

NORMAL PHYSIOLOGICAL VALUES OF SOME ORGANIC AND INORGANIC COMPONENTS OF BLOOD PLASMA IN YELLOW HUNGARIAN LAYING HENS

VITINGER, EMŐKE Ms. — KOVÁCSNÉ GAÁL, KATALIN Ms. —
VITINGER, NIKOLETTA Ms. — ORBÁN, ANNA Ms.

SUMMARY

Reference values for some plasma biochemical parameters were established in the native Yellow Hungarian laying hens. A total of 18 parameters were examined include: total protein, albumin, globulin fractions (α -, β_1 -, β_2 -, γ -globulin), albumin/globulin ratio, triglyceride, cholesterol, glucose, sodium, potassium, chloride, calcium, phosphorus, magnesium, plasma iron and total iron binding capacity (TIBC). Results of 56 week-old laying hen blood samples ($n=64/96$) were used to calculate mean, standard deviation and coefficient of variation for each parameter investigated. The values were evaluated on the basis and means described for avian species in general. The obtained parameters were within normal physiological range reported in the literature. Only two parameters tended to the lower limit (plasma iron) or the upper limit (TIBC) of the range presented in the literature.

Values obtained in the present study will contribute the maintenance of the breed, preserve its genetic stock, and establish a baseline for further studies.

ÖSSZEFOGLALÁS

Vitinger E. – Kovácsné Gaál K. – Vitinger N. – Orbán, A.: A VÉRPLAZMA NÉHÁNY SZERVES ÉS SZERVETLEN KOMPONENSÉNEK VIZSGÁLATA SÁRGA MAGYAR TOJÓTYÚKOKBAN

A szerzők vizsgálataik során az őshonos sárga magyar tyúkállomány vérplazmájának analízisét végezték el. Összesen 18 paraméter vizsgálatának eredményét tartalmazza a közlemény, melyek a következők: összfehérje, fehérje frakciók (albumin, α -, β_1 -, β_2 -, γ -globulin), albumin/globulin arány, triglicerid, koleszterin, glükóz és a legfontosabb elektrolitok (nátrium, kálium, klorid, kalcium, foszfor, magnézium, vas és a teljes vaskötő kapacitás (TVK)). Egyes paraméterek esetében 64, másokban 96 tojótyúk (56 hetes korú) eredményéből átlag, szórás és CV% értékek meghatározására került sor. A kapott adatokat a szakirodalomban madarakra közölt eredményekhez hasonlítva megállapítható, hogy azok a határértékeken belül vannak, a vastartalom és a TVK azonban az irodalomban közölt minimum illetve maximum értékekhez közelít. A kapott adatok elősegítik az őshonos fajta jobb megismerését, megőrzését és további vizsgálatok alapjául is szolgálhatnak.

INTRODUCTION

Clinical haematology and blood biochemistry are relevant diagnostic tools and support valuable information on the metabolic profile and the state of health of birds. Utilisation of results of haematological and plasma biochemical examinations are limited mainly by the lack of data relevant to a given species or breed. Analysis of blood plasma is preferred to assess general health (Jain, 1993). As the clinical signs of morbidity in birds are frequently subtle, clinical chemistry is necessary to evaluate diseases, bacterial septicemia or nutritional deficiencies. Some parameters as glucose concentration or heterophils/lymphocyte ratio can be used as indicators of stress. Biochemical values can also prove useful as physiological indicators in conservation program.

In the past decades native bred are threatened by extinction in most of the developed countries. Increases in animal production require improved varieties, and animal improvement is certainly dependent upon genetic diversity. The local, native breeds usually had one or more adapting characteristics to their living environment in the past. They had good tolerance and resistant to diseases. The genetic stock of these breeds can be used to refresh the genetic stock of the 'industrial animals'. Because of the mentioned reasons, preservation and utilization of genetic resource of local breed is one of the research missions in animal sciences.

Native Yellow Hungarian chickens have been bred at the Faculty of Agriculture and Food Sciences, University of West Hungary in Mosonmagyaróvár since 1950. The aim of the breed-reservation is to maintain the original characteristic of Hungarian Yellow breed without gene-loss. The breed maintained in the Faculty is one of the two pure-blooded stocks in Hungary. Demand of costumers turned definitely to the traditional animal breeding and animal products. Native domestic breeds can take a prominent part in producing excellent quality, healthy meat. Studying of valuable characteristics of this species will contribute the maintenance of the breed and preserve its genetic stock.

