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## Changes in concentration and fractions of blood serum proteins of chickens during fattening

Natalija Filipović\*, Zvonko Stojević, Suzana Milinković-Tur,  
Blanka Beer Ljubić, and Maja Zdelar-Tuk

*Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

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**FILIPOVIĆ, N., Z. STOJEVIĆ, S. MILINKOVIĆ-TUR, B. BEER LJUBIĆ, M. ZDELAR-TUK: Changes in concentration and fractions of blood serum proteins of chickens during fattening. Vet. arhiv 77, 319-326, 2007.**

### ABSTRACT

This paper describes research into concentration of total proteins and individual protein fractions in the blood serum of Ross broiler chickens during fattening period. Blood for analysis was taken at the 14<sup>th</sup>, 28<sup>th</sup> and 42<sup>nd</sup> days of age by wing vein puncture. Concentration of total serum proteins was detected by spectrophotometry. Electrophoresis on gelled cellulose-acetate tapes determined the shares of individual fractions: albumins, alpha 1-, alpha 2-, beta 1-, beta 2- and gamma globulins, and their absolute concentrations were calculated from the concentration of total proteins. A statistically significant growth of total proteins concentration was established during the experiment ( $P < 0.0001$ ). Changes of concentration of individual fractions did not always follow the quantity of total proteins in the blood serum. Concentration of albumins in blood did not show oscillations during fattening, unlike alpha 1-globulins, whose concentration showed a statistically significant increase by the 28<sup>th</sup> day of fattening ( $P < 0.0001$ ), and which then decreased considerably by the 42<sup>nd</sup> day compared to the 28<sup>th</sup> day ( $P < 0.05$ ). Concentration of alpha 2-globulins did not show significant growth until the 42<sup>nd</sup> day in comparison to the 14<sup>th</sup> day ( $P < 0.01$ ). Fractions of beta 1-, beta 2- and gamma globulins showed a significant increase during all trial periods (from  $P < 0.05$  to  $P < 0.0001$ ). The ratio of albumins to globulins showed a statistically significant decrease on the 28<sup>th</sup> and the 42<sup>nd</sup> days, compared to the 14<sup>th</sup> day of the experiment ( $P < 0.0001$ ). The given results are related to the role of protein metabolism in the rapid growth of chickens during fattening, and may serve as part of metabolic profile in evaluation of production patterns and health condition of chickens during fattening.

**Key words:** blood serum, total proteins, protein fractions, broiler chickens

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### Introduction

Blood plasma proteins play roles in the maintenance of colloid osmotic pressure, as a rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis,

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\*Contact address:

Natalija Filipović, DVM, Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Heinzelova 55, Croatia, Phone: +385 1 2390 172; Fax +385 1 2441 390; E-mail: natalija.filipovic@vef.hr

in transport of minerals and hormones, in build of enzymes and immune system in the organism. Therefore, blood plasma proteins have an exceptional significance in homeostasis maintenance. Total concentration of blood serum proteins of birds is about the same as half its value in mammals. In mammals it is 50-70 g/L (KANEKO, 1997), while in birds it is approximately 40 g/L (GRIMINGER and SCANES, 1986). Its cause is most probably the extremely high concentration of glucose in bird blood, approximately 14 mmol/L (GYLSTORFF, 1983) as an exceptionally osmotic active substance, which in total the maintenance of colloid-osmotic pressure diminishes protein concentration. The relation between individual fractions of proteins reflects the functional, metabolic and health status of birds. The importance and reliability of the routine application of serum protein electrophoresis in diagnostics of various disorders in birds was pointed out by WERNER and REAVILL (1999). The same authors also pointed out the importance of determining the referent values that are specific for species, age and sex of birds. Recent researches on concentration of proteins in bird blood mostly indicate total values and ratio of albumins to globulins (GRIMINGER and SCANES, 1986), as well as individual fractions in different pathological conditions (EL JACK et al., 1978).

