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DYNAMICS OF SERUM PROTEIN CONTENT AND PRODUCTIVITY OF CHICKENS WITH DIFFERENT TONUS OF THE AUTONOMOUS NERVOUS SYSTEM

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Abstract. *The main role in maintaining the functioning of the body, its growth, and its development belongs to protein. It is involved in the formation of the muscular skeleton and is s part of enzymes, neurotransmitters, hormones. The effect of the autonomic nervous system on total protein metabolism has not been sufficiently studied. It is known that the autonomic nervous system is a structure that is responsible for the homeostasis and stability of the whole organism. It participates in the regulation of the heart, endocrine and external secretion glands, gastrointestinal tract, excretory organs, and more.*

In our studies, it was found that in chickens of Cobb 500 strain with different tones of the autonomic nervous system during the growing period from the 35th to the 60th day, different contents of total protein, albumin, and globulins were observed and different body weights were recorded. Vagotonic chickens showed the lowest protein metabolism at the age of 35 and 45 days ($P < 0.05–0.001$) compared with sympathicotonics and normotonics, which tended to increase between 35 and 60 days of rearing compared with other groups of birds, where the studied protein fractions on the contrary decreased.

Correlations between total protein, albumin, and bird body weight had a high linear relationship in all groups of chickens ($P < 0.05–0.001$) and a negative relationship

between the 45th and 60th days of rearing in sympathicotonics and normotonics. In birds with a predominance of the parasympathetic tone of the autonomic nervous system, this correlation maintained its direction with high reliability (P < 0.05) between body weight and total protein on the 60th day of rearing.

Keywords: *sympathicotonia, vagotonia, normotonia, protein metabolism, albumin, globulin*

Introduction

The study of the dynamics of the influence of different tones of the autonomic nervous system on the metabolism of total protein and its components in the serum of chickens has not been thoroughly studied. Its fluctuations are influenced by numerous factors, such as age, diet, keeping animals, their genetic characteristics. In recent decades, breeders have bred high-yielding poultry crosses that have high feed conversion, rapid growth, and development. Therefore, the study of the peculiarities of the metabolic processes of protein as a major component involved in the growth of the organism is an extremely important issue today.

Analysis of recent researches and publications

Serum protein is a central link in all biochemical processes in animals and humans (Derho & Sereda, 2016). Albumin and globulins that are part of it are synthesized directly in the liver and immune organs (globulins). The functions of these proteins are difficult to overestimate because they provide transport of metabolites, biologically active substances, are a plastic material for the needs of the body, there are protective immune complexes, etc. (Gotovsky et al., 2018). The content of these proteins in the body depends on the state and activity of the immune system (Filipović et al., 2007), nutritional feed and content of all necessary substances, synthesizing capacity of the liver (Zaefarian et al., 2019), and directly the needs of the body (Filipović et al., 2007).

Effective metabolism of various compounds in animals is ensured by numerous biochemical processes that occur and are regulated under the influence of neuro-humoral factors (Postoi et al., 2020; Reutov & Chertok, 2016). The autonomic nervous system (ANS) through its numerous array of reflexes is able to regulate the work of almost all physiological functions of the body (Abboud & Singh, 2017). It consists of three divisions – sympathetic, parasympathetic (Wehrwein et al., 2016), and enteral (intestinal) (Volkova, 2015). Their centers are located at different levels of the nervous system – the cortex of the large hemispheres, hypothalamus, cerebellum, basal ganglia, and spinal cord (Gibbons, 2019). The activity of the sympathetic and parasympathetic divisions mostly has different directions, which allows them to precisely regulate any processes in the body of living beings (McCorry, 2007). For example, increased activity of the sympathetic branch of the ANS causes an increase in glucose output and utilization (Karpovskyi et al., 2013), the development of anti-inflammatory response in the body by activating T and B lymphocytes, and NO secretion (Padro & Sanders, 2014; Abboud & Singh,

