



Revista Brasileira de Ciência Avícola

ISSN: 1516-635X

revista@facta.org.br

Fundação APINCO de Ciência e Tecnologia
Avícolas
Brasil

Silva, PRL; Freitas Neto, OC; Laurentiz, AC; Junqueira, OM; Fagliari, JJ
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Revista Brasileira de Ciência Avícola, vol. 9, núm. 4, outubro-diciembre, 2007, pp. 229-232
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

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Blood Serum Components and Serum Protein Test of Hybro-PG Broilers of Different Ages

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■ Keywords

Broiler, serum biochemistry, serum protein test.

■ Acknowledgments

The authors thank FAPESP for providing financial support, and Renata Lemos Nagib Jorge and Paulo César Silva for their technical cooperation.

ABSTRACT

Blood serum samples of HYBRO PG broilers were analyzed, with 30 samples collected from 21-day-old broilers (G1), 30 from 35-day-old birds (G2), and 30 from 42-day-old birds (G3), with the aim of establishing normal values of some blood serum parameters. The activities of the enzymes gamma-glutamyl-transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), serum levels of total calcium, calcium ion, phosphorus, sodium, potassium, magnesium, chlorides, creatinine, uric acid, triglycerides, cholesterol, total protein, albumin, total and indirect and direct bilirubin, and electrophoretic profile of serum proteins in acrylamide (SDS-PAGE) and agarose gel were determined. There was no influence of age on total bilirubin and albumin levels. All the other evaluated parameters presented differences in at least one age group. Protein electrophoretic profile also changed as a function of age. The obtained results can be considered as normal for the studied ages, and therefore be used as references for the interpretation of laboratory exams of broilers of this genetic line in the evaluated ages.

INTRODUCTION

In the last few years, the poultry industry has shown extraordinary growth in Brazil, which is currently one of the main global producers and exporters of broilers.

When an animal population grows, there is often a proportional increase in disease incidence. Diseases present such a wide variety of symptoms that the physical exam is not sufficient to provide a diagnosis.

Blood components may be influenced by physiological factors, such as age and species, and by pathological factors (Szabo *et al.*, 2005; Lloyd & Gibson, 2006). The loss of cell membrane integrity caused by hypoxia or traumatism causes enzymes to leak to the extracellular fluid, where they are measured, allowing to determine the degree of cell or tissue lesions. These analyses are discussed in several studies with domestic animals (Jain, 2000), but only a few specifically refer to broilers (Kaneko *et al.*, 1997).

The evaluation of the levels of total protein and its fractions supply the information required to interpret the occurrence of dehydration, infections, immune diseases, and inflammatory responses.

The determination of blood component values using laboratory exams is an important procedure to aid the diagnosis of several diseases and dysfunctions, as they provide reliable results, and may also give inputs for research studies on nutrition, physiology, and pathology (Bounous *et al.*, 2000).

This study aimed at determining the levels of blood serum components of broilers of different ages, including serum protein test in



acrylamide gel (SDS-PAGE), in order to provide useful data for the diagnosis and prognosis of broiler diseases.

MATERIAL AND METHODS

Ninety blood serum samples were collected from HYBRO-PG broilers, being 30 samples collected at 21 days of age (G1), 30 collected at 35 days of age (G2), and 30 collected at 42 days of age (G3). Broilers were fed a corn and soybean meal-based diet, which supplied the nutritional requirements recommended by Rostagno (2000). The ages chosen for blood collection coincided with diet change (21 and 35 days of age), and with market age (42 days of age).

Blood samples were collected by puncture of the jugular vein in 21-day-old broilers, and axillary vein in 31- and 42-day-old broilers, using 5-ml plastic syringes, and 25 X 7 mm needles.

The activities of the enzymes gamma-glutamyl-transferase (modified Szasz' method), aspartate aminotransferase (Reitman-Frankel' method), creatine kinase (IFCC method), alkaline phosphatase (Bowers and McComb's method), lactate dehydrogenase (pyruvate-lactate method), serum levels of total calcium (Labtest method), calcium ion (ion-selective method), phosphorus (Basques-Lustosa's method), magnesium (Tonks' method), sodium (ion-selective method), potassium (ion-selective method), chlorine (Labtest method), creatinine (Basques-Lustosa's method), uric acid (Trinder's enzymic method), triglycerides (Trinder's enzymic method), cholesterol (Trinder's enzymic method), albumin (bromocresol green method), total protein (biuret method), total and direct bilirubin (Sims-Horn's method) were determined using of commercial kits (LABTEST). Samples reading were performed using spectrophotometry (LABQUEST semi-automatic spectrophotometer) with light wave length adequate for each test. Indirect bilirubin content was calculated by subtracting direct bilirubin level from total bilirubin level. Protein fractions were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli (1970).

