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Changes in Blood Chemistry in Broiler Chickens during the Fattening Period

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The aim of the study was to determine the changes in the selected biochemical serum parameters in male Ross 308 broilers during the fattening period. The birds were kept under standard farm conditions and they were fed on commercial mixtures. The blood for analysis was taken from the jugular vein on the 14th, 21st, and 42nd days of age. The concentration of serum protein (total protein, albumins, uric acid, creatinine), lipid (TG, TCHL, HDL) and mineral (Ca, P_i, Mg, Fe) indices were determined. The measurements were carried out with the use of Epoll 20 photometer. The content of LDL and VLDL lipoprotein fractions was calculated on the basis of the Friedewald equation. Most of the estimated parameters, except for LDL and P_i, were age-dependent ($P<0.05$). Total protein, albumins and total Ca levels showed a constant increase between the 14th and 42nd days of life. A lower ($P<0.05$) concentration of TG, TCHL, HDL, VLDL, Mg and Fe was determined at the end of the fattening period compared to 14-day-old broilers. A significant decrease of TG, VLDL, Mg and Fe content was noted already in the first age range (days 14-21) while in the case of TCHL and HDL a significant decrease was found between the 21st and 42nd days of fattening. The obtained results may be helpful in the evaluation of changes in the metabolic profile, health condition and production patterns in growing broiler chickens reared under farm conditions.

Key words: Broiler chickens, age, blood serum, biochemical indices.

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The evaluation of blood biochemistry in birds allows for the identification of metabolic alterations due to many endo- and exogenous factors including the genetic type, husbandry conditions, season, sex and age (ROSS *et al*. 1978; BOWES *et al*. 1989; MELUZZI *et al*. 1992; KRASNODĘBSKA-DEPTA & KONCICKI 2000; HARR 2002; JURÁNI *et al*. 2004; RAJMAN *et al*. 2006). Moreover, the biochemical blood parameters provide valuable information on the health state and are often helpful in revealing health disorders already in the preclinical stage. Blood analyses, widely used in large domestic animal medicine, are not commonly utilized for avian diagnostics, because no physiological reference values are available. In commercial conditions, when broiler chickens are fed on standard mixtures, the breeding line and age of birds seem to be the main factors influencing the intensity of metabolism which is reflected in changes of the blood parameters. The effect of age (during the first six weeks) on the biochemical profile of young growing broilers was examined in several studies, however, the values obtained are extremely variable (ROSS *et al.* 1978; BOWES *et al*. 1989; MELUZZI *et al.* 1992; KRASNODĘBSKA-DEPTA & KONCICKI 2000; SILVA *et al*. 2007). According to KRASNODĘBSKA-DEPTA and KONCICKI (2000), the considerable individual differences in biochemical indices in birds are the main reason for the wide physiological ranges specified for chickens.

The primary objective of the study was to examine 13 biochemical parameters of protein, lipid and mineral metabolism in growing male broiler chickens (Ross 308) in relation to age. Results of this experiment will broaden the current knowledge on the physiological changes in the biochemical profile in meat-type chickens reared under farm conditions during fattening.

Material and Methods

Animals

The study was conducted under a research protocol approved by the Local Ethical Committee in Bydgoszcz, Poland.

The experiment was carried out in a typical commercial broiler farm in the Kujawy-Pomerania region. Fattened Ross 308 chickens were reared from 1 to 42 days of age according to the technological recommendation for this breed. The birds were kept on litter at a density of 21 birds per square meter with light 24 hours a day during the whole experimental period. The hen house temperature began at 33°C and was gradually reduced to 20°C over a six-week period. The birds were fed a typical mixed maize, wheat and soybean diet. Chickens were fed up to the 21st day of age with commercial starter diets (starter I up to day 13 and starter II from day 14 to 20), from day 21 to the 34 with a commercial grower, and from day 35 until the end of fattening with commercial finisher diets. The content of crude protein (%) and metabolisable energy (MJ kg^{-1}) in the diets was as follows: starter I 21.5, 12.6, starter II 20.5, 12.9, grower 19.5, 13.3, finisher 18.5, 13.4. The mixtures met the nutrient requirements for broiler chickens (NRC 1994). Feed and water were provided *ad libitum*. In the whole rearing period, no clinical symptoms of disease or abnormal mortality were observed.

