, and had access to veterinary care. All personnel who work with the animals were sufficiently trained both in the experimental procedure and ethical handling of the animals. All researchers participating in this animal experimentation had also been trained in handling avian species.

Animals and housing

The research was performed at the poultry farm Benboulaid (15 km from Constantine, Eastern Algeria). Experiments were performed on 240 broilers of two strains: 120 heavy fast growing broilers Arbor Acres Plus strain and 120 lighter weight fast growing broilers Isa15 strain. Two separate battery brooders were used, however, the animals were housed in equivalent conditions according to the technological recommendations for meat type hybrids.

Diet

For both groups, food and water were provided *ad libitum*. Broilers were divided into 2 categories according to their age: young broilers included animals in the starter and grower phase, up to the age of one month, and adult broilers included animals in the finisher phase, over one month old. The National Livestock Feeding Office borrowed food formulations for broilers. A starter diet was offered to birds of the two groups during the period 1-11 days. A grower diet was offered from 12 days until the end of the fattening period. Ingredients and composition of broiler diet for both strains are reported in Table 1.

Table1. Ingredients and composition of broiler diet

	Starter diet	Grower diet
Ingredients		
Maize, %	50	62
Soybean meal (45 % CP), %	24	26
Wheat bran, %	23	8.50
Limestone, %	1	0.90
Dicalcium Phosphate, %	1	1.60
Mineral-vitamin complex, %	1	1
Chemical composition		
Metabolizable energy, kcal/kg	2823.75	2908.53
Crude protein, %	22.11	20.32
Lysine, %	1.24	1.12
Methionine, %	0.52	0.47
Methionine+Cysteine, %	0.93	0.83
Total calcium, %	1.10	1
Total phosphorus, %	0.70	0.69
Available phosphorus, %	0.45	0.44
Sodium, %	0.17	0.17
Chloride, %	0.30	0.30

Inclusion and exclusion criteria

Animals included in our study were in good health, showing no sign of visible pathology. In order to limit the risk of inter-individual variability, each group of individuals selected had neighboring weights. No abnormal mortalities were observed.

Partitioning criteria

Statistical methods allow a reference population to be divided into more homogeneous subsets, but only those likely to have an influence on clinical interpretation should be retained. In our study, age was used as a partition criterion. For each strain, chickens were divided into 6 lots of 20 individuals. 60 young chickens under one month of age: 20 of 14 days, 20 of 21 days and 20 of 28 days were included, and 60 adult chickens over one month of age; 20 of 35 days, 20 of 42 days, 20 of 57 days for Arbor Acres Plus strain and 20 of 59 days for Isa15 strain were selected. Strain was also used as a partition criterion in this paper: Isa15 and Arbor Acres Plus were designated for the study.

Blood samples

At 14, 21, 28, 35, 42 and 57 days of age for Arbor Acres strain Plus, and at 59 days of age for Isa15 strain, 20 birds per strain were investigated. From the jugular vein, blood for analysis was extracted and collected into heparinized polystyrene tubes. Blood plasma was isolated immediately after sampling by centrifugation at 3,000 rpm for 10 minutes and analyzed.

Biochemical analysis

All clinical chemistry analyses were carried out on the ARCHITECT ci 8200 Integrated System (Abbott Diagnostics, Illinois, USA). Colorimetric methods were used for analysis of total proteins (Tietz, 1995), creatinine (Fabiny and Ertingshausen, 1971), calcium (Henry et al., 1974), magnesium (Burtis and Ashwood, 1994), phosphorus (Fisk and Subbarow, 1925), and iron (Tietz, 1986). Enzymatic methods were employed for analysis of uric acid (Trivedi, 1978), glucose (Farrance, 1987), cholesterol (Allain et al., 1974), triglycerides (Fossati and Prencipe, 1982), ASAT (Bergmeyer et al., 1978). Sodium, potassium, and chloride were determined electrochemically (Tietz, 1986).