Serum components were expressed as means and standard deviation, which were submitted to statistical analysis. Means were compared by the test of Tukey after sample homogeneity was verified (Zar, 1999). Significance was considered at 5% probability level. Serum protein test results were expressed as lower and upper limits.

RESULTS AND DISCUSSION

Table 1 shows the means and standard deviations of the serum activities of the enzymes AST, ALP, GGT, LDH, and CK of healthy broilers of different ages: 21 days of age (G1), 35 days of age (G2), and 42 days of age (G3). Serum activity of all enzymes changed as a function of bird age. AST serum activity progressively increased with age, and LDH and CK activities were higher at older ages, which is consistent with the observations of other studies (Sandhu *et al.*, 1998; Agawane & Lonkar, 2004). This is possibly due to the increase of liver metabolism and to the significant muscle development that usually happens during this period, as observed by Szabo *et al.* (2005) in turkeys of commercial strains. ALP serum activity, as opposed to AST, LDH, and CK, was higher at 21 days of age due to a higher bone development, as compared to older ages. The activity of GGT was not different between extreme ages (G1 and G3), but it was higher at 35 days of age, a phase when there is rapid body development due to higher feed intake and feed gain (Almeida *et al.*, 2006), with a concurrent significant increase of liver metabolism. The differences detected among groups relative to serum enzymes are probably due to physiological changes that are normal in these different ages. Nevertheless, birds were not tested for mycotoxins, which may also influence these parameters and it is frequently present in farms.

Table 1 - Means and standard deviations of serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), and gamma-glutamyl-transferase (GGT) of broilers at 21 days of age (G1), 35 days of age (G2), and 42 days of age (G3).

Enzyme	G1	G2	G3
AST(U/mL)	173.19±17.84c	228.95±49.66a	272.94±53.38b
ALP (U/L)	18.164±6.961a	3.853±1.969b	5.103±2838b
LDH (U/L)	2.089±696b	2.028±625b	3.007±1167a
CK (U/L)	2.485±1.122b	13.019±7.905a	13.205±7.838a
GGT(U/L)	26.86±8.7b	37.32±8.8a	21.28±9.5b

Different small letters in the same row indicate significant difference among groups (P<0.05).

Table 2 shows the means and standard deviations of mineral serum levels. There were changes in the levels of Ca, P, Mg, Na, and K, with the highest values obtained at 35 days of age. Chlorine serum level was the lowest in G2. Calcium and phosphorus level at 21 days of age were similar to those observed in the control group in the study of Oguz *et al.* (2000), whereas sodium and potassium levels at 42 days of age were different from those analyzed in healthy



broilers by Oguz *et al.* (2002). Harr (2002) stressed that blood mineral results levels may be different as a function of the methodology applied, and breed and genetic line may also influence these parameters (Gyenis *et al.*, 2006). The higher mineral levels observed in the sera of 35-day-old birds are probably due to the higher nutrient supply (Rajman *et al.*, 2006), and to the more intense metabolism that commonly occurs at this age (Almeida *et al.*, 2006). Therefore, the obtained results suggest that the different blood mineral components usually vary with age due to physiological changes that happen at each development phase.

Table 2 - Means and standard deviations of total calcium, calcium ion, phosphorus, magnesium, sodium, potassium, and chlorine of broilers at 21 days of age (G1), 35 days of age (G2), and 42 days of age (G3).

Component	G1	G2	G3
Total calcium (mg/dL)	9.54±1.65b	11.06±2.07a	8.85±1.35c
Calcium ion (mmol/L)	0.49±0.22a	0.51±0.006a	0.4±0.06b
Phosphorus (mg/dL)	6.23±0.75b	6.81±0.98a	6.12±0.83b
Magnesium (mg/dL)	2.14±0.17b	2.47±0.47a	1.99±0.11b
Sodium (mmol/L)	136.4±2.22c	150.1±16.42a	142.9±9.05b
Potassium (mmol/L)	3.94±0.81b	4.12±1.17a	3.48±0.89b
Chlorine (mEq/L)	107.8±7.29ab	106.5±6.04b	111.41±4.6a

Different small letters in the same row indicate significant difference among groups (P<0.05).

Serum levels of creatinine, uric acid, triglycerides, cholesterol, total protein, albumin, as well as total, direct and indirect bilirubin levels are presented in Table 3. It is observed that age did not influence total bilirubin and albumin levels, but all other components changed as a function of age. Creatinine level at 35 days of age was higher than in the other age groups, and no difference was detected between G1 and G3. The level of this catabolite directly related to muscle volume and activity, and therefore its lower blood levels in old and young chickens (Rajman *et al.*, 2006; Agawane & Lonkar 2004; Sandhu *et al.*, 1998). Uric acid level was higher in 21-day-old birds, and there was no difference between G2 and G3. the highest dietary protein level is supplied in the starter phase of production, and a study (Szabo *et al.*, 2005) found a direct relation between the amount of ingested protein and uric acid serum level. Rajman *et al.* (2006) found higher uric acid levels in broiler breeders during the rearing phase, at 30 and 44 days of age, as compared to older breeders.