Chemical analysis

Blood samples for analysis were collected in the early morning from 10 male chickens (n=10) on the 14th, 21st and the 42nd days of age from the jugular vein after decapitation. The intensity of the gastrointestinal tract development and duodenal enzymes secretion in growing broiler chicks were taken into account in the choice of days for blood collection (days 14 and 21). The most intensive changes in the intestinal morphology and pancreas activity occur during the period of 21 days post-hatching (NOY & SKLAN 1995). The final day of sampling (42nd) coincided with the end of the fattening period. The mass of the selected male broilers was similar to the average body weight of the flock. After coagulation, blood samples were centrifuged at 3,000 rpm for 10 min and the obtained serum was stored in a freezer at -20°C for analyses.

In the blood serum, the content of total protein, albumin, uric acid, creatinine, triglycerides (TG), total cholesterol (TCHL), HDL fraction, calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg) and iron (Fe) were determined with an Epoll 20 photometer using the original Alpha Diagnostics kits. The content of LDL and VLDL lipoprotein fractions in the blood serum was calculated on the basis of the Friedewald equation (FRIEDEWALD *et al.* 1972).

Statistical analysis

The results were analysed statistically with the use of the Statistica 8.0 PL program (STATSOFT, INC. 2008) for the windows operating system with one way analysis of variance (ANOVA). The *post hoc* Tukey test was applied. The significance of differences was set at $P<0.05$.

Results and Discussion

The content of selected biochemical indices in the blood serum of Ross 308 broiler chickens during the fattening period is presented in Table 1.

Table 1

Biochemical indices in the blood serum of broiler chickens ($\bar{x}$ ± SD)

Index	Age of broilers			
	14d	21d	42d	P<
Total protein, g l^{-1}	33.00^{a} ± 3.71	38.80^{b} ± 2.90	47.80^{c} ± 1.99	0.05
Albumin, g l^{-1}	11.70^{a} ± 2.06	13.10^{a} ± 1.73	17.10^{b} ± 1.97	0.05
Uric acid, mmol l^{-1}	0.550^{a} ± 0.11	0.371^{b} ± 0.06	0.500^{a} ± 0.11	0.05
Creatinine, μmol l^{-1}	$26.52^{a,b}$ ± 4.17	22.10^{a} ± 4.66	27.40^{b} ± 5.02	0.05
Triglycerides, mmol l^{-1}	1.34^{a} ± 0.32	0.79^{b} ± 0.16	0.83^{b} ± 0.07	0.05
Total cholesterol, mmol l^{-1}	$4.13^{a,b}$ ± 0.61	4.44^{a} ± 0.48	3.71^{b} ± 0.71	0.05
HDL, mmol l^{-1}	1.74^{a} ± 0.23	1.80^{a} ± 0.18	1.40^{b} ± 0.13	0.05
LDL, mmol l^{-1}	1.78 ± 0.52	2.28 ±0.38	1.93 ± 0.65	NS*
VLDL, mmol l^{-1}	0.61^{a} ± 0.15	0.36^{b} ± 0.07	0.38^{b} ± 0.03	0.05
Calcium, mmol l^{-1}	2.21^{a} ± 0.58	2.73^{b} ± 0.23	3.09^{b} ± 0.17	0.05
Phoshorus, mmol l^{-1}	2.63 ± 0.38	2.83 ± 0.21	2.77 ± 0.26	NS
Magnesium, mmol l^{-1}	1.30^{a} ± 0.14	1.03^{b} ± 0.10	1.05^{b} ± 0.12	0.05
Iron, μmol l^{-1}	36.98^{a} ± 5.77	19.79^{b} ± 4.40	24.92^{b} ± 3.97	0.05