Systematic researches into changes of concentration of proteins and their fractions in blood serum of broiler chickens during fattening have not been conducted to date. In relation to the foregoing, the object of our study was to investigate changes of protein concentration and their fractions in blood serum of chickens during the fattening period. The given results could be used in evaluation of the health condition and production capability in poultry production.

### **Materials and methods**

An investigation was performed on Ross-line fattening chickens. One-day-old chickens were placed in wire cages with latticed floors and covered with cardboard during the first three days. Room temperature began at 32 °C and was gradually reduced to 24 °C over a four-week period. The chickens were then kept at room temperature, with lighting 24 hours a day during the whole experimental period. Feed and water were given *ad libitum*. Chickens were fed up to the 14<sup>th</sup> day of age with a commercial starter diet; from the 15<sup>th</sup> to the 29<sup>th</sup> days with a commercial grower, and from the 30<sup>th</sup> day until the end of fattening with a commercial finisher diet (TSH Sljeme, RC). Blood for analysis was taken on the 14<sup>th</sup>, 28<sup>th</sup> and the 42<sup>nd</sup> day by the wing vein punctures, and the blood serum was separated. Serum concentration of total proteins was determined by spectrophotometry, using the biuret method. The share of individual protein fractions was determined by electrophoresis in tapes of gelled cellulose-acetate (Cellogel<sup>®</sup>, MALTA Chemetron, Milan, Italy). Interrelation and absolute concentration of protein fractions were determined by Global-scan densitometer (MALTA Chemetron, Milan, Italy). Results were statistically evaluated. Significance of differences was verified by the Student T-test (Microsoft Excel 2002).

## Results

Concentration of total proteins in blood serum showed a constant increase during all three researched periods. Statistically significant differences ( $P < 0.0001$ ) were established between the first compared with the second and the third experimental periods. Concentration of albumins in blood serum did not change statistically during the fattening period. Concentration of alpha 1 globulins significantly increased in the second ( $P < 0.0001$ ) and the third ( $P < 0.001$ ) experimental periods, compared with the first period. At the same time a statistically significant decrease ( $P < 0.05$ ) was observed in the third experimental period compared with the second period.

Table 1. Concentrations of total protein, albumin, alpha 1-, alpha 2-, beta 1-, beta 2- and gamma globulins in the blood serum of Ross broiler chickens during fattening period (g/L and albumins/globulins ratio) (mean  $\pm$  SD)

	14 <sup>th</sup> day	28 <sup>th</sup> day	42 <sup>nd</sup> day
Total proteins	27.00 $\pm$ 2.24	33.60 $\pm$ 2.07****	35.60 $\pm$ 3.66****
Albumins	15.24 $\pm$ 1.14	15.25 $\pm$ 0.99	15.90 $\pm$ 1.26
Alpha 1 globulins	1.84 $\pm$ 1.84	5.60 $\pm$ 0.78****	4.33 $\pm$ 0.94*** a
Alpha 2 globulins	2.91 $\pm$ 0.49	3.35 $\pm$ 0.78	3.99 $\pm$ 0.94**
Beta 1 globulins	1.58 $\pm$ 0.42	2.03 $\pm$ 0.43*	2.42 $\pm$ 0.71**
Beta 2 globulins	2.31 $\pm$ 0.37	3.53 $\pm$ 0.95**	4.80 $\pm$ 1.20**** a
Gamma globulins	3.13 $\pm$ 0.37	3.84 $\pm$ 0.51****	4.16 $\pm$ 0.72****
Albumins/globulins ratio	1.30 $\pm$ 0.11	0.83 $\pm$ 0.08****	0.82 $\pm$ 0.15****

Significance of differences comparing 14-day-old chickens on the level \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ . Significance of differences regarding 28 old chickens at the level <sup>a</sup> $P < 0.05$ .