2017), increased antibacterial and lysozyme activity of the blood (Karpovskyi et al., 2016). The sympathetic system directly affects the growth of bone tissue. Strengthening of the alveolar bone of the upper jaw and a decrease in tooth mobility was observed in rats treated with selective β-blockers (Uchibori et al., 2020). The increased influence of the sympathetic system provides a decrease in osteoblast activity, which may lead to a decrease in skeletal strength (Elefteriou, 2008; Dimitri & Rosen, 2017). Studies (Postoi et al., 2019) found that pigs with a different tone of the ANS have varying degrees of the intensity of protein metabolism in the body. Animals with a predominance of sympathetic and parasympathetic divisions had a higher urea content and a decrease in total serum protein during the first seven days after exposure to technological stress. Also, pigs with balanced ANS tone took less time to establish baseline levels of test substances in serum compared to other groups. Other studies have shown the effect of the ANS on the state of the antioxidant system in pigs. Animals dominated by the sympathetic branch had significantly higher superoxide dismutase activity compared to other groups. The ratios of antioxidants superoxide dismutase to catalase (SOD/CAT) and superoxide dismutase to glutathione peroxidase (SOD/GPO) were lower in vagotonic pigs and sympathicotonics (Skrypkina et al., 2016). Determination of the influence of the ANS on the activity of the enzymatic link of antioxidant protection showed a significant effect in sympathicotonic pigs (Skrypkina et al., 2016).

One of the main functions of the ANS is the implementation of precise regulation of the cardiovascular system. The activity of the heart as the main target of the sympathetic and parasympathetic divisions is provided by regulating the rhythm and heart rate, vascular tone, and blood supply, which is necessary for the needs of the body (Albarado-Ibañez, 2019). Such control is not a simple predominance of sympathetic or parasympathetic influence but is a complex interaction of circulatory reflexes, the functioning of baro- and chemoreceptor zones in the vessel walls, as well as various molecular and hormonal factors (Taralov et al., 2016). The effect of the ANS on the cardiovascular system can be described as maintaining balance with various changes in the external and internal environment of the body, as confirmed by numerous studies. For example, in rats and weather-sensitive humans, reduced atmospheric pressure and hypoxia increase the parasympathetic effect on the vessels of the brain, which is necessary to maintain oxygen homeostasis and maintain energy-dependent processes in nerve cells (Volkova, 2015). In waterfowl, there is a well-defined evolutionary antagonism of the mechanism of action of the parasympathetic system. During immersion under water and emergence of reflex apnea, there is a blocking of a sympathetic branch of the ANS with the subsequent decrease in heart rate. This allows birds to spend more energy on swimming and oxygen for the body's biochemical needs (Shereshkov et al., 2010). Studies in rats have shown that early experimental desympathization leads to an increase in the diameter of blood vessels, delays the formation of their multidimensional structure, and the preservation of a predominantly linear orientation, which is characteristic of early development. There is also a decrease in the distance between the vascular wall and the area of the neuromuscular synapse, which

indicates a change in the level of metabolic reactions (Kovrigina & Filimonov, 2013). Identical data on vascular diameter were obtained in heifers with different ANS tones. They increased the Kernogan index (the ratio of the thickness of the middle membrane of the vascular wall to the width of the lumen of the vessel) in parallel with the growth of sympathicotonia in animals and vice versa (Demus, 2010).

The inter regulatory work of the sympathetic and parasympathetic divisions of the ANS ensures the precise functioning of tissues and the regulation of the metabolism of proteins, carbohydrates, fats, minerals, and biologically active substances. The ANS provides constant adaptation to changes in the internal and external environment. As a result of violation or imbalance of these departments of the ANS in the body, there are persistent disorders of hemodynamics, functioning of endocrine organs, catabolic or anabolic processes begin to predominate (Wehrwein et al., 2016; Zhukov et al., 2020). At present, the effect of the ANS on protein metabolism has not been sufficiently studied (McCorry, 2007; Postoi et al., 2019). Therefore, research in this direction is relevant.

Purpose. To determine the effect of different ANS tones on serum protein metabolism in chickens during the period of their rearing.

