

original article UDC 636.597.087.72:612.015:546.23 doi: 10.32718/ujvas6-1.02

Effects of selenium on metabolic processes in the body of ducklings and their productive qualities

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Article info Received 23.01.2023 Received in revised form 27.02.2023 Accepted 28.02.2023

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Abstract

Today, indicators controlling poultry's mineral nutrition have significantly increased. However, the physiological need of various types and technological poultry groups for certain mineral elements that perform essential biochemical functions in the body has yet to be definitively established. This also applies to such an element as Selenium, which, according to modern classification, is recognized as an indispensable biotic ultramicroelement with a broad spectrum of biological action. The inclusion of Selenium in the composition of mixed feed for young poultry changes the direction of physiological and biochemical processes in the body and improves metabolism and, as a result, contributes to the increase in their live weight, viability, feed conversion, slaughter, and meat qualities, improvement of organoleptic indicators of meat, its amino acid composition, energy, and biological value. We conducted comprehensive scientific studies to deepen and expand modern ideas about the biological role of Selenium, its influence on the productive qualities, and internal indicators of ducklings in the postembryonic period of ontogenesis. One of the tasks was to study the causal relationship between the level of consumption of Selenium with feed by ducklings and the studied indicators. Experimental studies were conducted on ducklings of the Ukrainian white breed. Following existing standards, feeding ducklings daily to 56 days of age was carried out with complete mixed feeds, balanced in essential nutrients and biologically active substances. Ducklings of the first control group did not receive selenium supplementation in mixed feed. Poultry of experimental groups (2-4) was additionally injected with different amounts of Selenium, respectively, by 0.2 mg/kg, 0.4, and 0.6 mg/kg. When conducting a correlation analysis of the obtained experimental data, it was found that between the economically useful and interior features included in the analysis, there are different levels and directions of the relationship, which may vary depending on the level of selenium consumption by ducklings. A scheme of modeling the effect of Selenium on the ducklings' organism raised for meat is proposed.

Keywords: Selenium; content; mixed feed; ducklings; productive qualities; correlations.

Citation:

Sobolev, O. I., Gutyj, B. V., Sobolieva, S. V., Kuzmenko, P. I., Liskovich, V. A., Melnychenko, A. P., & Melnychenko, Y. O. (2023). Effects of selenium on metabolic processes in the body of ducklings and their productive qualities. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6(1), 10–17.

1. Introduction

Poultry farming is one of the most successful branches of animal husbandry, developing rapidly and dynamically. Poultry farming is leading in providing humanity with dietary and high–calorie food products, particularly meat and eggs, and industry-crude materials (Prokopyshyn, 2019; Ravikumar et al., 2022; Gržinić et al., 2023).

The production of the maximum amount of competitive products of high quality is possible only under conditions that consider the poultry's biological characteristics, its physiological state, the direction of productivity, and the influence of external factors on it. At the same time, various types of poultry currently used in industrial poultry farming are distinguished by productive qualities, the intensity of metabolic processes in the organism, the need for metabolic energy, nutrients, and biologically active substances (Barszcz et al., 2022). In addition, farm poultry is very sensitive to the adverse effects of technological stress factors, such as high planting density, changes in the microclimate in industrial premises, diet composition, vaccination, and transportation, leading to metabolic disorders, suppression of the body's immunobiological reactivity and, as a result, a decrease in its productivity and product quality (Shevchuk et al., 2018).

Number 1

The rational and balanced feeding of poultry is the most significant factor affecting its growth and development, health status, productivity indicators, and profitability of poultry farming. The high productivity of poultry leads to a relatively high need for nutrients. The modern system of normalized poultry feeding satisfies its needs for metabolic energy, nutrients, and biologically active substances, including macro- and microelements (Tufarelli, 2021).