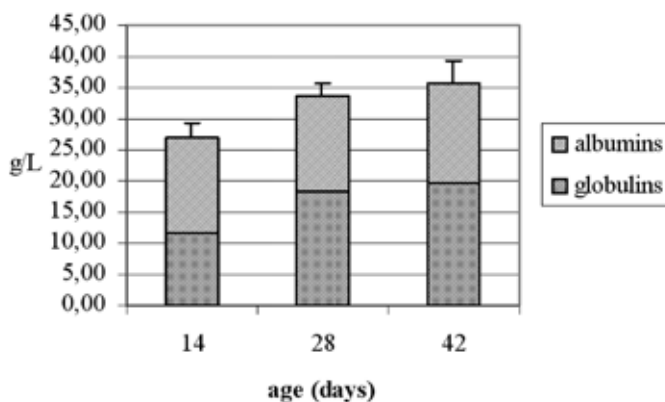


Fig. 1. Changes of total proteins concentration in blood serum of chickens during fattening period (g/L and ratio of albumins to globulins) (mean  $\pm$  SD)

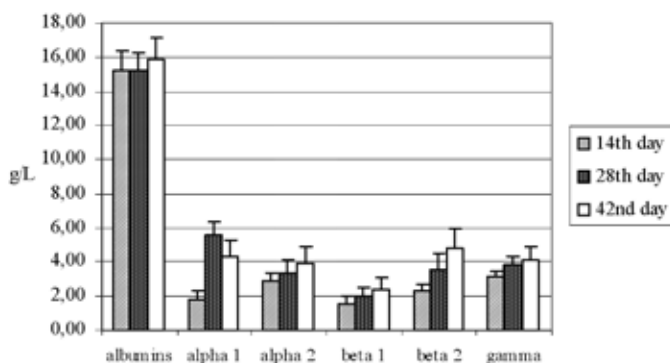


Fig. 2. Changes of concentrations of albumins, alpha 1-, alpha 2-, beta 1-, beta 2- and gamma globulins in blood serum of chickens (mean  $\pm$  SD)

Concentration of alpha 2 globulins gradually increased during the whole experimental period. However, a statistically significant ( $P < 0.01$ ) increase was observed between the 14<sup>th</sup> and 42<sup>nd</sup> days of fattening. Concentration of beta 1 globulins in blood serum statistically significant increased in the second ( $P < 0.05$ ) and the third ( $P < 0.01$ ) experimental periods compared with the first period. Concentration of beta 2 globulins showed a statistically significant increase in all three experimental periods (from  $P < 0.05$  to  $P < 0.0001$ ). Concentration of gamma globulins showed a statistically significant ( $P < 0.01$ ) increase in the second and the third experimental period compared with the first period. Ratio of albumins to globulins showed a statistically significant decrease on the 28<sup>th</sup> and the 42<sup>nd</sup> days of the experimental period compared to the 14<sup>th</sup> day of the experiment ( $P < 0.0001$ ).

## Discussion

Blood serum proteins are a significant indicator of the health condition and production features of the organism because of their numerous roles in the physiology. Among numerous factors that influence the concentration of serum proteins, age plays an important role in the physiology. Generally, concentration of proteins is significantly lower in young animals than in adults (RODGERS and GASS, 1983; KANEKO, 1997). MORGAN and GLICK (1972) pointed out an almost two-fold increase of serum proteins in chickens from the 1<sup>st</sup> to the 12<sup>th</sup> week of age. Similar data for Japanese quails were found in the research of ATWAL et al. (1964). In researches on pheasants, MULLER (1995) proved an increase of concentration of total proteins in blood with ageing from hatching to the 1<sup>st</sup> year of age. Results of our own researches are in accordance with the quoted data. Concentration of total proteins in serum of chickens has shown significant growth with ageing (from the