Statistical analysis

Data review was carried out on the theory of reference values according to the approved guidelines of the American Society for Veterinary Clinical Pathology (ASVCP) (Friedrichs et al., 2012). Statistical research was performed using Reference Value Advisor version 2.1 (Geffre et al., 2011). The following approach was used to define reference intervals: values were included as continuous variables and their distribution determined. When the p-value Anderson-Darling was less than 0.05, normality was challenged. Outliers were tested using Dixon-Reed's and Tukey's tests (Reed et al., 1971). Reference intervals were calculated using the non-parametric method when at least 120 reference values were available, with the 2.5th and 97.5th fractiles as the lower and upper reference limits. 90% confidence intervals around these limits were determined non-parametrically. When the number of reference individuals was less than 120, reference intervals were calculated by non-parametric or Robust method when the p-value symmetry test for robust was greater than 0.05, and 90%, confidence intervals were measured by the bootstrapping method (Friedrichs et al., 2012). To determine if these variables should be stratified based on age, a one-way analysis of variance was used to evaluate significant differences attributable to age for all measured analytes, and data obtained for plasma biochemical variables from young chickens (less than 1 month) and adult chickens (more than 1 month) were compared. Separate reference intervals were computed for parameters that displayed statistical variation due to age. T-test for independent samples was used to evaluate significant differences attributable to strain for all measured analytes. Calculated p values ≤0.05 were considered statistically significant. Statistical analysis was performed using Statview 1992-98 SAS Institute. Inc.

Results

Biochemical results for Isa15 strain and Arbor Acres Plus strain are presented in Table 2. All data are reported as mean±SD. In Isa15 strain, a significant age-related decrease in plasma uric acid, glucose, cholesterol, triglycerides, phosphorus, potassium and iron was established. Also, a significant age-related increase in plasma calcium and ASAT was reported. In Arbor Acres Plus strain, a significant age-related decrease in plasma glucose, cholesterol, triglycerides, and phosphorus was observed. Also, a significant age-related increase in plasma total protein, calcium and ASAT was obtained. A significant difference between the two strains was reported for plasma uric acid, glucose, cholesterol, phosphorus, magnesium and iron. Existing biochemical reference intervals for the domestic fowl *Gallus gallus* are detailed in Table 3. Reference values for investigated biochemical variables in Isa15 strain and Arbor Acres Plus strain are summarized in Tables 4 and 5, respectively. Calculation of separate reference intervals for young and adult animals was necessary for most estimated parameters in both strains. Reference intervals are presented for both ages using non-parametric method in Isa15 strain and Arbor Acres Plus strain. Robust method was used for potassium in young Isa15 chickens, and phosphorus in young Arbor Acres Plus strain. One outlier was detected each time for creatinine and triglycerides in Isa15 strain, and for Iron and Phosphorus in Arbor Acres Plus strain.

Table 2. Comparison of biochemical analytes (mean±SD) that showed significant difference ($p<0.05$) between young and adult chickens, and between Isa15 and Arbor Acres Plus strain

Analyte	Unit	Young Isa15 strain Mean±SD	Adult Isa15 strain Mean±SD	Young Arbor Acres Plus strain Mean±SD	Adult Arbor Acres Plus strain Mean±SD
Total proteins	g/L	26.85±2.23	27.24±8.23	25.96±10.41	31.12±5.36**
Creatinine	mg/L	2.52±0.34	2.38±0.33	2.58±0.52	2.44±0.50
Uric acid	mg/L	40.91±16.44	33.54±10.66**	67.22±20.20$^{≠≠}$	66.04±29.30$^{≠≠}$
Glucose	g/L	2.44±0.17	1.92±0.63**	2.33±0.56$^{≠}$	1.72±0.70**$^{≠}$
Total Cholesterol	g/L	1.14±0.17	0.75±0.30**	1.33±0.23$^{≠}$	0.80±0.37**$^{≠}$
Triglycerides	g/L	0.71±0.25	0.60±0.24*	0.88±0.27	0.53±0.22*
Calcium	mg/L	92.55±21.30	103.76±4.69**	83.63±26.67	110.69±16.11**
Magnesium	mg/L	20.70±1.85	21.25±2.96	20.43±3.60	17.68±6.27$^{≠}$
Phosphorus	mg/L	63.79±6.30	57.55±13.60**	61.90±10.59$^{≠}$	52.37±17.51**$^{≠}$
Iron	µg/dL	98.21±21.46	66.11±22.77**	97.25±23.55	81.43±31.03$^{≠}$
Sodium	mEq/L	146.71±2.12	146.55±7.38	148.50±7.65	147.95±10.54
Potassium	mEq/L	5.21±0.70	4.89±0.86*	5.40±0.68	4.55±0.64
Chloride	mEq/L	113.44±2.96	112.24±6.36	112.17±5.01	112.01±7.44
ASAT	UI/L	201.11±70.12	233.75±17.88**	198.45±65.70	231.53±27.22**

