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INFLUENCE OF WATERING I, SE, S CITRATE ON GROWTH AND DEVELOPMENT OF CHICKEN-BROILERS

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Abstract. At the moment, an important issue for medicine and veterinary sciences is the lack of iodine and selenium in human and animal bodies. The problem of I, Se, and S deficiency in livestock has not been solved yet, which motivates the development of new effective compounds of these elements. Particularly noteworthy are the citrates of minor nutrient elements included in mineral premixes and feed additives used to balance mineral nutrition in the diets of animals and poultry. The purpose of this paper is to study the efficacy of different doses of I, Se, S citrate on the intensity of metabolic processes in the body of chicken broilers before and after the intragastric introduction of *E. coli*. It was found that watering of chicken broilers with I, Se, S citrate has antibacterial action. As a result of the conducted studies, it was revealed that the applied doses of I, Se, S caused both prophylactic (E2) and therapeutic (E4) effect on the development of colibacillosis in broiler chickens. This effect is more pronounced with higher doses of Iodine (20 μg/dm^3) in I, Se, S citrate for therapeutic purposes, which contributed to the 100% preservation of the chicken population. High metabolic activity of low doses of I, Se, S citrate with a content of 2.5 μg I dm^3 was noted. It provided the highest weight gain of chickens and reaching 780 g on the 35th day of growing, or 121.5% versus control and 788.4 g (119.9% versus control) on 42nd day of growing in the vivarium. The obtained data indicate a positive influence of watering I, Se, S citrate with a content of 20 μg I/dm^3, which was characterised by 100% preservation of chickens throughout the growing period

Keywords: nano-materials, chicken broilers, iodine, selenium, sulphur, citrate, *E. coli*, biological action, biochemical indices, blood serum

ВПЛИВ РІЗНИХ ДОЗ ВИПОЮВАННЯ I, SE, S ЦИТРАТУ НА РІСТ І РОЗВИТОК КУРЧАТ-БРОЙЛЕРІВ

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Анотація. На сьогодні важливим питанням для медицини та ветеринарії є відсутність I і Se в організмі людини та тварин. Проблема нестачі I, Se та S у тваринництві ще не вирішена, що мотивує розробку нових ефективних сполук цих елементів. Особливої уваги заслуговують цитрати мікроелементів, що входять до мінеральних премісів і кормових добавок та використовуються для балансування мінерального живлення у раціонах тварин і птиці. Метою статті було вивчити ефективність дії різних доз цитратів I, Se та S до та після внутрішньошлункового введення *E. coli* на інтенсивність обмінних процесів в організмі курчат-бройлерів в окремі періоди росту та розвитку. Було встановлено, що випоювання цитратів I, Se, S курчатам-бройлерам демонструє їх антибактеріальну дію. У результаті проведених досліджень було встановлено, що використані дози I, Se, S викликали як профілактичний (Е2), так і лікувальний (група Е4) вплив на розвиток кишкової палички у курчат-бройлерів. Ця дія є більш вираженою в період застосування високої дози йоду (20 мкг/дм^3) у I, Se, S цитраті з лікувальною метою, що сприяло 100 % збереженню поголів'я курчат. Відзначена висока метаболічна активність низьких доз I, Se, S цитрату із вмістом 2,5 мкг I/дм^3, що забезпечило найбільший приріст маси тіла курчат і досягнення його на 35-й день вирощування 780 г, або 121,5 % до контролю та 788,4 г (119,9 % до контролю) на 42-й день вирощування в умовах віварію. Отримані дані вказують на позитивний вплив випоювання I, Se, S цитрату із вмістом 20 мкг I/дм^3, що характеризувалось 100 % збереженістю курчат впродовж усього періоду вирощування

Ключові слова: наноматеріали, курчата-бройлери, Йод, Селен, Сульфур, цитрат, *E. coli*, біологічна дія, біохімічні показники, сироватка крові