In a recent study, Vitér (1996) reported the haematological parameters such as red blood cell, white blood cell, differential leucocyte counts, thrombocytes, hemoglobin and hematocrit values of this breed. The object of this study was to determine the physiological range of some other biochemical values and to compare this data to levels measured in other stocks or varieties in avian species.

MATERIALS AND METHODS

A total of 64 or 96 (according to the blood parameter) Yellow Hungarian laying hens about 56 weeks of age were used in these experiments. Laying hens were kept on litter in a naturally lightened broiler house. Hens were fed ad libitum with commercial layer feed produced by Lajta Hanság Ltd. Water was given ad libitum as well. Blood samples (5 cm³) were collected from *vena cutanea ulnaris* (Nickel *et al.*, 1977) using Na₂EDTA solution or heparin (according to the blood parameter) as an anticoagulant. The biochemical parameters were evaluated within short time as possible using commercial kits (Sigma-Aldrich Chemi-

cal Co.). Parameters and methods utilized for determining of blood chemistries (Gaál, 1999): total protein by biuret method (Hitachi-912 automated analyzer); protein fractions (albumin, α -, β_1 -, β_2 -, γ -globulins) by cellulose acetate electrophoresis (Biomidi analyzer); glucose analysis by GOD-POD (colorimetric test 115A); triglyceride by GPO-PAP (colorimetric test 701882); cholesterol by CHOD-PAP (monotest cholesterol 290319); sodium and potassium analysis by flame emission spectrophotometry; chloride analysis by mercury-rodanid method; calcium analysis by crezolphthalein-komplexon method; total phosphorus content by molibden-blue-komplexon method; magnesium by spectrophotometric komplexon method (test 595 A); iron and total iron binding capacity by spectrophotometric analysis (Hitachi-912 automated analyzer).

The mean, standard deviation and coefficient of variation (CV%) were obtained from levels detected in each individual, for each parameter investigated by Sváb (1981).

RESULTS AND DISCUSSION

Plasma biochemical and electrolyte values with mean, standard deviation and coefficient of variation are shown in *Table 1*. The obtained results were within the physiological range of variation presented in the literature. Evaluating the values of standard deviation and coefficient of variation, very satisfying (<20%) results were ascertained for blood biochemistry parameters except for γ -globulin, triglyceride, cholesterol and glucose (<20%). The majority of discrepancies can be attributed to individual differences, which probably exist among laying hens in the egg production period.

Total protein

Total protein content of Yellow Hungarian laying hens was 50.4 ± 7.3 g/l. Data was within the range reported for birds by Campbell (1997) (*Table 1*). Blood plasma contains a complex mixture of proteins with different structures and properties. Total plasma protein is made up by albumins, globulins, immune globulins, proteohormones, etc. In female birds, a considerable increase in plasma total protein concentration occurs before the production period. It could be attributed to an estrogen induced increase of globulins. The estimation of this parameter has been recognized as a valuable test for evaluating the general nutritional status of birds (van Wyk *et al.*, 1998). Lower total protein values are commonly associated with malnutrition.

Albumin

The obtained albumin value of the laying hens was 17.2 ± 2.1 g/l, and it was within the range reported for birds in the literature (Campbell, 1997). Albumin constitutes 30–40% of the total plasma protein. The main metabolic role of this fraction is its general transport function. Lowered albumin concentration may be present in undernourished birds due to the fact that albumin can be utilized as protein reserve and a carrier of several nutrients.

Table 1.