*when compared with age class, #when compared with strain; *: signifcant ($p<0.05$), **: very significant ($p<0.01$); #: signifcant ($p<0.05$), ##: very significant ($p<0.01$).

Table 3. Validating study of existing biochemical reference intervals for the domestic fowl, *Gallus gallus*[1]

Analyte	Kaneko et al., 2008	Weiss and Wardrop, 2010	Carpenter, 2013	Board et al., 2018	Heatley and Russel, 2020
Total protein, g/L	56.0	-	33-55	39-70	52.0±6.0
Creatinine, mg/L	1-4	-	-	-	1.29±0.02
Uric acid, mg/l	-	25-81	-	9-89	65±12
Glucose, g/L	1.67	-	-	1.74-2.39	2.18±0.23
Tot. Cholesterol, g/L	1.83	-	-	-	1.45±0.2
Calcium, mg/l	284	-	132-237	≥109	110.4±4.0
Phosphorus, mg/L	78.1	62-79	41-57	16-72	36±10
Sodium, mmol/L	-	141-152	131-171	133-151	141-157
Potassium, mol/L	-	-	3-7.3	3.2-6.1	4.0-6.0
ASAT, UI/L	174.8	-	-	118-298	261.2±151.8
Triglycerides, g/L	-	-	-	-	0.76±0.23
Chloride, mEq/L	-	-	-	-	112±1.5

[1]: Means in parentheses, Ranges with means ± standard deviations in hooks

Table 4. Biochemical reference intervals for broiler chickens of Isa15 strain[1]