Triglycerides and total cholesterol present a similar pattern of variation as a function of age, with higher values in G1 and G3 birds, as observed in other studies (Sandhu *et al.*, 1998; Oguz *et al.*, 2002). The higher values of these components in G1 correspond to their low mobilization by tissues and to their intense synthesis

by the liver (Szabo *et al.*, 2005). At 42 days of age, the increase of these values is related to the higher dietary energy supply (Rajman *et al.*, 2006). The lower triglycerides and cholesterol levels at 35 days of age are due to the bird's high energy requirement at this stage caused by high body development (Almeida *et al.*, 2006).

Table 3 - Means and standard deviations of serum levels of creatinine, triglycerides, uric acid, cholesterol, total protein, albumin, total bilirubin, direct bilirubin, indirect bilirubin of broilers at 21 days of age (G1), 35 days of age (G2), and 42 days of age (G3).

Component	G1	G2	G3
Creatinine (mg/dL)	0.49±0.06b	0.59±0.05a	0.46±0.05b
Uric acid (mg/dL)	9.2±2.13a	5.3±1.14b	4.59±1.01b
Triglycerides (mg/dL)	130.8±28.03b	97.11±25.16b	132.52±33.94a
Cholesterol (mg/dL)	140.16±20.34a	128.9±16.59c	129.42±20.36b
Total protein (g/dL)	2.96±0.37b	3.19±0.44a	3.23±0.23a
Albumin (g/dL)	1.72±0.29a	1.75±0.33a	1.72±0.21a
Total bilirubin (mg/dL)	0.39±0.13a	0.4±0.14a	0.41±0.12a
Direct bilirubin (mg/dL)	0.05±0.04c	0.09±0.05b	0.16±0.07a
Indirect bilirubin (mg/dL)	0.33±0.09a	0.31±0.09b	0.25±0.05c

Different small letters in the same row indicate significant difference among groups (P<0.05).

The evaluation of total protein serum levels did not reveal any differences between G2 and G3; however, at 21 days of age, its level was lower, due to the high requirement for growth. These data are compatible with those presented above for uric acid level, main catabolite of protein metabolism, which presented the highest serum levels at 21 days of age. Direct and indirect bilirubin levels were different in the three studied ages, and their variation is probably linked to variation in liver metabolism as a function of age.

Table 4 shows the results of the serum protein test obtained in acrylamide gel SDS-PAGE). Electrophoretic fractioning performed by this technique allowed the identification of 24 proteins, out of which 11 were identified by their name, including acute-phase proteins and immunoglobulins, whereas the remaining 13 were identified on molecular weight basis.

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Table 4 - Molecular weight (MW; Kilodalton, Kd) and lower limit (LL), mean (X) and upper limit (UL) of serum levels (mg/dL) of proteins in the serum protein test obtained in acrylamide gel (SDS-PAGE) of broilers at 21 days of age (G1), 35 days of age (G2), and 42 days of age (G3).

Protein	PM (KD)	G1			G2			G3		
		LL	X	UL	LL	X	UL	LL	X	UL
NI*	232	5	15	32	1	9	18	4	10	18
IgA	175	2	5	14	2	5	9	2	5	12
NI	143	1	4	18	0	5	10	0	3	7
NI	130	3	15	31	3	8	16	2	7	25
NI	117	0.3	2	9	1	4	11	0.3	2	5
Ceruloplasmin	110	3	12	31	0	11	19	3	22	50
Reactive-C protein	105	5	18	30	2	14	27	0	6	25
NI	98	0	8	34	4	8	15	00	0	0
Phosphorylase	94	0	9	40	0	4	19	2	59	152
Transferrin	85	0.9	14	40	2	19	49	7	15	28
Hemopexin	82	0.4	4	50	0	3	24	1	4	37
NI	79	55	119	183	40	116	187	0	66	144
Albumin	65	1.836	2.106	2.900	1.958	2.283	2.751	1.361	2.126	2.696
NI	58	5	9	22	0.6	6	13	2	10	17
NI	53	3	8	18	3	9	14	10	23	43
NI	47	0	2	8	0	1	6	0	3	18
Haptoglobin	45	4	10	15	4	9	15	6	13	21
Acid glycoprotein	40	2	6	18	0	6	11	3	8	33
NI	38	3	10	22	6	15	29	7	18	40
IgG light chain	36	252	523	707	492	662	855	400	752	1.009
NI	32	2	7	23	3	8	17	3	9	18
NI	28	0	5	17	0	5	9	2	5	17
Hemoglobin	16	30	50	97	30	49	82	35	80	103
NI	13	0	9	66	0	0	0	0	0	0

* Protein identified only by molecular weight. There are presently no purified proteins to nominally identify these fractions.

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