Materials and methods of research

Studies of protein metabolism depending on the tone of the ANS were performed on broiler chickens Cobb 500 strain, aged 35, 45, and 60 days in accordance with international bioethical requirements set out in Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes (Louhimies, 2002) and the Law of Ukraine On Protection of Animals from Cruelty (Law of Ukraine, 2006). Determination of the ANS tone was performed by the method of variation pulsometry (Tybinka, 2011). At least 100 consecutive cardio intervals were recorded for 20–30 s with a portable electrocardiograph (EK3T 01-RD, Russia) (Studenok et al., 2020). Alligator electrodes were placed according to standard electrocardiography (ECG) protocols in poultry in the shoulders and legs (Tybinka, 2011; Reddy et al., 2016). Experimental animals, depending on the tone of the ANS were divided into 3 groups: sympathicotonics, in which the indicators of the mode of the duration of the cardiac cycle (Mo) were 0.14–0.16 s, the amplitude of the mode (Amo) – > 45%; normotonics (Mo – 0.16–0.17 s, Amo – 40–45%); vagotonics (Mo – 0.18–0.21 s, Amo – < 40%) (Studenok et al., 2020). Venous blood for biochemical studies was taken from the subcutaneous vein of the shoulder after a 2-hour fasting diet (Bayer et al., 2012). The venipuncture site was cleaned of dirt, disinfected with 70% aqueous solution of ethyl alcohol, skin anesthesia was performed with gel (Kategel, Austria) based on lidocaine (Bayer et al., 2012). The content of total protein in blood serum was determined by biuret reaction (Levchenko et al., 2002), albumin-method with bromocresol green (Levchenko et al., 2002) on a semi-automatic biochemical analyzer (Biosystems A15, Spain) using a set of reagents (Pointer Scientific, USA) according to the manufacturer's instructions, and the content of globulins – by calculating the difference between the content of total protein and albumin. Statistical processing of the results was performed in the Statistica 6 program

with the determination of averages and their errors, the probability of difference between groups, correlation (Pearson's method) and analysis of variance of the data (one-way analysis of variance) was performed in Microsoft Excel.

Results of the research and their discussion

The dynamics of the content of total protein and its fractions in blood serum of chickens with balanced ANS tone in the period of 35–60 days of rearing was characterized by a decrease (Fig. 1–3). In particular, the content of total protein (Fig. 1) decreased by 2.20 g/L (3.0%), albumin – by 1.10 g/L (5.5%), globulins – by 1.05 g/L (4.4%) within the trend.

A similar pattern was observed in chickens with a predominance of the sympathetic tone of the ANS. The content of total protein was the same for 35- and 60-days-old with a slight increase at the age of 45 days (1.50 g/L; 3.6%). The content of globulin fraction (Fig. 3) at the age of 45 days also increased by (1.20 g/L; 5.2%) with a subsequent decrease to the 60th day. The content of albumins (Fig. 2) increased slightly by 0.60 g/L (3.1%).

With age, there is a balance of metabolic processes, the transition from intensive growth, and an increase in muscle mass in the plateau of stable indicators (Filipović et al., 2007; Pi-

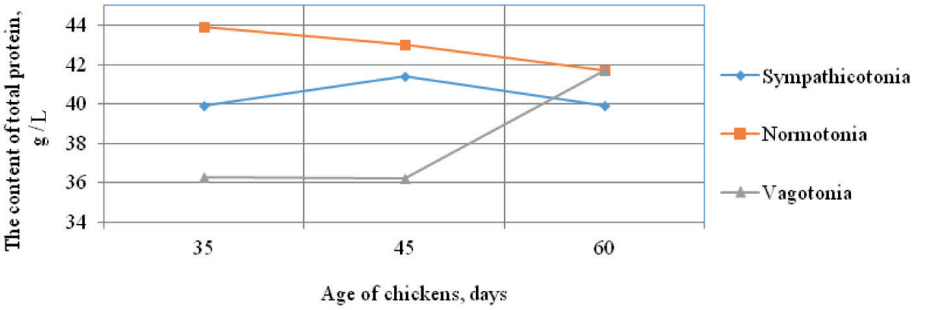

Fig. 1. Dynamics of the total protein content in serum of chickens with different tones of the autonomic nervous system, n = 8