Analyte	Age class	N	Median	Min-Max	Lower limit of reference interval	Upper limit of reference interval	90% CI for lower limit	90% CI for upper limit	Method used
Total protein, g/L	B	120	27.1	8.0-38.3	10.5	36.8	8.0-12.6	36.1-38.3	NP/NP
Creatinine, mg/L	B	119	2.5	2.0-3.42	2.0	3.0	2.0-2.0	3.0-3.4	NP/NP
Uric acid,	Y	60	40.8	17.0-88.0	17.6	84.9	17.0-20.0	68.8-88.0	NP/BS
mg/l	A	60	32.2	16.0-54.3	16.5	52.6	16.0-18.0	50.4-54.3	NP/BS
Glucose,	Y	60	2.5	2.07-3.03	2.1	2.9	2.1- 2.2	2.7- 3.0	NP/BS
g/L	A	60	2.3	0.49-2.83	0.5	2.7	0.5- 0.8	2.4- 2.8	NP/BS
Total cholesterol, g/L	Y	60	1.2	0.81-1.52	0.8	1.5	0.8-0.8	1.1- 1.5	NP/BS
	A	60	0.9	0.19-1.5	0.2	1.4	0.2- 0.3	1.1- 1.4	NP/BS
Triglycerides,	Y	60	0.7	0.25-1.75	0.3	1.4	0.3-0.3	1.0-1.8	NP/BS
g/L	A	60	0.6	0.24-1.44	0.3	1.3	0.2-0.3	1.1-1.4	NP/BS
Calcium,	Y	60	100.0	36.8-18.4	40.1	118.4	36.8-61.1	115.5-118.4	NP/BS
mg/l	A	60	103.0	95.4-13.6	96.0	113.2	95.4-98.3	111.4-113.6	NP/BS
Magnesium, mg/L	B	120	21.0	15.5-25.7	16.9	24.2	15.5-17.4	24.2-25.7	NP/NP
Phosphorus,	Y	60	64.0	46.0-75.0	46.3	74.6	46.0-53.5	74.0-75.0	NP/BS
mg/L	A	60	62.8	16.5-68.0	17.6	68.0	16.5-23.7	65.7-68.0	NP/BS
Iron,	Y	60	101.5	46.0-76.0	47.1	157.6	46.0-62.9	123.9-176.0	NP/BS
μg/dL	A	60	63.5	23.0-96.0	24.6	96.0	23.0-28.5	93.0-96.0	NP/BS
Sodium, mmol/L	B	120	147.0	122.0-159.0	125.1	156.0	122.0-136.0	152.0-159.0	NP/NP
Potassium,	Y	60	5.3	4.46-8.3	3.9	6.7	3.4- 4.4	6.2-7.1	R/BS
mmol/L	A	60	4.8	3.2-5.6	3.3	5.6	3.2- 3.4	5.4-5.6	NP/BS
Chloride, mmol/L	B	120	113.9	90.0-122.0	95.1	120.0	90.0-103.0	118.0-122.0	NP/NP
ASAT	Y	60	224.0	42.0-298.0	43.1	293.3	42.0-73.0	278.0-298.0	NP/BS
UI/L	A	60	233.0	196.0-310.0	197.1	294.3	196.0-212.2	255.0-310.0	NP/BS

[1]: presented stratified by age class, or presented overall, depending on if a difference between groups was detected;
[2]: B- both young and adult, Y- young, A- adult, NP- non-parametric method, R- robust method, BS- bootstrapping method.

Table 5. Biochemical reference intervals for broiler chickens of Arbor Acres Plus strain[1]

Analyte	Age class	N	Median	Min-Max	Lower limit of reference interval	Upper limit of reference interval	90% CI for lower limit	90% CI for upper limit	Method used
Total protein,	Y	60	29.1	8.4-44.9	9.1	44.2	8.4-11.3	38.8- 44.9	NP/BS
g/l	A	60	30.8	20.3-47.7	21.6	47.5	20.3-23.8	41.1- 47.7	NP/BS
Creatinine, mg/L	B	120	2.4	2.0-4.91	2.0	3.7	2.0-2.0	3.3-4.9	NP/NP
Uric acid, mg/l	B	120	63.7	22.0-139.8	23.1	112.1	22.0-32.0	99.0-139.8	NP/NP
Glucose,	Y	60	2.4	1.21-4.08	1.3	3.8	1.2- 1.5	3.1- 4.1	NP/BS
g/L	A	60	1.9	0.58- 3.41	0.7	3.2	0.6- 0.8	2.7- 3.4	NP/BS
Total cholesterol,	Y	60	1.3	0.91- 1.85	0.9	1.8	0.9-1.0	1.6- 1.9	NP/BS
g/L	A	60	0.9	0.21- 1.5	0.2	1.5	0.2- 0.3	1.4- 1.5	NP/BS
Triglycerides,	Y	60	0.8	0.39- 1.87	0.4	1.6	0.4-0.5	1.3-1.9	NP/BS
g/L	A	60	0.5	0.17- 1.61	0.2	1.3	0.2-0.3	0.9-1.6	NP/ BS
Calcium,	Y	60	89.2	30.7-111.9	33.4	111.4	30.7-38.9	110.0-111.9	NP/BS
mg/l	A	60	109.4	43.4-158.6	63.2	154.1	43.4-95.9	133.6-158.6	NP/BS
Magnesium, mg/L	B	120	19.9	7.3-35.6	7.3	28.7	7.3-7.9	25.4-35.6	NP/NP
Phosphorus,	Y	60	61.4	29.8-99.2	40.1	82.7	35.7-45.1	77.5-87.9	R/BS
mg/L	A	60	58.0	15.1-79.0	15.9	76.7	15.1- 21.2	72.6-79.0	NP/BS
Iron, μg/dL	B	120	96.5	21.0-161.0	26.1	144.9	21.0-30.0	133.0-161.0	NP/NP
Sodium, mmol/L	B	120	147.5	122.0-189.0	125.1	163.0	122.0-134.0	161.0-189.0	NP/NP
Potassium, mmol/L	B	120	5.1	3.2-6.39	3.3	6.4	3.2-3.5	6.1-6.4	NP/NP
Chloride, mmol/L	B	120	112.1	90.0-141.0	95.1	123.2	90.0-101.6	122.0-141.0	NP/NP
ASAT	Y	60	223.0	52.0-297.0	59.9	296.5	52.0-73.2	278.0-297.0	NP/BS
UI/L	A	60	228.0	100.0-289.0	150.9	288.5	100.0-200.0	266.0-289.0	NP/BS