Organic and inorganic parameters of plasma in Yellow Hungarian laying hens

		n	$\bar{x} \pm sd$	CV%	Reference range(1)	
Total protein(2)	g/l	96	50.4±7.3	14.5	30–55	(Campbell, 1997)
Albumin	g/l	64	17.2±2.1	12.2	15–25	(Campbell, 1997)
α globulin	g/l	64	5.5±0.7	12.7	Total globulin content: 20–40	(Lumeij, 1997)
β_1 globulin	g/l	64	5.6±0.7	12.5		
β_2 globulin	g/l	64	4.9±0.6	12.2		
γ globulin	g/l	64	17.7±4.1	23.2		
A/G ratio(3)		64	0.6±0.04	7.1		
Triglyceride(4)	g/l	64	13.0±3.4	26.3	10.8–18.2	(Mori et al., 2000)
Cholesterol(5)	mmol/l	64	2.7±0.7	27.4	2.0–2.9	(Mori et al., 2000)
Glucose(6)	mmol/l	64	13.8±3.0	21.9	11.0–25.0	(Lumeij et al., 1990)
Sodium(7)	mmol/l	96	147.4±8.6	5.8	135–155	(Gaál, 1999)
Potassium(8)	mmol/l	96	3.9±0.6	15.4	3–6	(Gaál, 1999)
Chloride(9)	mmol/l	96	111.3±7.4	6.6	100–115	(Gaál, 1999)
Calcium(10)	mmol/l	96	5.8±4.1	17.8	5.0–7.5	(Campbell, 1997)
Phosphorus(11)	mmol/l	96	1.3±0.7	16.7	0.6–1.4	(Campbell, 1997)
Magnesium(12)	mmol/l	96	1.1±0.3	11.1	0.8–2.0	(Gaál, 1999)
Iron(13)	μ mol/l	96	17.6±2.6	15.0	15–40	(Gaál, 1999)
TIBC(14)	μ mol/l	96	66.1±5.4	8.2	50–68	(Gaál, 1999)

A/G: albumin/globulin ratio(3), TIBC: total iron binding capacity(14)

1. táblázat: A vérplazma szerves és szervetlen komponensei sárga magyar tojótyúkokban referencia értékek(1), összfehérje(2), albumin/globulin arány(3), triglicerid(4), koleszterin(5), glükóz(6), nátrium(7), kálium(8), klorid(9), kalcium(10), foszfor(11), magnézium(12), vas(13), teljes vaskötő kapacitás(14)

Globulins

Reference values of this fraction of birds are about 20–45 g/l (Lumeij, 1997). This group is a mixture of α -, β_1 -, β_2 - and γ -globulins and each fraction fulfils important biological functions. For example, α - and β -globulins are synthesized by the liver and constitute part of lipoproteins and vitellogenin. Globulins are necessary for yolk production and are transported via plasma to the ovary where they incorporated in the oocytes. The γ -fraction is synthesized by the B-lymphocytes as a response to antigenic stimuli, hence elevated levels indicate chronic infections or inflammation. Values of globulin fractions obtained in Yellow Hungarian laying hens are shown in Table 1.

Albumin/globulin (A/G) ratio

A/G ratio, as calculated in this study, was 0.56 ± 0.04 in laying hens at the final weeks of the egg production period. An A/G ratio of <1 indicates that globulins are found in higher concentrations than albumin. The A/G ratio is a parameter, which has been applied very extensively in the past as an index of differentiation among species in birds (Sturkie, 1986).

Triglyceride

Triglyceride values of plasma ranges between 10.8 g/l (Mori et al., 2000) and 18.2 g/l (Shafey et al., 2003) in the literature. The obtained values of Yellow Hungarian hens were well within the range (13.0 ± 3.4 g/l). During lipid metabo-

lism, triglyceride molecules are released from very low density lipoproteins (VLDL) and adipose tissue and used for energy supply of muscles or metabolites of gluconeogenesis. Triglyceride concentration of the blood is about 10 times higher in egg laying period under control of estrogen. In a special way, triglyceride is transported directly to the developing oocyte by VLDL.

Cholesterol

The obtained cholesterol value of laying hens was 2.7 ± 0.7 mmol/l, and it was within the range of variation presented in the literature (2.0–2.9 mmol/l, *Mori et al.*, 2000). The main part of blood cholesterol (about 80%) is synthesized in the liver; the other part (20%) can be obtained from the diet. Cholesterol is the precursor of steroid hormones, vitamin D and bile acids and is a constituent of cell membranes and bile micelles. There is a tremendous increase in the amount of cholesterol in plasma during the egg-laying period. Cholesterol content of egg has many varied uses in the developing embryo.