INTRODUCTION

Minerals, in particular trace elements, play an important role in poultry feeding. They participate in protein, lipid, carbohydrate, and mineral metabolism, activate enzyme systems. [1; 2]. Iodine is characterised by versatile biological activity, provides a regulatory influence on almost all organs and systems of the body [3–6]. The stimulating effect of organic compounds of iodine and selenium [7] in various dietary supplements on the growth of broiler chickens, and quality of their meat was noted. Studies by other authors have proven that deficiency and hypothyroidism in chicken broilers may be the cause of ascites syndrome with hypoxemia, impaired cardiac and pulmonary function. The main function of selenium in the body – to participate in the work of antioxidant systems and hormonal metabolism of the thyroid gland [8]. In the body, iodine and selenium enhance the action of each other, normalise metabolic processes that protect the organism from harmful substances formed during the breakdown of toxins. Sulphur is indispensable in the process of protein biosynthesis. Sulphur acts as binding agent for heavy metals. It neutralises harmful toxins that have a negative influence on the liver and kidneys [9]. In poultry, S plays an important role in the processes of growth and development of the body, formation of feathers, and protective reactions, and is accumulated in the form of sulphates. The deficiency of this trace element inhibits the synthesis of sulphur-containing

amino acids, which is accompanied by a decrease in the poultry productivity with a decrease in the exchange of nitrogen compounds. Monogastric herbivores and poultry can synthesise sulphur compounds from the methionine of their tissues, except for thiamine and biotin.

To ensure the need of animal agriculture in mineral supplements, the most promising are the organic compounds of macro- and micronutrients, obtained with the help of nanotechnologies. The distinguishing characteristic of nanomaterials is due to their high chemical and biological activity and ability to influence metabolic processes [10]. Citrates of trace elements, which are part of mineral premixes and feed additives deserve special attention and are used to balance mineral nutrition in the rations of animals and poultry [11]. The level of assimilation and biological efficiency of such compounds is several times higher than the mineral salts of these elements. Literature data suggests the possibility of using chelates of biotic elements made by nanotechnology as highly active compounds in animal husbandry and veterinary medicine [12; 13].

Studies by other authors [14; 15] show the stimulating metabolic effect of the nanosized compounds of biotic mineral elements. There are numerous literature data on the possibility of the complex application of I and Se compounds to increase the resistance and productivity of the organism and prevent a number of diseases [16]. Physiologically adequate ratios of I, Se, S citrates for their co-application in laboratory animals has been experimentally determined. Therefore, the purpose of the study was to investigate the biological effects of I, Se, S citrate in different doses, for the supplementation of the broiler chickens' water throughout the entire production cycle.

MATERIALS AND METHODS

The study was conducted on 70 broiler chickens from the 1st to the 42nd day of life, formed in 7 groups, 10 in each. Control and experimental groups were kept in similar vivarium conditions for laboratory animals of the State Research Control Institute of Veterinary Preparations and Feed Additives (SCIVP) in compliance with hygiene requirements. Chickens of all groups received standard compound feed: starter, grower and finisher according to the technological map of growing and drinking water from portable drinkers.

Chickens of the first experimental group (E1) were watered from the 2nd day of the experiment until its completion. The drinking water with a content of 2.5 μg l/dm^3 in the form of I, Se, S citrate was obtained with nanotechnologies from the "Nanotechnology and Nanomaterials" LLC, Kyiv [17]. On the 7th day of life (d.l.), the chickens were injected intragastrically (i/g) with 1 ml/head of the archival strain of *E. coli* received from SCIVP with a concentration of 1 million microbial cells (m. c). Re-introduction of *E. coli* was carried out at the 15th day of life at a dose of 10 million m. c.

Chickens of the second experimental group (E2) from 2nd day of the experiment and until its completion were given drinking water containing 20 μg l/dm^3 in the form of I, Se, S citrate. *E. coli* was injected to chickens at th 7th and 15th day of life in the same doses as in E1. Chickens of third experimental group (E3) were given I, Se, S citrate with a content of 2.5 μg l/dm^3 from the 7th day of life after i/g injection of *E. coli*. Re-introduction of *E. coli* was carried out at the 15th day of life.

Chickens from fourth experimental group (E4) were watered with I, Se, S citrate with a content of 20 μg l/dm^3 from the 7th day of life after intravenous injection of *E. coli*. Re-introduction of *E. coli* was carried out at the 15th day of life. Chickens of the fifth experimental group (E5) were given water without I, Se, S citrate, with i/g injection of *E. coli* at the 7th day. Subsequent re-introduction of *E. coli* at the 15th day of life.

Chickens of the sixth experimental group (E6) from 2nd day of the experiment and until its completion were given drinking water containing 2.5 μg l/dm^3 in the form of I, Se, S citrate. Intragastric introduction of *E. coli* culture was not carried out. The control group received drinking water and compound feed in accordance with technological requirements.