[1]: presented stratified by age class, or presented overall, depending on if a difference between groups was detected;
[2]: B- both young and adult, Y- young, A- adult, NP- non-parametric method, R- robust method, BS- bootstrapping method.

Discussion

In order to correctly interpret laboratory analysis results, it is difficult to compare the values obtained with reference ranges established on populations that may be different. With this in mind, we considered that it would be useful to define reference intervals in strains of broilers reared in Algeria. The objective of the present paper was to provide physiological reference ranges for plasma analytes of young and adult broiler chickens and to investigate the variation in these values arising from differences between broiler strains. Arbor Acres chickens are heavy broilers selected on fast growth, and Isa15 chickens are lighter-weight fast-growing broilers (Abdullah et al., 2010). Reference intervals for biochemical variables have been established previously, and existing values are summarized in Table 3. The published ranges have been more restrictive, and the upper limits for uric acid, glucose, cholesterol, phosphorus and chloride in Arbor Acres Plus strain have exceeded those found in the literature. Values of the other variables were within the reference values proposed precisely for the upper limit. Lower limit of reference intervals fell out of the ranges for total protein, glucose, cholesterol, triglycerides, calcium, sodium and ASAT. This could be interpreted as differences in population characteristics in which other chicken genotypes could have been used, also diet and ages of individuals were not specially reported (Weiss and Wardrop, 2010; Carpenter, 2012; Board et al., 2018). Also, ranges are sometimes presented as means, or means with standard deviations (Kaneko et al., 2008; Heatley and Russel, 2020) and not as reference ranges. Additional reference intervals were established in this paper for magnesium and iron. In the present research, the influence of age was significant for the majority of the investigated variables in the both strains, which confirms that age specific reference intervals for broiler chickens are needed.

Laboratory evaluation of the avian kidney

By preserving water homeostasis and electrolyte balance, the avian kidney plays a major role in osmoregulation (Thrall et al., 2012). Because birds are uricotelic, they only have very small amounts of plasma urea, and the primary product of nitrogen catabolism is uric acid (Donsbough et al., 2010). It is a potent oxidative stress regulator (Rajman et al., 2006). Alvarez (2005) has confirmed that hyperuricaemia is a good predictor of renal failure especially when reference ranges are available. Age (Piotrowska et al., 2011) and diet (Szabo et al., 2005; Board et al., 2018) appear to be the two main factors that can influence blood uric acid levels in poultry. Generally, higher levels are detected in young birds in comparison to older chickens. In the present study, the high protein concentration typical of starter diet results in increased values in plasma uric acid in young Isa15 chickens. A marked influence of the strain was reported in this paper, and by Hartman et al. (2006) in domestic turkeys. Creatinine is a chemical waste molecule that is generated from muscle metabolism (Wyss and Kaddurah-Daouk, 2000). In birds, creatine is usually excreted by the kidney before it is converted to creatinine, therefore, plasma creatine may better detect a decreased glomerular filtration rate rather than creatinine concentrations (Thrall et al., 2012). They are directly proportional to muscle mass (Szabo et al., 2005; Rajman et al., 2006), and increases with age and muscle mass have been recorded. Piotrowska et al. (2011) found that from the 2nd to the 3rd week of age, the concentration of creatinine decreases slightly, and increases significantly afterwards. This was not documented in the present study. Indeed, no significant difference was found between young and adult chickens, or between strains.