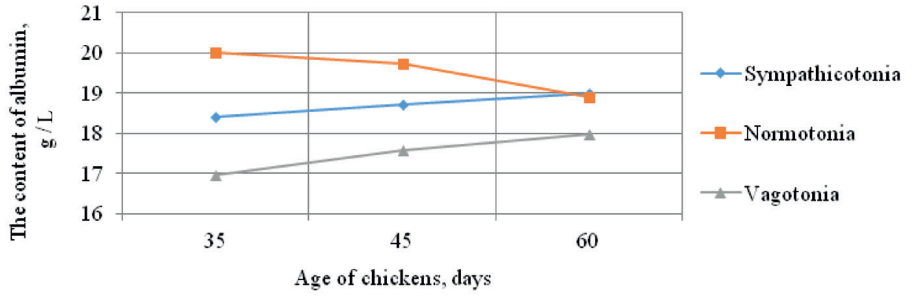

Fig. 2. Dynamics of albumin content in serum of chickens with different tones of the autonomic nervous system, n = 8

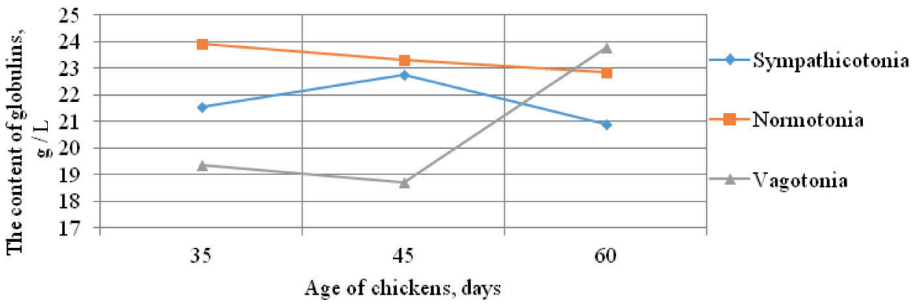

Fig. 3. Dynamics of globulin content in serum of chickens with different tones of the autonomic nervous system, n = 8

otrowska et al., 2011). This is especially noticeable in chickens with a predominance of the parasympathetic tone of the ANS, in which protein metabolism gradually increased and at the 60th day of age approached the results of animals with a dominant sympathetic and balanced tone of the ANS.

Vagotonic chickens had a steady increase in the content of total protein and its fractions. Thus, between the 45- and 60-day period, the content of total protein and globulins increased rapidly by 5.50 g/L (13.1%; $P < 0.001$) and 5.10 g/L (21.3%; $P < 0.001$), respectively. The albumin fraction showed only a tendency to increase the content by 1.00 g/L (5.6%).

It should be emphasized that at the age of 35 and 45 days normotonic chickens compared to vagotonic had a significantly higher content of total protein ($P < 0.001$); albumins ($P < 0.01$–0.001), and globulins ($P < 0.01$–0.001) in serum. A similar pattern concerning these proteins was observed in sympathicotonic chickens compared with vagotonics at the age of 35 ($P < 0.05$) and 45 ($P < 0.001$) days. Chickens with a predominance of parasympathetic tone compared with sympathicotonic had a significantly higher content of globulins only at the age of 60 days ($P < 0.01$).

The content of total protein and its fractions in different periods of rearing may depend on many factors: the level of feeding, protein content in feed, intensity and rate of growth and development of the organism (Filipović et al., 2007; Piotrowska et al., 2011), age of the animal (Kuznyak & Savchuk, 2017). Different ANS tone, in our opinion, also affects the fluctuations of total protein, albumin, and globulins because the dominant effect of the sympathetic branch of the ANS is accompanied by an increase in catabolic processes in animals and humans (Morozova et al., 2016), which may cause statistical differences between indicators. The authors (Morozova et al., 2016) note that in vagotonic animals, due to rapid growth, weight gain, and trophic effects of parasympathicotonia, registered a decrease in total protein and its components in serum. Similar data were obtained in vagotonic chickens at the 35th and 45th days of age (Fig. 1–3).