Today, the number of indicators controlling poultry's mineral nutrition has significantly increased. However, the physiological need of various types and technological poultry groups for certain mineral elements that perform essential biochemical functions in the organism has yet to be definitively established. This also applies to a biotic element such as Selenium (Sobolev et al., 2019; Meng et al., 2019; Martyshuk et al., 2020).

Analysis of the results of scientific research by scientists from different countries has shown that Selenium is a trace element with a relatively broad spectrum of biological action (Rayman, 2012; Sobolev et al., 2018; Qazi et al., 2018; 2019). According to the results of numerous scientific studies conducted on various species of animals and poultry, it was found that Selenium has immunostimulating properties (Dalgaard et al., 2018), antioxidant (Surai & Kochish, 2019), antiviral (Shojadoost et al., 2020), antitoxic (Ge et al., 2021), adaptogenic (Shakeri et al., 2020), radioprotective (Karami et al., 2018), anti-carcinogenic (Kuršvietienė et al., 2020) and other properties. At the same time, some aspects of the effect of Selenium on the poultry's organism have not yet been definitively clarified.

Already the first attempts to use Selenium as a mineral additive in poultry feeding allowed us to obtain results that prove the absolute need to develop optimal standards for the introduction of this trace element into mixed feed for various types and technological groups of poultry, including for ducklings raised for meat.

Today, it has already been proven that the inclusion of Selenium in the composition of mixed feeds for young animals of various types of poultry changes the direction of physiological and biochemical processes in the organism and improves the digestibility and metabolism of nutrients and, as a result, contributes to the increase in their live weight, safety, feed conversion, slaughter, and meat qualities, improving the organoleptic parameters of meat, its amino acid composition, nutritional and biological value (Wang et al., 2021; Cehmistrenko et al., 2022).

The norms recommended in different countries for introducing Selenium into mixed feed for ducklings have specific differences and range from 0.1 to 0.4 mg/kg of feed. The reason for the disagreement is that scientific studies were conducted on different breeds and crosses of ducks against the background of different diets, under different conditions, and using different selenium-containing compounds.

Until recently, domestic scientists recommended introducing Selenium in mixed feed for ducklings raised for meat at 0.1 mg/kg. However, such a dose corresponds only to the minimal physiological need of poultry for this trace element (Pardechi et al., 2020).

Further studies conducted by Ukrainian scientists have shown that ducklings have the best productive qualities at the rate of selenium introduction into the mixed feed of 0.4 mg/kg (Sobolieva et al., 2020; Sobolev et al., 2020) – scientists from Egypt (Abdel-Hamid et al., 2020).

European standards for introducing trace elements in mixed feed for ducklings provide for adding Selenium only at a dose of 0.14 mg/kg (Egorov et al., 2000).

Scientists from the Czech Republic believe that the guaranteed addition of Selenium to mixed feed for fattening ducklings should be 0.2 mg/kg of feed. At the same time, they note that this norm is indicative and can be adjusted based on recommendations for a specific breed or cross-country duck.

Italian scientists claim that Selenium should be introduced into mixed feed for ducklings raised for meat at 0.3 mg/kg (Bonomi et al., 2001).

When developing standards for introducing Selenium, scientists also consider the recommended maximum permissible levels in mixed feeds for young poultry on fattening, which differ in different countries. For example, in the United States, the maximum dose of Selenium in mixed feed for all poultry species is 0.3 mg/kg (FDA, 2003). According to the legislation of the European Committee, the concentration of Selenium allowed in poultry feed is 0.5 mg/kg (EFSA, 2012).

As can be seen from the above, the standards recommended by foreign and domestic scientists for the introduction of Selenium in mixed feed for ducklings raised for meat are contradictory and, in our opinion, they should be evaluated as indicative, requiring further clarification depending on the biological and regional characteristics of poultry feeding.