Every 7 days, body weight was controlled by weighing chickens, and livability, morbidity and feed activity were monitored daily. After watering with I, Se, S citrate for 35 and 42 days, the chickens were slaughtered by decapitation in accordance with the bioethical principles of animal treatment used for scientific purposes. Blood samples were taken during the slaughter. The content of protein, albumin, creatinine, calcium, inorganic phosphorus, urea, triacylglycerols, the activity of aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) were determined in the blood using the Humman sets (Germany) on a

biochemical analyser Humalyzer 2000 (Humman, Germany) according to the manufacturer's instructions. The resulting digital data was statistically processed using a standard statistical software package Microsoft EXCEL. The degree of reliability of comparative data was assessed by the Student's ratio.

RESULTS AND DISCUSSION

Analysis of data on the clinical condition and morbidity of chickens indicates a slight inhibitory action of *E. coli* at a dose of 1 million m.c. on the growth and development of poultry in the experimental groups. Within 7 days after hatching, two chickens died in the control group and the same number in E3, which received I, Se, S citrate after intravenous introduction of 1 ml of *E. coli*.

After 14 days, the number of dead chickens in these groups was increased to 5 due to E1 group (1 chicken). In the period from 14th to 21st day, 1 chicken in E5 group has died due to *E. coli* without watering with I, Se, S citrate. Thus, for 21 days of growing only 6 chickens have died, of which in the control group – 2 (20%), in the experimental 1,3,5 – 4 (13.3%), of which E1 – 1 – 3.3%, E3 – 2 – 6.7%, and E5 – 1 – 3.3%. Importantly, after the second intravenous injection of a higher dose – 10 million m.c. *E. coli*, the death of chickens was recorded only in the E5, which did not receive I, Se, S citrate. This may indicate the therapeutic and prophylactic action of I, Se, S citrate in chickens of E1–E4 groups at high doses of *E. coli* and the development of a pathological state in chickens of E5 group who did not receive I, Se, S. This is confirmed by the growth retardation (body weight of 1 chicken was 83–84% versus control), general inhibition of the organism and the excretion of watery droppings. Body weights were also low (88% at 21 days) in chickens of E2 group who received water with 20 μg I/dm^3 before and after i/g introduction of *E. coli*. This indicates a lower metabolic efficiency of I, Se, S citrate at the high dose, used prophylactically in E2 from the 2nd day of life. However, the use of low and high doses of I, Se, S after intragastric introduction of *E. coli* increased body weight accretion of chickens in E3 and E4 groups versus control group by 5.7 and 6.1% in the first 10 days after infection (14th day) while maintaining growth intensity at the level of 90.1 and 95.1% versus control on the 21st day of raising. It is important that the chickens of E6 group, which received a low dose of I, Se, S were characterised by high resistance of the body (100% survival rate) and a stable weight gain over the experimental period while maintaining these indicators at the level of 92–98% versus control for the 7th–21st day of growing.

On the day 28 of the study, the average body weight of chickens in E6 group exceeded the control group by 9.2% and amounted to 581.2 g. In poultry of E4 group, this figure was 506.1 g, and the control – 532 g. However, the liveability of chickens in the control group was 80% versus 100% in E4 and E6 groups. This indicates a complex positive influence on the weight gain and resistance of chicken's organism of low (E1 and E6 groups) and high (E2 group) doses of I, Se, S citrate in the first 28 days of rearing.

Importantly, in the following periods (28–35 days) the growth intensity of chickens of E6 was the highest (199 g of growth) among all experimental (increase in 83–91 g) and control (109.6 g) groups. The measurement of body weight of chickens of control and experimental groups on the 42nd day of rearing indicates a decrease in the growth rate in E5 group (body weight decrease from 531.3 g on the 35th day to 497 g on the 42nd day, or 6.5%) against the background of lower feed and water consumption (Fig. 1).