Note that our results correlate with studies to determine the effect of different ANS tones on the intensity of growth of chickens, which revealed the relationship between the dominance of the parasympathetic system of higher

productivity of chickens during the period of its rearing. A bird with a balanced tone, on the contrary, had the lowest absolute and average daily weight gain (Shnurenko et al., 2020). Vagotonics and sympathicotonics in comparison with normotonics are characterized by a higher content of essential and non-essential amino acids in blood serum (Studenok et al., 2020; Studenok et al., 2021). It should be noted that the globulin fractions, which consist of α-, β-, and γ-globulins can also increase due to chronic and acute inflammatory processes, changes in the environment, and the body's adaptation to them (Tothova et al., 2019). Some authors report a physiologically low level of γ-globulin fraction in chickens, which usually increases during the growth of poultry with other peptides (Povoznikov & Pustovaya, 2013). Similar studies have shown an increase in total protein, albumin, and globulin levels by day 32–42 of meat-fattening poultry, which occurs during periods of intense growth (Filipović et al., 2007; Piotrowska et al., 2011). Other publications suggest that fluctuations in the content of protein fractions in serum of chickens depend on the needs of the body and the period of their rearing. According to the author (Repko, 2015), the protein content increased during the period of intensive egg-laying and tended to decrease after it.

Interactions between the content of total protein, albumin, globulins and body weight of chickens in all experimental groups had a rectilinear relationship at the 35th day of age with a predominance in vagotonic chickens (Table 1).

They showed a close correlation between the content of total protein, albumin, and body weight ($P < 0.001$). Sympathicotonic chickens aged 45 and 60 days showed a sharp change in the direction of the correlation, mostly in the negative direction compared to the previous period. Normotonics at the age of 45 days had a significant ($P < 0.01–0.001$) close correlation between the content of total protein, globulins, and body weight, which changed to negative on the 60th day of life. In contrast, in birds with a predominance of excitability of the parasympathetic division of the ANS, a significant positive correlation was observed between the content of to-

1. Correlation of the content of protein fractions in serum with body weight of chickens in different age periods of rearing, r (n = 8)

Indexes	35 days			45 days			60 days		
	Total protein	Albumin	Globulins	Total protein	Albumin	Globulins	Total protein	Albumins	Globulins
Body weight (sympathicotonics)	0.792*	0.768*	0.690	-0.473	-0.513	0.240	-0.212	-0.262	-0.083
Body weight (normotonics)	0.789*	0.497	0.716*	0.908**	0.205	0.936***	-0.441	-0.473	-0.414
Body weight (vagotonics)	0.927***	0.886**	0.699	0.260	-0.103	0.385	0.707*	0.230	0.809*

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – reliability of correlation coefficients.

tal protein, globulins, and body weight at the age of 60 days.

The concentration of protein fractions in serum of chickens and their interaction with weight gain over the entire study period, in our opinion, depends on the neuro-humoral effect on the metabolism of these compounds, the intensity of animal growth, and diet. Similar results were obtained in studies (Demus, 2010; Demus, 2013), where animals with a predominance of the parasympathetic ANS tone had higher measurements, body weight, and size of individual heart structures compared to sympathicotonics. In fish (rainbow trout Oncorhynchus mykiss) the positive effect of the vagus nerve on the hypothalamic protein AMPKα2, which participates in the control of energy homeostasis throughout the body by regulating feed intake (increased productivity), thermogenesis, metabolism in the liver and muscles (Conde-Sieira et al., 2020). Other studies have shown a direct effect of vagotonia on weight gain and hunger due to the action of neuropeptides Y and AgRP on nutrient-sensitive areas of the hypothalamus and subsequent hyperphagia (Grechko et al., 2018).

The effect of different ANS tones on the content of protein components depending on the age of the bird is diverse and has a general tendency to decrease depending on the growth of chickens. This is evidenced by the results of analysis of variance with the definition of the main indicator of the force of influence η2x (Table 2).

