

UDC: 619:616-008.9:636.5.087.73

DOI: 10.18413/2500-235X-2017-3-1-105-113

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Zhrebnnenko S.V.¹****EFFICIENCY OF CAROTENE-CHLOROPHYLLIC COMPLEXES USE
IN A-HYPOVITAMINOSIS OF POULTRY**

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Abstract. As is known, poultry is very sensitive to the lack of vitamins in the case of stress or diseases, reducing food intake and reducing intestinal absorption of vitamins. Deficiency of carotene and vitamin A leads to various diseases of young animals, a drastic reduction in the productivity and fertility of adult animals. The principal purpose of this study was to investigate the possibility of using chloroprenol as a vitamin supplement in diets of broiler chickens for the prevention of A-vitamin deficiencies and improve the commercial quality of poultry products. In accordance with desired goal we have estimated the clinical-biochemical status of the broiler chickens under production-line conditions, defined the optimal dosages of chloroprenol for the broiler chicken, determined the effect of the drug on the morphological and biochemical parameters of blood, studied their effect on the natural resistance parameters of the body, accumulation of biological active substances in the liver, viability and productivity. It should be noted that before experimentation a significant increase in aspartate aminotransferase was found in the serum of all chickens, indicating toxic liver injury and vitamin A reduction – insufficient intake of this vitamin in the body of the poultry. After administration of the highest doses of chloroprenol (5.0 and 10.0 ml/l water) a significant increase in vitamin A was observed (on 24.2 and 27.3%) in the blood serum and (on 20.5 and 18.3%) in the liver. At the end of the experimental period phagocytic activity of pseudo eosinophiles was increased (23.6 and 26.1%) in comparison with the control. Based on the results of the research we have identified the optimal dose chloroprenol – 5.0 ml/l water, because the a higher dose does not give a significant increase in poultry weight and enhance the natural resistance, and the low dose is less effective. Therefore, we have substantiated efficiency of using chloroprenol for broiler chickens as a preventive agent for A-hypovitaminosis.

Keywords: carotene, chlorophyll, vitamin A, broiler chickens, hypovitaminosis, blood serum, liver, chloroprenol.

Introduction.

In the modern intensive technology of the livestock breeding hypovitaminoses are the leading cause of metabolic disturbance, of the natural resistance reduction and immune reactivity of the organism.

Disbalance of vitamins in the animal's diet leads to the abnormalities of the specific biochemical reactions in the body, morphological changes in organs and tissues, the development of clinical signs of diseases [1].

With an increase in the productivity of modern crosses of hens there is a need of stable income with feed the whole complex of nutrients and bioactive

substances, which is capable to provide the required level of energy in the body [2]. However, the problem of nutrition of modern poultry crosses has many aspects [3].

In these latter days, the scientists prefer carotenoid-containing preparations with increasingly frequently, as overdose of carotene, unlike vitamin A never causes toxic effects; in addition β -carotene has an effect on the commercial quality of the poultry products [4]. One of the most important functions of carotenoids and especially β fractions – is the ability to be converted in the body into vitamin A [5]. Excessive amounts of β -carotene does not pose any

toxicity of vitamin A because the body does not produce any of the β -carotene more vitamin A than is required [6].

Carotene protects the animal's body from the damaging effects of nitrate on hemoglobin, stimulates the nonspecific factors of natural resistance, defends from the carcinogenic effects of aggressive pro-oxidants – active forms of oxygen and free radicals, which are formed in the cells during the process of intracellular respiration [7, 8]. Established immunostimulatory role of carotenoids is determined [9].

Consequently, the formation of the national water-dispersible carotenoid preparations are now very relevant in the present time [10, 11] and especially from own material using the composition of dietary supplements [12, 13, 14]. It is especially important to use naturally occurring antioxidants [15], as antioxidants block the activation of proto-oncogenes and normalize immune status [16, 17].