$^{a, b, c}$ means in the rows with different letters differ significantly $P<0.05$

* not statistically significant

Total plasma proteins are a common parameter utilized to estimate the avian body condition. It is generally known that blood plasma proteins play key roles in the maintenance of colloid osmotic pressure, as a rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis, in transport of minerals and hormones, in forming enzymes and the immune system in the organism. Therefore, blood plasma proteins have an exceptional significance in homeostasis maintenance. Moreover, albumin, one of the main serum proteins, serves as the most favorable source of amino acids for synthesis of tissue proteins in the period of quick somatic growth of birds, especially under feed restricted conditions (YAMAN *et al.* 2000; FILIPOVIĆ *et al.* 2007). The content of protein in the blood serum of experimental chickens ranged from 33.00 to 47.80 g l^{-1} and showed a constant significant ($P<0.05$) increase from the 14^{th} to 42^{nd} day of age. Also, the serum albumin level demonstrated an increasing tendency and the highest ($P<0.05$) content (17.10 g l^{-1}) of this protein fraction was detected on the 42^{nd} day of fattening. The percentage of albumin fraction in total serum protein was relatively constant and it constituted 35.5, 33.8 and 35.8% of total protein on the 14^{th}, 21^{st} and 42^{nd} days of life, respectively. The data obtained in our experiment confirm results of earlier studies on protein profile changes in bird blood in relation to age (ROSS *et al*. 1978; MELUZZI *et al*. 1992; SZABO *et al*. 2005; RAJMAN *et al*. 2006; FILIPOVIĆ *et al*. 2007; SCHMIDT *et al*. 2007; SILVA *et al*. 2007). Investigations conducted on broiler chickens demonstrate that total protein level increases about 20-30% from the 14^{th} to 42^{nd} day of fattening (ROSS *et al*. 1978; BOWES *et al*. 1989; FILIPOVIĆ *et al*. 2007). At the same age range, ROSS *et al*. (1978) and BOWES *et al*. (1989) showed a 10-15% increase of serum albumin concentration. In the present study, the concentration of albumin followed the increase of total protein and it was more intensified than in the investigations cited above. However, other experiments on chickens revealed a relatively constant level of albumin in broiler blood over a period from the 2^{nd} to 6^{th} week of age (MELUZZI *et al*. 1992; FILIPOVIĆ *et al*. 2007; SILVA *et al*. 2007). The total blood protein and its fractions can be extremely variable in birds. Changes in its levels depend on many external and internal factors and result from the physiological role of blood proteins. The increasing concentration of total proteins and albumin between the 14^{th} and 42^{nd} day of age is probably a direct consequence of high demand for amino acids which are utilized for very intensive somatic growth (STURKIE 1970; SZABO *et al*. 2005; FILIPOVIĆ *et al*. 2007).

Uric acid is the major avian nitrogenous waste product and an important antioxidative agent (STURKIE 1970; DAWSON *et al*. 1991; HARR 2002; JURÁNI *et al*. 2004). Any changes in protein catabolism are mainly reflected in serum uric acid concentrations. In the present study, comparable levels of this metabolite were found on the 14^{th} and 42^{nd} days of fattening (0.550 and 0.500 mmol l^{-1}) while its lowest ($P<0.05$) content (0.371 mmol l^{-1}) was detected in the blood of 21-day-old broilers. It is well documented that in birds the blood uric acid content can vary greatly under standard conditions depending on the bird species, diet and protein dietary quality (STURKIE 1970; ROSS *et al*. 1978; BOWES *et al*. 1989; SZABÓ *et al*. 2005; SCHMIDT *et al*. 2007). Earlier studies on poultry revealed age-dependent changes in the blood uric acid concentration. Generally higher levels were detected in birds during the early rearing period in comparison to older individuals (ROSS *et al*. 1978; BOWES *et al*. 1989; SZABÓ *et al*. 2005; SILVA *et. al,* 2007). According to SZABÓ *et al*. (2005), there is a direct relationship between the amount of ingested protein and the serum uric acid level. The high protein concentration typical of commercial diets used in the starter phase of broiler rearing results in a higher serum uric acid content (SZABO *et al.* 2005). On the basis of the available literature, it is difficult to explain why in the present study the significant increase of the uric acid content was detected at the end of the fattening period (42^{nd} day). Probably, factors other than those described above can also influence the level of this metabolite in the blood of broiler chickens. On the other hand, no significant effect of age on its serum concentration was found by RAJMAN *et al.* (2006) in growing meat-type chickens and by SCHMIDT *et al*. (2007) in pheasants, additionally confirming the great variability in serum uric acid content in birds.