The growing period of 35 days was characterized by a significant effect of vagotonia and normotony on the content

2. The effect of different ANS tones on the content of protein fractions in serum of chickens, η2x (n = 8)

Indexes	35 days		
	Sympathicotonia	Normotonia	Vagotonia
Total protein	0	0.39**	0.37**
Albumin	0	0.26*	0.25*
Globulins	0	0.37**	0.35**
Indexes	45 days		
	Sympathicotonia	Normotonia	Vagotonia
Total protein	0	0.32**	0.65***
Albumin	0	0.19*	0.19*
Globulins	0.1	0.22*	0.61***
Indexes	60 days		
	Sympathicotonia	Normotonia	Vagotonia
Total protein	0.08	0	0
Albumin	0.04	0	0.12
Globulins	0.24*	0	0.15

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – reliability of the main indicator of the force of influence η^2_x.

of all fractions of protein in serum ($P < 0.05–0.01$) and the complete absence of such effects in sympathicotonics with a subsequent slight increase to the 60th day and a significant effect on the content globulins ($P < 0.05$). In contrast, the period of 45 and 60 days in vagotonic animals differed in the pronounced effect of the parasympathetic branch of the ANS on the content of blood proteins ($P < 0.05–0.001$).

The ratio of albumin to globulin (A/G) in 35-day-old chickens did not differ much in different experimental groups with a slight predominance in vagotonics by 3.4%. Chickens of 45-days-old with a predominance of the parasympathetic ANS tone had a significantly higher A/G ratio compared to sympathicotonics by 13.7% ($P < 0.05$) and within the trend – with normotonics by 9.5%. The 60-days-old bird had opposite ratios of protein fractions. In sympathicotonic chickens, A/G ratio was statistically higher than in normotonics by 8.8% ($P < 0.05$) and vagotonics by 16.5% ($P < 0.01$) (Fig. 4).

The authors (Taldykina & Semenyutin, 2021) in their research claim that the decrease in the albumin fraction of proteins may be associated with their use as a plastic material during the intensive growth of chickens. Instead, there are conflicting data that suggest that based on the detected increase in the ratio of albumin to globulins due to the former, we can indirectly talk about increased meat productivity, and globulins – increased egg production (Povoznikov & Pustovaya, 2013; Pushkarev et al., 2021). After all, the higher the value of A/G, the more intense the protein metabolism, which, in turn, affects the state of the whole organism (Pushkarev et al., 2021).

It should be noted that protein metabolism also depends on higher nervous activity, which performs the finest regulation of physiological processes in the body, including through the ANS. Studies by domestic authors have established a direct effect of different types of higher nervous activity on protein metabolism and productivity of pigs and cattle (Danchuk et al., 2020). Animals with a strong balanced higher nervous activity type had a higher content of total protein and albumin compared to a weak one; productivity in these animals also prevailed over the latter. The content of essential amino acids (valine, proline,

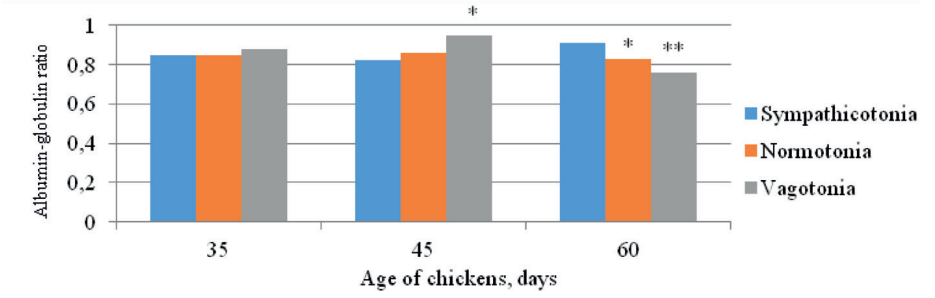

Fig. 4. Albumin-globulin ratio in different periods of rearing in chickens with different tones of the autonomic nervous system, n = 8

Note: * $P < 0.05$; ** $P < 0.01$ – compared with sympathicotonics.

and glycine) in individuals with a weak type of higher nervous activity was lower compared to other groups (Vasyliv et al., 2020).