Electrolytes

In the plasma of birds, sodium is the primary osmotically active electrolyte and potassium is the major intracellular cation. Consequently, with hemolysis, an artifactual rise in plasma potassium concentration occurs. The largest extracellular anion is chloride. Thus, chloride and sodium represent the main osmotically active components of plasma (Thrall et al., 2012). After potassium, magnesium is the most important intracellular ion (Bowes et al., 1989). The present paper revealed the highest levels of potassium in young chickens. An age-dependent gradual decrease in blood potassium content in relation to dietary intake has been reported (Hochleithner, 2013). No significant difference was observed for plasma sodium, magnesium and chloride. Concerning magnesium, its dosage in poultry has been a subject of few studies. Bowes et al. (1989) showed that plasma magnesium concentrations are directly related to its incorporation in the diet, and that, therefore, plasma levels of magnesium decrease with age. A significant difference was reported between the two strains for plasma magnesium.

Calcium and phosphorus

About 50% to 80% of the calcium in avian plasma is biologically inactive and consists of protein-bound calcium and complexed calcium. Total calcium concentration is therefore influenced by plasma protein concentrations. Ionized calcium represents the biologically active form (Stanford, 2005; De Matos, 2008). A crucial function in the body is played by organic and inorganic phosphorus that is complexed with calcium to form hydroxyapatite; the main component of the beak and bones, and also functioning as a storage form. Calcium and phosphorus are generally considered together, the calcium:phosphorus ratio remains above 1 in healthy individuals. If it is less than 1, it may be a sign of kidney disease (Harr, 2006). The present study reported the lowest levels of calcium and the highest levels of phosphorus in young chickens. The results of previous investigations are diversified. Some of them showed a clear increasing tendency in the first weeks of age, and other studies revealed only fluctuations in calcium and phosphorus concentrations (Bowes et al., 1989; Ansar et al., 2004; Szabo et al., 2005; Talebi, 2006; Silva et al., 2007; Zheng et al., 2020). Szabo et al. (2005) clarified that the low calcium levels observed just after hatching in young animals were probably due to the eggshell's progressive degradation. Stockham and Scott (2008) found that elevated phosphorus levels in young animals are likely to be the result of increased osteoblastic activity during rapid bone development. Thrall et al. (2012) also reported that young growing birds tend to have higher plasma phosphorus concentrations. A significant difference was detected between the two strains for plasma phosphorus.

Laboratory evaluation of plasma proteins

In the diagnosis of gastrointestinal, hepatic, renal, or infectious disease, total proteins represent important complementary constituents, and are essential to maintain homeostasis (Piotrowska et al., 2011). Age of birds is one of the most significant factors that can impact the amount of plasma proteins in chickens and higher levels are normally seen in adult birds, probably due to a rise in the level of several immunoglobulins (Szabo et al., 2005; Rajman et al., 2006; Schmidt et al., 2007; Silva et al., 2007; Filipović et al., 2007; Vergneau-Grosset et al., 2016; Zheng et al., 2020). This was described in our study for the two strains, but the increase of total proteins was not significant in Isa15 strain. No significant difference was found between the two strains. This is contrary to the findings of Ibrahim et al. (2012) who reported a significant serum total proteins difference among genotypes of chickens.