In addition, the analysis of literature sources showed that experimental data on the effect of different doses and compounds of this trace element on the productivity of ducklings and product quality are also quite contradictory and are only sometimes supported by theoretical, statistical, and economic calculations. All this confirms the need for additional scientific research on the development, theoretical and experimental justification of the optimal rate of selenium introduction into mixed feed for ducklings raised for meat to increase their productivity, feed efficiency and improve product quality.

Many factors cause the poultry organism's ordinary course of physiological and biochemical processes. It is formed by a chain of causal phenomena that change in time and space (Sobolev et al., 2019). To assess these processes, it is necessary to establish their development's causes, sources, and trends. At the same time, there is a need to study the dependencies that connect these processes with each other and with other factors (features). The presence of connections between traits is manifested at all levels of the organization of living organisms (Gutyj et al., 2019).

Modern zootechnical Science uses various methods to assess certain factors' influence on the productivity level of animals and poultry. However, a special place among them is occupied by correlation analysis.

The task of correlation analysis is to establish the strength (or tightness) of the relationship between individual traits, identify unknown causal relationships, and assess the factors that most impact the effective trait. The correlation coefficient is one indicator that determines the degree of dependence between features (Sobolev et al., 2022).

Since each productivity indicator is a complex practical feature associated with other, sometimes undesirable correlations, the absence of such an analysis can reduce the effectiveness of poultry feeding, breeding work, etc.

The study of correlations between quantitative and qualitative characteristics allows us to determine which factors can be used to more effectively increase the productivity of poultry and the quality of its products and, on this basis, formulate correct theoretical and practical conclusions.

In this regard, our research aimed to study the causal relationship between the level of selenium intake with the feed of ducklings and the studied indicators.

2. Materials and methods

Experimental studies were conducted on ducklings of the Ukrainian white breed. For the scientific experiment, 400 one-day ducklings were divided according to the principle of analogs into four groups of 100 heads each. The experiment's duration corresponded to the technological cycle of raising ducklings for meat and amounted to 56 days.

Following existing standards, feeding ducklings daily to 56 days of age was carried out with complete mixed feeds, balanced in essential nutrients and biologically active substances. Ducklings of the first control group did not receive selenium supplementation in mixed feed. Poultry of experimental groups was additionally injected with different amounts of Selenium in mixed feed according to the experimental scheme (table 1).

Table 1

Scheme of scientific experience

Group	Selenium supplement to mixed feed, mg/kg	
1 control group	Complete mixed feed – CMF	
2 experimental group	CMF + 0.2	
3 experimental group	CMF + 0.4	
4 experimental group	CMF + 0.6	

Sodium selenite (Na_2SeO_3) was used as a source of Selenium with a coefficient of conversion of the element to the salt of 2.20.

During the scientific research, various methods were used that made it possible to identify genuine relationships between the studied phenomena and processes, in particular, zootechnical (indicators of poultry growth, its viability), physiological (digestibility of nutrients, balance of nitrogen and Selenium), pathology-anatomical (slaughter and meat qualities, development of the gastrointestinal tract), chemical (the content of dry matter, protein, fat, ash and Selenium in biological objects), toxic-biological (biological value of meat), hematological (the content of red blood cells, white blood cells, hemoglobin, total protein, immunoglobulins, glutathione) and statistical (mathematical processing of research results).

In the course of experimental studies, the following indicators of the productivity of ducklings were determined:

live weight (g) – individual weighing (on electronic scales) at the beginning and end of the growing season;

- liveability for the rearing period (%) - by daily accounting of poultry that has left the group, with the establishment of the reasons for disposal.

In order to study the effect of additives of different doses of Selenium in mixed feed on the digestibility of essential nutrients, the balance of nitrogen and Selenium in the organism of ducklings. Against the background of scientific experiments, a physiological experiment was conducted following the generally accepted method. The amount of digested nutrients and the balance of nitrogen and Selenium were determined by the difference between the intake of nutrients and minerals from feed and their excretion in feces and droppings. When determining the digestibility of crude protein, the separation of nitrogenous substances of feces from uric acid and its salts was carried out by the chemical method proposed by M. I. Dyakov.