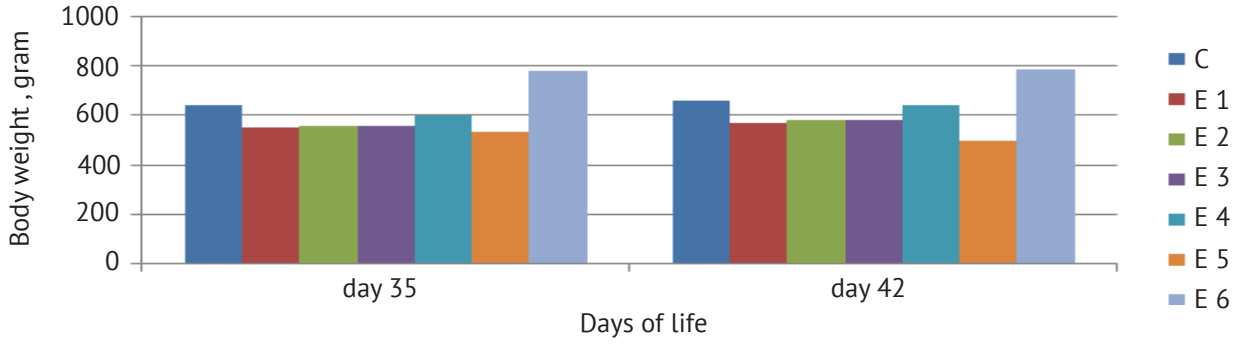

Figure 1. *Body weight of broiler chickens*

However, in all other experimental groups, the increase in body weight of chickens during the last stage of growing was increased compared to the 35th day, including 1.1% (E6) and 7.9% (E4). This may indicate the development of subacute or chronic colibacillosis in chickens of E5 group (did not receive I, Se, S citrate), which led to a decrease in feed intake, digestive disorders with the excretion of watery droppings.

The development of *E. coli* apparently inhibited the absorption of nutrients in the intestines. However, the mortality of chickens in the E5 group remained at the level of the E1 group, which may indicate low virulence of the archival strain of *E. coli*. Thus, the obtained results may indicate the antibacterial action of I, Se, S citrate, which is more pronounced with higher doses of I, Se, S for therapeutic purposes.

This contributed to 100% survival rate of the chickens in the E4 group and the achievement of body weight of 597.4 g (93.1%) on the 35th day, and 644.6 g on the 42nd day (98% versus control). High metabolic activity of I, Se, S citrate in a small dose was noted, which provided the highest weight gain of chickens of E6 group (780 g, or 121.5% versus control) on the 35th day of growing and 788.4 g (119.9%) on the 42nd day of growing in vivarium conditions.

The analysis of biochemical indicators of blood shows a decrease in urea content ($P < 0.05$) in broiler chickens of E1, E2, E3 groups on the 35th day of growing (Table 1).

Table 1. *Biochemical indicators of blood of broiler chickens at the age of 35 and 42 days during watering with I, Se, S citrates and the introduction of E. coli (M±m, n=5)*

Indices	Group	Days of life	
		35	42
Creatinine, mmol/L	C	34.8±2.16	30.0±2.34
	E1	35.5±0.85	29.8±1.88
	E2	33.3±1.32	30.7±1.48
	E3	33.5±1.82	34.4±2.80
	E4	36.9±1.00	32.4±1.86
	E5	38.1±1.28	34.0±3.26
	E6	38.7±1.30	28.8±1.88
Urea, mmol/L	C	1.9±0.14	1.5±0.11
	E1	1.5±0.06*	1.5±0.05
	E2	1.3±0.11*	1.5±0.08
	E3	1.3±0.10*	1.3±0.11
	E4	1.7±0.07	1.6±0.06
	E5	1.6±0.03	1.8±0.06
	E6	1.7±0.12	1.7±0.07
Calcium, mmol/L	C	1.1±0.05	2.2±0.07
	E1	1.1±0.12	2.1±0.19
	E2	1.5±0.12*	1.8±0.04**
	E3	1.2±0.05	2.2±0.08
	E4	1.1±0.12	2.0±0.06
	E5	1.2±0.02	1.9±0.08*
	E6	1.5±0.04***	2.1±0.04
Inorganic phosphorus, mmol/L	C	1.3±0.09	2.1±0.09
	E1	1.2±0.04	2.4±0.13
	E2	1.5±0.05	2.1±0.12
	E3	1.4±0.07	1.7±0.09*
	E4	1.5±0.16	2.1±0.09
	E5	1.5±0.06	1.9±0.06
	E6	1.5±0.11	2.0±0.12
Triacylglycerols, mmol/L	C	1.2±0.04	1.7±0.08
	E1	1.4±0.04	0.8±0.04***
	E2	2.1±0.12***	0.9±0.02***
	E3	1.3±0.02	1.1±0.03***
	E4	1.3±0.04	1.1±0.06***
	E5	1.2±0.05	1.0±0.04***
	E6	1.2±0.05	1.3±0.05**