Another important indicator of protein metabolism is creatinine, a byproduct of phosphocreatine breakdown in skeletal muscle. Its concentration is directly proportional to muscle mass, related to age, physical activity and like the majority of blood chemistry constituents, is influenced by diet (WYSS & KADDURAH-DAOUK 2000; SZABÓ *et al.* 2005; RAJMAN *et al.* 2006). In the present study, the concentration of creatinine decreased slightly ($P>0.05$) from the 2^{nd} to 3^{rd} weeks of age and afterwards a significant increase of its level was detected between the 3^{rd} and 6^{th} weeks of age. In an experiment on growing turkeys, the highest levels of creatinine were detected on the 3^{rd} day of life as an effect of muscle activity in thermogenesis and at the end of the fattening period as a direct consequence of large muscle growth (SZABÓ *et al.* 2005). In our study, the highest content of creatinine determined in 6-week-old broilers is also a re-

sult of intensive muscle growth between the 21st and 42nd days of fattening. However, other studies on broiler chickens did not reveal an unambiguous influence of age and feeding treatment on the level of serum creatinine (BOWES *et al.* 1989; RAJMAN *et al.* 2006).

Lipid metabolites are strongly associated to energy metabolism and reflect its fluctuation occurring during the growth period. In the present study, the serum triglyceride concentration peaked on the 14th day of life, reflecting intensive lipid metabolism and transport in the experimental broiler chickens. At the subsequent blood sampling (21st day) a significant decrease of triglyceride level was determined and remained at a similar level until the end of the fattening period. Age-related changes, with a tendency of serum triglyceride to decrease significantly from the 2nd to 7th weeks of life, were also observed in chickens by PEEBLES *et al.* (1997a) and KRASNODĘBSKA-DEPTA and KONCICKI (2000). However, other authors in experiments on growing chickens fed standard diets observed quite the opposite, i.e. a pattern of serum triglyceride increasing with age (MELUZZI *et al.* 1992; PEEBLES *et al*. 1997b). Pronounced alterations in lipid blood metabolites were also found in an experiment on growing turkeys by SZABÓ *et al.* (2005). According to the authors, in turkeys the sudden decrease of serum triglyceride content after the 3rd day of life results from a decline of the specific activity of the intestinal fatty acid binding protein (FABP) in the early posthaching period.

In the present experiment, significant age-related differences were also found in the concentration of total cholesterol, HDL and VLDL fractions. Both the total and HDL cholesterol levels showed identical time-patterns during the rearing period with a significant ($P<0.05$) decrease between the 21st and 42nd days of life. The highest level of VLDL fraction was noted in the youngest birds and a significant decline of its content recorded on the 21st day was maintained until the end of the fattening period. There were no significant differences in the concentration of LDL cholesterol. The results of previous studies concerning changes in lipid serum metabolites in relation to age of birds are strongly diversified. Similar to our results, PEEBLES *et al.* (1997a) also detected the tendency of total and HDL cholesterol content to decrease with age in meat type chickens. However, other authors did not show any significant association between age and cholesterol concentration in broilers in the period from the 2nd to 6th weeks of life (ROSS *et al.* 1978, BOWES *at al.* 1989; MELUZZI *et al.* 1992; KRASNODĘBSKA-DEPTA & KONCICKI 2000; SARIKHAN *et al.* 2009). In the present experiment, the main fraction of serum total cholesterol consisted of a low density lipoproteins (43, 51 and 52% on the 14th, 21st and 42nd days respectively). HDL and VLDL constituted 38-42 and 8-15% of total cholesterol. According to HERMIER and DILLON (1992) and PEEBLES *et al.* (1997a), high density lipoproteins are the main fraction of total cholesterol in the blood of birds, a finding not confirmed in our experiment or by other authors (KARAMOUZ *et al.* 2009; SARIKHAN *et al*. 2009). It should be pointed out that both cholesterol and triglyceride serum levels are genetically dependent. This may be one of the reasons for their great variability revealed by different researchers in experiments on growing chickens (KRASNODĘBSKA-DEPTA & KONCICKI 2000).