It should be noted that the justification of these observations is based on the statement about the multifunctionality of blood proteins (Derho & Sereda, 2016). Their concentration in serum directly depends on the intensity of metabolism and the needs of other tissues. Proteins are also used as carriers of biologically active substances, from which hormones and enzymes are synthesized (Filipović et al., 2007; Piotrowska et al., 2011).

Thus, the studies found a relationship between different ANS tones and poultry productivity, total protein, albumin, and globulins in serum. The highest positive effect of parasympathicotonia on the content of test substances and weight gain in the period of 35–60 days of age was noted.

Conclusions and future perspectives

A significant difference in the content of total protein, albumin, and serum globulins in chickens with different tones of the autonomic nervous system in different periods of rearing is a manifestation of the influence of the autonomic nervous system on protein metabolism.

The influence of the dominant tone of the parasympathetic division of the autonomic nervous system is characterized by an increase in total protein and its fractions in the period from the 35th to the 60th day of growing chickens. In normotonics, the studied indicators, on the contrary, tend to decrease. In sympathicotonic chickens, significant fluctuations in the content of serum proteins are mostly absent.

The established correlation between the content of total protein, albumin, globulins and body weight of chickens indicates a direct relationship between protein metabolism and productivity of chickens, which depends on the tone of the autonomic nervous system in different periods of rearing.

A significant effect of parasympathetic and balanced autonomic nervous system tone on the content of protein fractions in serum of chickens aged 35 and 45 days, as evidenced by the albumin-globulin ratio, which decreases to the 60th day of rearing with a parallel significant correlation of total protein and albumin, body weight of the bird.

In the future, it is planned to investigate the effect of different autonomic nervous system tones on the content of micro- and macroelements, hormones in serum of chickens.

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Студенок А. А., Шнуренко Е. О. , Трокоз В. О., Карповський В. І. (2021).
ДИНАМІКА ВМІСТУ БІЛКА В СИРОВАТЦІ КРОВІ ТА ПРОДУКТИВНОСТІ КУРЕЙ ІЗ РІЗНИМ ТОНУСОМ АВТОНОМНОЇ НЕРВОВОЇ СИСТЕМИ
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Анотація. *Головна роль у підтриманні функціонування організму, його рості та розвитку належить білку. Він бере участь у формуванні м'язового каркасу та входить до складу ферментів, нейромедіаторів, гормонів. Вплив автономної нервової системи на ме-*

таболізм загального білка вивчений не достатньо. Відомо, що автономна нервова система – це структура, яка відповідає за гомеостаз та сталість усього організму. Вона бере участь у регулюванні роботи серця, залоз внутрішньої та зовнішньої секреції, травного тракту, органів виділення тощо.

У проведених нами дослідженнях було встановлено, що в курей кросу Кобб 500 в різним тонусом автономної нервової системи впродовж періоду вирощування з 35 до 60 доби спостерігався різний вміст загального білка, альбуміну, глобулінів та реєструвалася неоднакова маса тіла. Кури-ваготоніки демонстрували найнижчі показники білкового обміну у віці 35 та 45 діб ($P < 0,05–0,001$) порівнюючи з симпатикотоніками та нормотоніками, які мали тенденцію до зростанняи в період між 35 та 60 добою вирощування порівнюючи з іншими групами птиці, де досліджувані білкові фракції навпаки знижувались.

Кореляційні взаємозв'язки між загальним білком, альбуміном та масою тіла птиці мали високу лінійну залежність в усіх групах курей ($P < 0,05–0,001$) та негативну залежність у період між 45 та 60 добою вирощування в симпатикотоніків та нормотоніків. У птиці із домінуванням парасимпатичного тонусу автономної нервової системи така кореляція зберегла своє направлення із високою достовірністю ($P < 0,05$) між масою тіла та зальним білком на 60 добу вирощування.

Ключові слова: *симпатикотонія, ваготонія, нормотонія, метаболізм білка, альбумін, глобуліни*