Laboratory evaluation of glucose metabolism

Birds have high metabolic rate and higher plasma glucose concentrations than mammals (Koochakasaraie et al., 2010). Hepatic glycogenolysis preserves the normal blood glucose concentrations. Birds do not decrease their glucose utilization during fasting, but fat depletion and protein mobilization are associated with the greatest energy loss (Thrall et al., 2012). Compared to young broilers, higher glucose levels in adults are in line with previous studies in birds that have also shown a steady age-dependent decrease in plasma glucose concentrations (Hernandez and Margalida, 2010; Zheng et al., 2020). Between Isa15 and Arbor Acres Plus strain, there was a significant difference in plasma glucose. This difference can reflect the greater efficiency of the Arbor Acres strain's utilization of energy.

Laboratory evaluation of the avian liver

Enzymes activities: The aminotransferases, including alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) are a group of enzymes that, by amino acid transfer, catalyze amino acid interconversion (Harr, 2006). ALAT is present in the liver, however, the specific diagnostic value of this enzyme is low in poultry. In certain cases, animals with serious liver damage have normal ALAT activity. This is reflecting the low activity of this enzyme in liver cells. Also, in most avian species, normal ALAT activity is lower than the sensitivity of most commercial analyzers. Its activity in erythrocytes is 1.6 times higher than in plasma, so hemolysis can lead to higher levels of this enzyme (Lumeij, 1987; Harris, 2009). Important ASAT activity has been observed in various poultry tissues including liver, heart and muscle. High values of this enzyme are commonly associated with damage in the muscle or liver. In combination with other basic tests, the activity of this enzyme offers better information; for example, creatine kinase amount can be investigated to exclude any muscle damage capable of causing an increase in the level of ASAT (Board et al., 2018). In the present paper, ASAT activity levels were higher in adult chickens. Elevated plasma levels of ALAT and ASAT activity have previously been reported in adult animals (Hochleithner, 2013).

Cholesterol and triglycerides: Cholesterol is a major lipid, it is an essential steroid for the renewal of all cell membranes. In its synthesis, the liver plays an essential role (Hochleithner, 2013). Triglycerides represent the liver's energy reserves and are involved in multiple metabolic pathways (Piotrowska et al., 2011). Higher plasma cholesterol and triglyceride values were observed in young compared to adult chickens in the present study, and a significant difference was identified for cholesterol levels between Isa15 and Arbor Acres Plus strain. Plasma triglyceride concentrations are generally higher at young ages indicating a normally intensive early metabolism and transport of lipids (Noble and Cocchi, 1990). Similar to our findings, a general reduction in blood triglycerides and cholesterol with age was observed by Peebles et al. (1997). Krasnodebska-Depta and Koncicki (2000) detected the tendency of triglycerides to decrease with age, and did not show any correlation between age and cholesterol concentration in broilers. Szabo et al. (2005) described a sudden decrease of blood triglyceride levels at the early life of turkeys as a result of decline of the intestinal fatty acid binding protein (FABP).

Iron metabolism: Via its involvement in hemoglobin production and its function in tissue respiration, iron plays an important role. A few anecdotal reports describe an increase in serum iron levels in poultry compared to control animals (Harr, 2006). A decrease in sideremia with age is reported by Piotrowska et al. (2011). The authors suggested that the level of erythropoiesis decreases with age explaining the lower needs for iron in older chickens. Mohanna and Nys (1998) reported that iron utilization in chickens decreased rapidly before the 21^{st} day and only fluctuations were observed thereafter. A significant difference was observed between the two strains for plasma iron.

Conclusion

For many years, the reference limits will remain a valuable "tool" to guide the interpretation of biochemistry examinations by the veterinarian. They constitute one of the components of the medical decision which is multifactorial. According to the approved recommendations of the American Society for Veterinary Clinical Pathology (ASVCP) on the theory of reference values, the present paper allowed us to determine reference ranges for selected plasma biochemical variables in young and adult Isa15 and Arbor Acres Plus broiler chickens. Updating these values will certainly help to detect anomalies earlier, and to assess the quality of functioning of certain organs by a correct interpretation of the obtained biochemical profile.

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Conflicts of interest

The authors have declared no conflict of interest.

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