At the end of the rearing period, at the age of 56 days, a

controlled slaughter of ducklings was performed. During the anatomical disassembly of duckling carcasses, medium muscle tissue samples (thigh, lower leg, and chest muscles) were taken for chemical analysis.

The following methods carried out chemical analysis of mixed feed, manure, and meat:

- a mass fraction of total humidity by drying the suspension to a constant mass in a drying cabinet (DSTU ISO 6496: 2005, 2006; DSTU ISO 1442: 2005, 2008);

a mass fraction of nitrogen and crude protein - according to Kjeldahl (DSTU ISO 937:2005, 2007; DSTU ISO 5983-1:2014, 2015);

 a mass fraction of crude and total fat by extraction with ethyl alcohol in the Soxlet apparatus (DSTU ISO 6492:2003, 2005; DSTU ISO 1443:2005, 2008);

 a mass fraction of crude fiber by intermediate filtration (DSTU ISO 6865:2004, 2006);

a mass fraction of crude and total ash by ozolizing the suspension in a muffle furnace (DSTU ISO 5984: 2004, 2006; DSTU ISO 936: 2008, 2008);

- selenium content by method of atomic emission spectrometry with inductively coupled plasma (ICP-AES), which is based on the study of the emission spectra of free atoms and ions under the influence of an excitation source (arc, spark, flame, plasma) in the wavelength range of 150–800 nm, on the Optima 210 DV device from Perkin Elmer (USA). The WinLab32 software controls the operation of the spectrometer.

The content of nitrogen-free extractive substances (NFE) was determined by the calculation method using the formula namely:

NFE = 100 - (crude ash + crude fat + crude fiber + crude protein)

The caloric content (energy value) of duckling meat was determined by the calculation method using the formula namely:

$$C = [D - (F + A)] \times 4.0 + (F \times 9.0),$$

Where: C is the caloric content of 100 g of meat of natural moisture, kcal; D is the proportion of dry matter in meat, %; F is the proportion of fat in meat, %; A is the proportion of ash in meat, %.

The relative biological value of duckling meat was determined by a micrometer using a test organism of ciliate Tetrachymena pyriformis, strain WH_{14} (Mikitjuk et al., 2004).

At the end of the rearing period, blood samples were taken from the experimental poultry. Blood in ducklings was obtained before morning feeding by puncture from the axillary vein using a heparinized tubeless needle, observing the rules of asepsis and antiseptics.

The following methods were used in the blood test:

- shaped blood elements (red blood cells and white blood cells) by melange method;

- hemoglobin by hemoglobin cyanide method;

- the total protein in blood serum by refractometric method;

- the total amount of immunoglobulins in blood serum by photoelectrocolorimetric method (Levchenko et al., 2004);

- total glutathione by the iodometric method (Forman et al., 2009; Averill-Bates, 2023).

The computer program for statistical processing, Microsoft Excel 2010, was used for the mathematical processing of the obtained results. Variance analysis (one-way ANOVA) was used to identify a statistically significant difference between the mean values in the experimental groups.

3. Results and discussion

At the initial stage of experimental research, we set out to determine the actual selenium content in mixed feed for

Table 2

Selenium consumption with duckling feed

ducklings raised for meat. Our research (Sobolev, 2009) it was found that in mixed feeds for ducklings, the average selenium content is 0.075 mg/kg (concentration range is 0.060–0.093 mg/kg), which is considered insufficient even to meet the minimum physiological need (0.1 mg/kg) of this type of poultry in this trace element (table 2). Hence, to eliminate its deficiency in the diets of ducklings, there is an urgent need to enrich mixed feeds with Selenium.