It is known, that carotene and vitamin A deficiency is not observed in the organism of the animals after administration of the green fodder and grass meal, so we have focused on coniferous chlorophyll-carotene paste, which representing an emulsion of the pine needle extract with content of chlorophyll (1%) and carotene (0.2%). However, the preparation is difficult to administer in the chicken's rations caused by paste-like consistency of it. Therefore, water-dispersed form of the preparation (called chloroprenol) was designed by the chemical scientists from CJSC "Petrohim" (Belgorod) to facilitate industrial use of chlorophyll-carotene paste.

We have studied the pharmacological efficacy of the preparation according to use perceptiveness of it in the poultry breeding, as a compound substituted grass meal, and thus it prevents hypovitaminoses of broiler chickens and improves commercial quality of the meat.

The main purpose of the present research was the studying pharmaco-toxicological effect of chloroprenol on the broiler chickens for the rationale possibility to using of it as the prophylactic agent for A-hypovitaminosis of broiler-chickens.

For achieving the purpose the following goals were set:

- to monitor the biochemical status of broiler chickens in industrial environments;
- to identify the optimal dose of chloroprenol;
- develop modes of use of the drug and keeping its cost low for its use in the poultry industry.

Chloroprenol – is water-dispersed chlorophyll-carotenoid preparation, it has olive or dark-green colour with characteristic smell of pine, dissolves in water to form an emulsion, it contains in its composition 1.0% of chlorophyll, 0.1% of carotene,

0.03% of vitamin E, 0.01% of vitamin K, 1.5% of phytosterols; 6% of polyprenols; 0.7% of squalenes; 3% of trace elements; 4% of waxlike compounds, and also ethereal oils, aldehydes, alcohols and etc.

Materials and methods.

The pharmacological efficacy of chloroprenol was determined on broiler chickens cross «Hubbard».

The nature of the influence of the chloroprenol on the organism was assessed by the clinical parameters, changes in protein, carbohydrate and mineral metabolism, general non-specific resistance of the organism, intensity of the growth and productivity of the chickens.

Experimental studies were carried under production conditions of the livestock farms of Belgorod region.

Group formation was carried out according to the principle of analogues.

Biochemical parameters were determined by the conventional methods. Hematology Analyzer "Hitachi" was used.

Lysozyme activity in serum was determined by turbidimetric method [18], the phagocytic activity – by phagocytic neutrophil count of 100 cells, bacterial growth-inhibitory activity of blood serum – by I. M. Karput' [19].

Obtained in all the experiments the digital material was subjected to statistical processing on a personal computer by conventional methods of variation statistics with calculation of the student's argument (td). The difference among the compared values was considered significant at $p \leq 0.05$.

Results and discussion.

The assessment of the clinical state of the chickens was carried out in "Prioskolye" Novooskolskiy district of Belgorod region.

For the research two groups of broiler chickens were selected, which are included 10 and 20-day-old to 60 goals each.

Clinical state of chickens was assessed as satisfactory. However, in each age group, about 10% of chicks were lagging behind in growth and development, had a poor appetite, we noted ruffled feathers cover depression. In the study of morphological parameters of blood it was found in chickens that in both age groups, the number of red blood cells and white blood cells were consistent with the physiological norm.

In the analysis of blood biochemical composition of broilers 7-14-day-old (Table 1) it was found a violation of calcium-phosphorus ratio, and the phosphorus concentration was higher than the physiological norm almost 1.5-2 times, concentration of calcium was at lower limits of the normal range.

Table 1

Biochemical blood parameters of broiler chicken

Parameters	Chicken age, days		Normal range
	10	20	
Protein, g/l	30.2±1.48	36.7±1.49	43-59
Ca, Mmol/L	3.32±0.28	3.67±0.45	3.75-6.75
P, Mmol/L	4.27±0.38	5.65±0.49	4.0-6.90
Bilirubin mg/dl	1.29±0.14	1.57±0.19	0.1-0.35
Glucose, Mmol/L	11.36±0.82	11.58±0.77	4.44-7.77
Cholesterol, Mmol/L	1.25±0.22	1.30±0.24	1.0-1.4
Vitamin A mcg/ml	0.30±0.06	0.34±0.075	
AST u/L	129.8±3.25	176.8±4.24	15.3-55.3
ALT u/L	48.3±1.22	58.4±2.37	21.7-46.5

The total protein content was below the physiological normal range by 1.5-2 times.