Glucose

Glucose concentration in laying hens was 13.8 ± 3.0 mmol/l. Values were similar to those previously published by *Roskopf et al.* (1982) and *Lumeij and Overduin* (1990) (11.0–25.0 mmol/l). Avian blood glucose values are much higher than those of mammals. Glucose metabolism is modulated by insulin and glucagon but there are species differences in the way in which birds regulate blood glucose. The insulin content is about 1/6 than that of mammals, while glucagon content is about 2 to 5 times higher (*Lumeij*, 1997). Absorption of glucose from the small intestine is more intensive to that of mammals (*Husvéth*, 2000). A persistent, marked hyperglycemia is reported during stress with glucocorticosteroid excess (*Siegel*, 1995). Diabetes mellitus caused by hyperglycemia is rarely observed in birds but blood glucose levels below 8.3 mmol/l should be considered serious and mortal.

Sodium, potassium and chloride

The obtained sodium values were 147.4 ± 8.6 mmol/l, potassium values were 3.9 ± 0.6 mmol/l and chloride values were 111.3 ± 7.4 mmol/l in Yellow Hungarian laying hens. These data were within the range reported in the literature (*Gaál*, 1999).

The predominant intracellular and extracellular anions and cations in birds are similar to those in mammals. The plasma osmolarity is preserved by these ions derived from their corresponding electrolytes present in blood plasma. Definite alterations in the values of plasma electrolytes in conjunction with plasma osmolarity can usually be assumed the changes of homeostatic stability.

Calcium and phosphorus

The mean plasma calcium level was 5.8 ± 1.0 mmol/l in laying hens. In laying cycle, the calcium value of blood is usually increased and ranges between 5.0 and 7.5 mmol/l (*Campbell*, 1997). Blood calcium metabolism of birds is mediated by parathormone, calcitonin and activated vitamin D₃ (1,25 dihydrocholecalciferol). Vitamin D₃ regulates the calcium-binding protein (CaBP) synthesis in

the intestines, which increases the rate of mucosal uptake of calcium. Calcium plays an important role in a wide variety of metabolism. Estrogen release and the onset of egg production in laying hens drastically increase calcium concentrations in blood. Under the control of estrogen, mobile calcium storage is created in medullary bones, which is used as a calcium reserve for eggshell formation (Rudas and Frenyó, 1995). The increase in the plasma calcium is associated with an increase of protein content.

Normal plasma phosphorus concentration for Yellow Hungarian laying hens was 1.3 ± 0.2 mmol/l. Reference values of birds reported in the literature range between 0.6 and 1.4 mmol/l (Lumeij, 1997). Approximately 80% of the total quantity is found in the skeletal system, with the remainder widely distributed throughout the body. The main functions in the body are: compound the cell membranes as part of phospholipids, importance in many functions of energy metabolism and activator of many enzyme systems. Plasma levels of phosphorus are responsive to dietary changes. Hypophosphatemia may result from nutritional deficiency of phosphorus. Rare causes of hyperphosphatemia include severe tissue trauma and osteolytic bone disease (Campbell, 2004). A factitious hyperphosphatemia can occur when blood plasma is not promptly separated from the cells and phosphorus can be released from red blood cells.

Magnesium

The levels of magnesium were 1.1 ± 0.1 mmol/l in blood samples. These values were similar to those presented in the literature (Table 1.). The main part of magnesium content of the body is found in the skeleton, the other part is found in the various tissues of the body, mainly in muscle. Some of the most important functions of magnesium include: metabolism of carbohydrates, activator or inhibitor of many enzymes, important role in muscle contraction. Less than 1% of all the magnesium content of the body can be found in the blood plasma.

Iron and total iron binding capacity (TIBC)

The obtained iron values in Yellow Hungarian laying hens were 17.6 ± 2.6 $\mu\text{mol/l}$. These data tended to the lower limit of the range presented in the literature (15 to 40 $\mu\text{mol/l}$) (Gaál, 1999). Iron content of plasma elevated three times higher before the onset a laying period. Transported iron of plasma is evaluated by measuring the plasma iron and TIBC. The TIBC represents the total amount of the iron transport protein called transferrin in plasma. The obtained TIBC values are 66.1 ± 5.4 $\mu\text{mol/l}$ as TIBC values ranges 50 and 68 $\mu\text{mol/l}$ in the literature (Gaál, 1999).