	Group			
Indicator	1	2	3	4
	control	experimental	experimental	experimental
Selenium content in the mixed feed, mg/kg	0.075	0.275	0.475	0.675
Selenium consumption during the growing period				
(56 days), mg/head	0.736	2.711	4.664	6.634
Selenium intake per day, mg/head	0.013	0.048	0.083	0.118

According to the experiment scheme, introducing an additional amount of Selenium in the composition of mixed feeds for ducklings contributed to an increase in the level of its consumption by poultry of experimental groups. Thus, the average daily intake of Selenium with ducklings of the second experimental group was higher by 0.035 mg/head, the third by 0.060, and the fourth by 0.105 mg/head, compared to young animals of the control group, where the same indicator was 0.013 mg/head.

All biological processes in the poultry organism are somehow connected and depend on each other. Studying these relationships, their nature, strength, and other features makes it possible to objectively assess the quantitative and qualitative results of experimental studies.

When conducting a correlation analysis of the obtained experimental data, we set the following tasks: to characterize the measure of dependence between the level of selenium consumption by poultry and effective traits with a constant value of all other factors, as well as to determine the values of the relationship between the traits included in the analysis.

We calculated the corresponding correlation coefficients to establish a causal relationship between the level of selenium consumption by ducklings and the studied indicators (table 3).

Correlation analysis primarily showed the presence of a solid direct statistically probable (P < 0.05) relationship between the level of selenium intake and its assimilation in the organism of ducklings (r = 0.89) and, as a result, with its accumulation in the chest and leg muscles of young animals (r = 0.97 and r = 0.95, respectively).

Numerous strong (r > 0.75) positive links have also been established (although not always likely) between the level of selenium intake by ducklings and their digestibility of crude fat and crude feed fiber, the absorption of nitrogen in the body, the concentration of white blood cells, total protein and glutathione in the blood of young animals, the weight of gutted carcass, the content of protein in the chest muscles of ducklings, and ash in the leg muscles, the biological value of both types of muscle tissue and the length of the small intestine.

Average strength correlations were found between the level of selenium consumption and the live weight of duck-

lings at 56 days of age (r = 0.66), the digestibility of crude feed protein (r = 0.69), some indicators of meat productivity, namely, the weight of the half-patered carcass (r = 0.73) and the weight of edible parts of the carcass (r = 0.64).

Table 3

Relationship between selenium intake in ducklings and trait variables

Indicator	$r\pm m_r$
Digestibility of feed nutrients:	
crude protein	0.69 ± 0.510
crude fat	0.92 ± 0.279
crude fiber	0.90 ± 0.304
nitrogen-free extractives	0.20 ± 0.693
Nitrogen uptake	0.80 ± 0.420
Selenium assimilation	0.89 ± 0.323
Blood content:	
red blood cells	0.41 ± 0.646
white blood cells	0.82 ± 0.404
hemoglobin	0.41 ± 0.645
total protein	0.87 ± 0.343
immunoglobulins	0.24 ± 0.686
total glutathione	0.89 ± 0.322
Weight of semi-patched carcass	0.73 ± 0.480
Weight of gutted carcass	0.78 ± 0.442
Weight of edible parts of the carcass	0.64 ± 0.546
Muscle weight	$\textbf{-0.02}\pm0.707$
Contents in the chest muscles:	
protein	$1.00\pm 0.045^{**}$
fat	-0.52 ± 0.602
ash	0.12 ± 0.702
Selenium	$0.97 \pm 0.178^{*}$
Caloric content of chest muscles	$\textbf{-0.53} \pm 0.598$
The biological value of chest muscles	0.93 ± 0.266
Contents in the leg muscles:	
Protein	0.45 ± 0.633
Fat	-0.04 ± 0.706
ash	0.87 ± 0.350
Selenium	$0.95 \pm 0.213^{*}$
Caloric content of leg muscles	0.12 ± 0.702
The biological value of leg muscles	0.80 ± 0.420
Length of the small intestine	0.88 ± 0.331
Live weight of ducklings	0.66 ± 0.530
Liveability of poultry	$\textbf{-0.09} \pm 0.704$

Note. Probability of difference: * - P < 0.05; ** - P < 0.01.