Minerals are essential for broiler growth and they are involved in many digestive, physiological and biosynthetic processes within the body. Calcium is mainly needed for the ossification of bones, regulation of muscle activity and catalization of enzyme and hormone systems while phosphorus is an important constituent of nucleic acids and phospholipids. Magnesium is vitally involved in the metabolism, mostly as a catalyst of a wide array of enzymes, and iron is an essential constituent of haemoglobin, mioglobin and cytochrome enzymes (UNDERWOOD & SUTTLE 1999). The results of the present study showed a significant ($P<0.05$) relationship between age and concentration of serum calcium, magnesium and iron while there were insignificant differences in the level of phosphorus. The lowest Ca level was noted on the 14th day and later a significant ($P<0.05$) increase was noted on the 21st day. In older birds (3-6-week-old) a slight further ($P>0.05$) increase was detected. The results of previous studies concerning serum Ca and P contents in relation to the age of chickens are diversified. Some of them showed a clear increasing tendency in the first 2-4 weeks of age while others revealed only fluctuation ($P>0.05$) in Ca and P concentrations during the entire fattening period (ROSS *et al*. 1978; BOWES *et al*. 1989; MELUZZI *et al.* 1992; ANSAR *et al*. 2004; TALEBI 2006; SILVA *et al*. 2007). A significant increase ($P<0.05$) of Ca content between the 14th to 21st day may be related to the rapid bone formation and mineralization which occur in the first days after hatching (days 4-18) (WILLIAMS *et al*. 2000). In the present experiment, the highest Mg concentration was recorded in 2-week-old broilers, subsequently a significant decrease was noted (days 14-42). Our findings are in agreement with previous studies on meat-type chickens which also revealed an age-dependent gradual decline in serum Mg content (ROSS *et al*. 1978; BOWES *et al*. 1989). The highest level of Fe in the blood of the experimental birds was noted on the 14th day of life and afterwards a significant decline ($P<0.05$) occurred. According to UNDERWOOD and SUTTLE (1999),

serum iron values in poultry vary with age. The intensification of erythropoesis decreases with age explaining the lower requirement for Fe in older chickens compared to younger ones. The results obtained in our experiment support a tendency showed in the balance trial on growing chicks performed by MOHANNA and NYS (1998) who recorded the highest retention of Fe during the first days of life. Iron utilization decreased rapidly before the 21st day and thereafter only slight changes were observed until the 50th day of life.

In conclusions, the results of the present study carried out on broiler chickens in the fattening period (days 14-42) indicate that most of the estimated parameters of protein, lipid and mineral metabolism are age dependent. Only the concentration of LDL cholesterol and inorganic phosphorus did not show significant changes. It should be pointed out that the values of the determined indices were within the wide range of physiological values specified for growing broiler chickens (ROSS *et al*. 1978; BOWES *et al*. 1989; MELUZZI *et al*. 1992; KRASNODĘBSKA-DEPTA & KONCICKI 2000). The obtained results may be helpful in the evaluation of changes in the metabolic profile, health condition and production patterns in rapidly growing chickens during the fattening period.

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