The obtained iron content of laying hens approached to the lower as TIBC tended to the upper limit of the range presented in the literature. These data expect latent iron deficiency when the iron content of plasma is still within the normal range but the signs of the exhausted organism begin to develop.

CONCLUSION

An increasing demand exists to study the complete blood count and biochemistry values of birds similarly to those of mammals. Blood parameters are

often influenced by species, age, gender nutritional status, season or physiological status of the animal. This makes interpretation of blood parameters and biochemistry results challenging. Reference values reported in the literature are only general guidelines that can be used as decision levels. Therefore, blood biochemistry examinations under a given set of environmental, nutritional or production periods can constitute a more meaningful set of reference values, specific for that species or breed. The obtained values are important, as they are the first report of the haematophysiological parameters of this native specific breed. Values obtained in the present study will contribute towards a better understanding of this unique breeding line and establish a baseline for further studies.

REFERENCES

- Campbell, T.W.(1997): Avian hematology and cytology, Ames, IA, Iowa State University Press
- Campbell, T.W.(2004): Blood Biochemistry of Lower Vertebrates. In: Proc. 55th Ann. Meet. Am. Coll. Vet. Path., Middleton WI., USA
- Gaál, T.(szerk.)(1999): Állatorvosi laboratóriumi diagnosztika. Sik Kiadó, Budapest
- Husvéth, F.(szerk.)(2000): Gazdasági állatok élettana az anatómia alapjaival. Mezőgazda Kiadó, Budapest
- Jain, N.C.(1993): Essentials of hematology. Lea and Febiger, Philadelphia, 61.
- Lumeij, J.T.(1997): Avian Clinical Biochemistry. In: Kaneko,J.J. – Harvey,J.W. – Bruss,M.L.(eds): Clinical Biochemistry of Domestic Animals. (5th ed.) Academic Press, San Diego, California. 857–884.
- Lumeij, J.T. – Overduin, L.M.(1990): Plasma chemistry reference values in Psittaciformes. Avian Pathol.,19. 235–244.
- Mori, A.V. – Mendon, X. – Watanabe, C.(2000): Effects of cholestyramine and lovastatin upon plasma lipids and egg yolk cholesterol levels of laying hens. Braz. J. Vet. Res. Anim. Sci., 37. 84–89.
- Nickel, R. – Schummer, A. – Seiferte, E.(eds.)(1977): Anatomy of the Domestic Bird. Verlag Paul Parey, Berlin-Hamburg
- Roszkopf, W.J. – Woerpel, R.W. – Roszkopf, G. – Van de Water, D.(1982): Hematologic and blood chemistry values for common pet avian species. Vet. Med. Small Anim. Clin., 77. 1233–1239.
- Rudas, P. – Frenyó, V.(szerk.)(1995): Az állatorvosi élettan alapjai. Springer Hungarica, Budapest
- Shafey, T.M. – Dingle, J.G. – McDonald, M.W. – Kostner, K.(2003): Effect of Type of Grain and Oil Supplement on the Performance, Blood Lipoproteins, Egg Cholesterol and Fatty Acids of Laying Hens. Int. J. Poult. Sci., 2. 200–206.
- Siegel, H.S.(1995): Stress, strains and resistance. Br. Poult. Sci., 36. 3–22.
- Sturkie, P.D.(ed.)(1986): Avian Physiology. (4th ed.). Springer-Verlag, Berlin
- Sváb, J.(1981): Biometria módszerek a kutatásban. Mezőgazdasági Kiadó, Budapest
- Vitinger, E.(1996): Az őshonos sárga magyar tyúkfajta kvantitatív és kvalitatív vérképe. Állattenyésztés és Takarmányozás, 45. 465–471.
- van Wyk, E. – van der Bank, H. – Verdoom, G.H.(1998): Dynamics of haematology and blood biochemistry in free-living African white backed vulture (*Pseudogyps africanus*) nestlings. Comp. Biochem. Phys., A 120. 495–508.

Érkezett: 2005. június
 Szerzők címe: Nyugat-Magyarországi Egyetem, Mezőgazdaság- és Élelmiszertudományi Kar
 Authors' address: University of West Hungary, Faculty of Agriculture and Food Sciences
 H-9200 Mosonmagyaróvár, Vár u. 4.
 E-mail: vitinger@mtk.nyhme.hu