Indicators such as the content of red blood cells and hemoglobin in the blood of ducklings, as well as the content of protein in the leg muscles, had weak correlations (0.25 < r < 0.50) with the level of selenium consumption by poultry.

At the same time, correlation analysis revealed inverse statistically unlikely relationships between the level of selenium intake in poultry and its muscle weight (r = -0.02), muscle fat content (r = -0.52 to -0.04), the caloric content of chest muscles (r = -0.53), and liveability of poultry (r = -0.09).

Correlation analysis also allowed us to establish that between the productive qualities of poultry, indicators of digestibility and assimilation of nutrients and biologically active substances of feed, and some interior features, there are different levels and directions of interrelation, which may vary depending on the level of consumption of Selenium by ducklings (table 4).

Table 4

Relationships between the studied indicators in ducklings for the action of Selenium $(r \pm m_r)$

Indicator	Live weight of ducklings	Liveability of poultry	Length of the small intestine
Live weight of ducklings	-	—	0.90 ± 0.309
Digestibility of feed nutrients:			
crude protein	$0.96 \pm 0.197^*$	_	0.81 ± 0.416
crude fat	0.85 ± 0.375	_	$0.99\pm 0.082^{**}$
crude fiber	0.89 ± 0.316	_	$0.98 \pm 0.155^{*}$
nitrogen-free extractives	0.89 ± 0.329	_	0.60 ± 0.567
Nitrogen uptake	0.91 ± 0.301	_	0.83 ± 0.393
Selenium assimilation	0.74 ± 0.473	_	$0.98 \pm 0.137^{*}$
Blood content:			
red blood cells	0.71 ± 0.497	$0.85 \pm 0,371$	_
white blood cells	0.92 ± 0.276	$0.46 \pm 0,629$	_
hemoglobin	0.77 ± 0.447	0.81 ± 0.418	_
total protein	0.84 ± 0.379	0.41 ± 0.646	_
immunoglobulins	0.59 ± 0.573	0.93 ± 0.285	_
total glutathione	$0.97 \pm 0.168^{*}$	0.22 ± 0.690	_
Weight of semi-patched carcass	$0.99\pm 0.075^{**}$	_	_
Weight of gutted carcass	$1.00\pm 0.056^{**}$	_	_
Weight of edible parts of the carcass	0.91 ± 0.297	_	_
Muscle weight	0.38 ± 0.655	_	_

Note. Probability of difference: $^* - P < 0.05$; $^{**} - P < 0.01$

In particular, it was found that an increase in the live weight of ducklings when feeding them mixed feeds enriched with Selenium is associated with changes in the intensity of metabolism in the body. This is confirmed by a firm (r > 0.75) relationship between the live weight of ducklings and their digestibility of crude protein (P < 0.05), crude fat, crude fiber, and nitrogen-free extractives of the feed, as well as the absorption of nitrogen.

Indicators of digestibility and assimilation of feed nutrients are interrelated mainly statistically probable (P < 0.05and P < 0.01) strong bonds (except the digestibility of NFE) with the length of the small intestine, which increased in poultry of the experimental groups. Probably, as a result of an increase in the length of the small intestine in ducklings, the time of passage of chyme through, and as a result increases, the duration of action of digestive juices on feed weights and hydrolytic enzymes on proteins increases. Consequently, the digestibility and assimilation of feed nutrients improve.

Comparison of the live weight of ducklings with the length of their small intestine revealed the presence of a high positive correlation between these indicators (r = 0.90).

In addition, the experimental poultry showed links between interior indicators and indicators of its productivity. Thus, the live weight of ducklings has a strong (r =from 0.77 to 0.97) correlation with the content of white blood cells, hemoglobin, total protein, and glutathione in the blood (P < 0.05), and average strength (r =from 0.59 to 0.71) with the content of red blood cells and immunoglobulins in the blood.