14<sup>th</sup> to the 42<sup>nd</sup> day) (Fig. 1). The described changes are related to the most important physiological role of blood proteins; e.g. as a source of amino acids for synthesis of tissue proteins. Growth of the organism, especially during fattening, as an extreme form of growth, demands an intensive supply of amino acids, whose transport form are proteins in blood, also energetic and other biologically active substances that are mostly transported by blood are attached to serum proteins. Because of increased needs, the liver increases the synthesis of serum proteins, which results in the growth of their concentration in blood. However, fluctuations of protein concentration in blood are not necessarily a reflection of their synthesis in liver. Apart from the extent of synthesis, the intensity of building into other tissues plays a significant role in concentration and their composition, the same as the actual needs of the organism; transport role, synthesis of hormones, enzymes. According to results of our researches the total proteins concentration in chicken's blood serum shows a constant increase during the fattening period. The given results can be explained in accordance to cited data, especially because chickens accumulate body mass extremely rapidly during fattening. Researches on pheasants showed that increase of total proteins does not always follow proportionally the increase of individual serum protein fractions, since their roles are different (MULLER, 1995). Fluctuations of concentrations of individual protein fractions, considering their function, can also be in relation to the rapid increase in body mass. Therefore, in evaluation of health and metabolic status, as well as total concentration of proteins it is important to determine the concentration of individual fractions in blood serum protein. With some bird species, depending on the type of electrophoresis, the electrophoretogram is characterized by the presence of the prealbumins fraction. GREEN et al. (1982) have determined the presence of prealbumins in ducks using cellulose acetate electrophoresis. Presence of prealbumins fraction was proved by TUREEN et al. (1966) only in chickens, KURLY (1978) in hens, HARRIS and SWEENEY (1969) during growth in cockerels, and ELLIOT and BENNET (1971) only in hens. In our researches on fattening chickens we did not discern presence of prealbumins fraction. Albumins serve as a depot of proteins and as a source of amino acids during insufficient intake by food, and also participate in transporting fatty acids, minerals and vitamins (GRIMINGER and SCANES, 1986). In chickens and quails it was determined that albumins also transport thyroid hormones (McNABB and HUGHES, 1983). In these researches we have determined that concentration of albumins follows the increase of concentration of total proteins during fattening (Fig 1.). However, this increase was not statistically significant, which could be explained by the rapid utilization of albumins as the most favourable source of amino acids for synthesis of tissue proteins. Alpha 1 and alpha 2 globulins are considered functionally unique. Mostly, they are a component of lipoproteins of plasma. Alpha lipoproteins (HDL) migrate to alpha 1 position during electrophoresis. Pre-beta lipoproteins (VLDL) and beta lipoproteins (LDL) migrate to alpha 2 position during electrophoresis separation on

cellulose-acetate (KANEKO, 1997). The significant increase of concentration of alpha 1 and alpha 2 globulins, which we determined during experimental fattening (Fig. 2), is most probably the consequence of increased transport of fatty substances in the organism to satisfy energy needs and in-built into tissue structures. Beta globulins appear in blood as a unique fraction, but through electrophoresis beta 1 (fast) and beta 2 (slow) fractions are separated. The importance of proteins of this fraction is the connection to iron metabolism: transferrin and ferritin, as transport and storage forms, and haemopexin, which ties them during physiological haemolysis. Components C3 and C4 of complement also migrate to the area of beta globulins (KANEKO, 1997). Since growth is proportionally related to an enlarged need for iron, especially for haemoglobin synthesis, that could explain the significant increase of beta 1 and beta 2 fractions of globulins in serum of chickens. This conclusion is supported by the fact that during the growth period the number of erythrocytes in birds greatly increases, and by the data that birds have the most intensive erythropoiesis in the animal kingdom, because of the very short life period of erythrocytes (28 to 35 days). Gamma globulins are synthesized in cells of the reticulo-endothelial system and participate in immunological reactions. Since the immune system gradually matures with ageing, it is to be expected that the quantity of gamma globulins in bird serum increases consequentially. This assumption is confirmed by researches on geese by LOSONCZY et al. (1972) in which they determined that the concentration of antibodies increased up to the age of two months, and after which they remained constant. During these researches we have established a statistically significant increase of gamma globulins concentration with ageing (Fig.2). Ratio of albumins to globulins is important in the interpretation of diagnostic significance of the electrophoretogram since it changes at different pathological stages (KANEKO, 1997). Therefore, establishing their referential values is also significant. The significant decrease of ratio of albumins to globulins which we detected on the 28<sup>th</sup> and the 42<sup>nd</sup> days of experimental fattening, compared to the 14<sup>th</sup> day, (Fig. 2) is in accordance with the researches of TUMBLESON et al. (1973), who established an increase in total proteins, as well as globulins fractions with ageing in cows. The increased share of globulins fraction that we determined in our researches is the result of an increase in all globulins fractions that we investigated: alpha 1-, alpha 2-, beta 1-, beta 2- and gamma globulins, which appeared in the period from the 14<sup>th</sup> to the 28<sup>th</sup> day of experimental fattening, and an additional increase of concentration of alpha 1- and beta 2- globulins, which appeared in the period from the 28<sup>th</sup> to the 42<sup>nd</sup> day of the experiment. These results are in accordance with the results of researches on pheasants by MULLER (1995), who established a reduction of albumins fraction share with aging, with a constant increase in alpha-, beta- and gamma- globulins up to the first year of age. Results of conducted researches indicate a very intensive protein metabolism in chickens during fattening. Some of these researches confirm facts that are already known. However, the changes of proteins concentration and their electrophoretic fractions in blood serum