Cholesterol, mmol/L	C	3.0±0.09	3.1±0.19
	E1	3.3±0.16	2.6±0.12
	E2	3.7±0.22*	3.2±0.14
	E3	3.2±0.17	3.3±0.23
	E4	4.2±0.32*	3.6±0.26
	E5	2.7±0.13	3.0±0.15
	E6	3.0±0.21	3.5±0.16
Albumin, g/L	C	12.6±0.63	11.4±0.50
	E1	11.4±0.61	11.7±0.30
	E2	11.9±0.19	13.4±0.63*
	E3	12.2±1.62	12.4±0.95
	E4	12.2±0.66	12.8±0.91
	E5	11.9±0.87	14.1±1.28
	E6	15.3±0.89*	14.0±1.32
Crude protein, g/L	C	22.5±0.86	22.7±1.02
	E1	22.2±0.80	20.4±0.73
	E2	22.8±0.65	23.0±0.75
	E3	21.2±1.02	23.6±1.07
	E4	21.7±0.25	23.9±0.69
	E5	24.4±0.29	24.5±0.27
	E6	23.4±1.04	24.2±1.00

Note: ** – P<0.05; ** – P<0.01; *** – P<0.001*

The Ca content increased on the 35th day in chickens of E2 (P <0.05) and E6 (P <0.001), but on the 42nd day it was lower (P <0.05) in chickens of E2 and E5 groups, and P – in E3 group. Characteristic is the low content of triacylglycerols in chickens of all experimental groups, which is more pronounced with the introduction of *E.coli* (E1–E5 group). The obtained results may indicate the inhibitory influence of the *E.coli* pathogen on the metabolism of nitrogen-containing compounds and triacylglycerols in the blood, which is more pronounced at low doses. However, the cholesterol level showed a tendency to a higher content in the blood of chickens of all experimental groups on the 35th day with a probable difference in E2 and E4 groups.

The elevated level of cholesterol in the blood of chickens watered both prophylactically (E2) and therapeutically (E4) with I, Se, S with intragastric introduction of *E. coli* indicates the stimulating influence of citrate of these compounds of elements on lipid metabolism with increased cholesterol synthesis in the first ten-day interval of the second month of growing. The absence of probable differences in the content of creatinine and total protein in all groups may indicate the compensatory ability of the body to normalise homeostasis under the influence of both low and high doses of I, Se, S citrate and the introduction of *E. coli*. Albumin-synthesising function of the body was higher in chickens of E6 group at low doses of I, Se, S, as evidenced by an increase in blood albumin on day 35 (P <0.05) and 42 (6.6%).

The evaluation of the transaminase activity of the blood indicates a pronounced enzymatic reaction-response of the body to I, Se, S on 35 day with an increase in AlAT and AsAT indexes (Table 2).

Table 2. *Aminotransferase activity in the blood of broiler chickens at the age of 35 and 42 days, after watering with I, Se, S citrates and the introduction of E. coli (M±m, n=5)*

Indices	Group	Days of life	
		35	42
AlAT, nkat/l	C	6.2±0.21	5.9±0.30
	E1	7.1±0.33	7.8±0.80
	E2	7.1±0.62	6.6±0.79
	E3	7.2±0.14*	7.1±0.35*
	E4	7.9±0.26**	5.6±0.65
	E5	7.5±0.21**	5.6±0.22
	E6	9.6±0.23***	4.0±0.22*
AsAT, nkat /l	C	226.5±17.03	224.1±0.60
	E1	256.9±19.96	173.6±4.87***
	E2	241.7±18.90	192.3±13.81
	E3	236.9±4.380	210.0±1.90***
	E4	259.5±11.63	218.2±16.17
	E5	248.0±4.05	223.6±16.92
	E6	286.5±8.98*	236.9±16.28

Note*: * – P<0.05; ** – P<0.01; *** – P<0.001*

On day 42, higher AlAT activity was maintained for chickens in groups E1–E3, but probably was decreased in E2. AsAT blood activity was lower in chickens of all experimental groups with a probable difference in E1 and E3 ($p < 0.05$). These noted differences in the transaminase activity in the blood of chickens in the experimental groups can be due to the applied doses of I, Se, S, and the period of their watering – before or after the introduction of *E. coli*.

CONCLUSIONS

As a result of the study, it has been discovered that the applied doses of I, Se, S citrates caused both prophylactic (E2) and therapeutic (E4) influence on the development of colibacillosis in broiler chickens. This influence is more pronounced when applying higher doses of I, Se, S citrate, which was characterised by 100% survival rate of chickens in E2 and E4 groups throughout the rearing period. In the blood samples from chickens of E6 group, which received I, Se, S similar to E1, but without the introduction of *E. coli*, a higher content of Ca, P, albumin, AsAT activity, better growth rate were observed on the 35th day, indicating the stimulating metabolic influence of a low dose of the considered compound.

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