A slightly different nature of the relationship was found between the studied blood parameters and the viability of ducklings. Thus, the preservation of young animals has a strong (r = from 0.81 to 0.93) correlation with the content of red blood cells, hemoglobin, and immunoglobulins in the blood and a weak (r = from 0.22 to 0.46) correlation with the content of white blood cells, total protein, and glutathione in the blood.

It should also be noted that there is a strong, mostly statistically likely correlation between the live weight of poultry at 56 days of age and their meat productivity, in particular, the weight of a semi-patched carcass (r = 0.99 at P < 0.01), the weight of the gutted carcass (r = 1.00 at P < 0.01) and the weight of edible parts of the carcass (r = 0.91).

Based on the data of correlation analysis, we propose a scheme of direct and indirect influence of Selenium on the metabolic processes in the organism of ducklings and their productive qualities, which are connected employing lines corresponding to the statistically significant relationship of the corresponding traits (fig. 1).

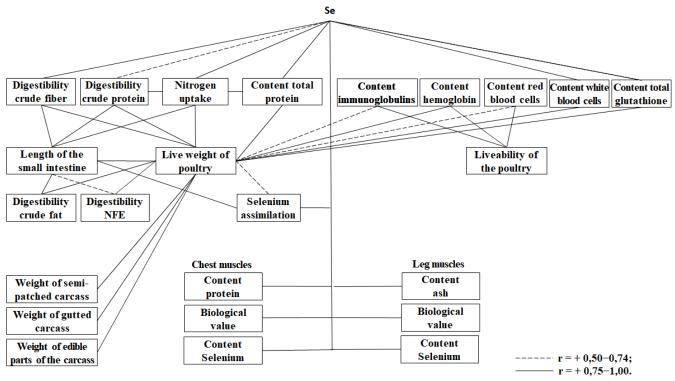


Fig. 1. Scheme of direct and indirect influence of Selenium on the metabolic processes in the organism of ducklings and their productive qualities

4. Conclusions

Based on a comprehensive assessment of the results of scientific research, it was found that the introduction of selenium additives in the composition of compound feeds for fattening ducklings contributes to the following:

- increasing live weight, preserving young animals, and reducing feed costs per unit of production;

- increased digestibility of crude protein, crude fat, crude fiber, and nitrogen-free extractives, which indicates the activation of enzymatic reactions in certain parts of the digestive system and more efficient use of feed nutrients for protein and fat synthesis;

– increasing the intensity of metabolic processes in the body, which improves absorption and increases the deposition of nitrogen in the body, which contributes to the formation of higher meat productivity in young animals;

- increasing the absorption of Selenium in the organism of ducklings and, as a result, increasing its content in the muscles of the chest and legs, which does not exceed the maximum permissible concentrations (MPC);

 increase in the weight of semi-patched carcass, patched carcass, and edible parts of the carcass due to better development of muscle tissue and skin with subcutaneous fat;

- improving the chemical composition, energy, and biological value of duckling meat;

- stimulation of hemocytopoiesis, as evidenced by the simultaneous tendency to increase, within the physiological norm, in the peripheral blood of young animals, the number of red blood cells, white blood cells, and hemoglobin content; activation of immune defense mechanisms, which is manifested in an increase in the level of total protein and the concentration of immunoglobulins in the blood serum; activation of the antioxidant defense system of the body, which is manifested in an increase in the content of total glutathione in the blood and its restored form. In addition, correlation analysis allowed us to determine the nature and degree of relationships between the level of selenium consumption by ducklings with feed and quantitative and qualitative indicators of its productivity. A scheme of modeling the effect of Selenium on the organism of ducklings raised for meat is proposed.

The results of the conducted research have contributed to developing the scientific concept of rational use of Selenium in poultry feeding and meat productivity.

Conflict of interest

The authors declare that there is no conflict of interest.

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