were investigated on the same chickens during crucial phases of fattening for the first time. The given results may serve primarily as part of the metabolic profile of chickens in fattening, important in evaluation of production patterns, as well as in evaluation of health condition (STOJEVIĆ et al., 2001).

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**FILIPOVIĆ, N., Z. STOJEVIĆ, S. MILINKOVIĆ-TUR, B. BEER LJUBIĆ, M. ZDELAR-TUK: Kretanje koncentracija ukupnih bjelančevina i njihovih frakcija u krvnom serumu pilića tijekom tova. *Vet. arhiv* 77, 319-326, 2007.**

**SAŽETAK**

Istraženo je kretanje koncentracija ukupnih bjelančevina i pojedinih frakcija bjelančevina u krvnom serumu tovnih pilića hibridne linije Ross. Krv za analizu uzimana je 14., 28. i 42. dana starosti, punkcijom krilne vene. Spektrofotometrijski je određena koncentracija ukupnih bjelančevina u krvnom serumu. Elektroforezom su određeni udjeli pojedinih frakcija: albumina, alfa 1-, alfa 2-, beta 1-, beta 2- i gama-globulina, a njihove apsolutne koncentracije izračunate iz ukupne koncentracije bjelančevina. Tijekom pokusa utvrđen je statistički značajan porast koncentracije ukupnih bjelančevina ( $P < 0,0001$ ). Kretanja koncentracija pojedinih frakcija nisu uvijek pratila ukupnu količinu bjelančevina u krvnom serumu. Koncentracija albumina u krvi nije kolebala tijekom tova, za razliku od alfa 1-globulina, čija je koncentracija statistički značajno porasla do 28. dana tova ( $P < 0,0001$ ), a potom značajno pala do 42. dana u odnosu na 28. dan ( $P < 0,05$ ). Koncentracija alfa 2-globulina značajno je porasla tek 42. dana u odnosu na 14. dan ( $P < 0,01$ ). Frakcije beta 1-, beta 2- i gama-globulina pokazale su značajan porast tijekom svih istraživanih razdoblja (od  $P < 0,05$  do  $P < 0,0001$ ). Omjer albumina prema globulinima bio je statistički značajno snižen 28. i 42. dana u odnosu na 14. dan pokusa ( $P < 0,0001$ ). Dobiveni rezultati dovode se u vezu s ulogom metabolizma bjelančevina u brzom prirastu tovnih pilića i mogli bi poslužiti kao dio metaboličkog profila, u procjeni proizvodne sposobnosti, kao i zdravstvenog stanja pilića tijekom tova.

**Cljučne riječi:** krvni serum, ukupne bjelančevine, frakcije bjelančevina, tovni pilići

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