As is known transamination enzymes indicate liver function. From the data presented in the table it is clear that their values exceed the physiological normal range 4-6 times and with the age it only aggravated the situation. Increased in serum glucose was also significantly high (by 2-3 times higher than the physiological range). But especially the high values were achieved by bilirubin levels (more 7.5 times than the physiological range).

Thus, the birds had impaired protein and mineral metabolism, which may be the consequence of an imbalance of minerals in the feed and their poor digestibility in the gastrointestinal tract. Disorders of mineral metabolism may be associated by possible imbalance of amino acids, and it is the cause of lower assimilation of protein. On violation of carbohydrate metabolism was evident by the increase of glucose in the blood and damage to the pancreas.

Toxic damage of the liver of chickens was accompanied by a significant increase of bilirubin and enzymes of transamination in the serum. Serum of birds had marked lack of vitamin A

From each age group were killed 6 chickens and in the liver we determined the content of vitamin A.

The results of this analysis are presented in Table 2.

The data table shows that in the liver of chickens in both age groups, there is a lack of vitamin A, the level 1.5-2.0 times lower than the physiological norm, indicating insufficient intake of the vitamin in body of a bird or its destruction by assimilation.

Table 2

The content of vitamin A in the liver of broiler chickens

Parameters	Chicken age, days.			
	7	Normal range	14	Normal range
Vitamin A, mcg/g	23.13±1.16	30-40	32.19±2.18	45-60

It should be noted that to mitigate these negative effects, it is necessary to introduce carotene in diet of chicken – and vitamin A therapeutic drugs for the prevention of hypovitaminosis. This drug is in our opinion is chlorophenol.

The experiment was formed on the principle of analogues on 4 groups of broiler chickens 7-day-old to 60-goal each. The birds were kept in the same broiler house, were exposed to work stress to the same degree and were fed on a diet adopted in the farm. The first group was the control group. The second, third and fourth were experimental groups and were given chlorophenol in addition to diet, in different doses according to the scheme of experience presented in Table 3. A formulation used with water for 20 days.

Table 3

Experimental design on broiler chickens

Groups	The applied drug	Dosage, ml/l water
1 – control	The main diet (MD)	-
2 – experimental	MD+ chlorophenol	2.5
3 experimental	MD+ chlorophenol	5.0
4 – experimental	MD+ chlorophenol	10.0

As a result of the research (Table 4) it is found that the highest average daily gains of chickens were in the third and fourth experimental groups (by 5.7 and 6.1% higher than the control). The lowest feed costs were also in these groups (by 9.2 and 8.6% below control).

The highest safety of the birds was also in the third and fourth experimental groups, which used the maximum dose of the drug. In the control and the second experimental group fell one chicken. At autopsy the dead chickens were found liver and pancreas damages.

Thus, the conducted research indicates the positive impact of chlorophenol on the safety and productivity of poultry, with the obvious advantage of maximum doses of the drug.

Table 4

The test results of chlorophenols on broiler chickens

Parameters	Groups			
	1 control	2 experimental	3 experimental	4 experimental
Number, goal at the beginning of the experiment	40	40	40	40
at the end of the experiment	39	39	40	40
distemper	1	1	-	-
safety, %	97.5	97.5	100	100
average daily gain, g	40.2	41.0	42.5	42.7
±for control, %	-	+1.9	+5.7	+6.1
the cost of feed per 1 kg gain, kg	1.40	1.33	1.27	1.28
±for control, %	-	-5.0	-9.2	-8.6

During the whole experimental period blood samples were taken of chickens to determine the morphological and biochemical parameters.

The results of hematological studies showed that the hemoglobin content, leukocytes and red blood cells in chickens of all experimental groups tended to

increase, however, statistically significant differences from control were not noted.

The composition of the leukogram (Figure 1) after the administration of chlorprenol also did not undergo significant changes.

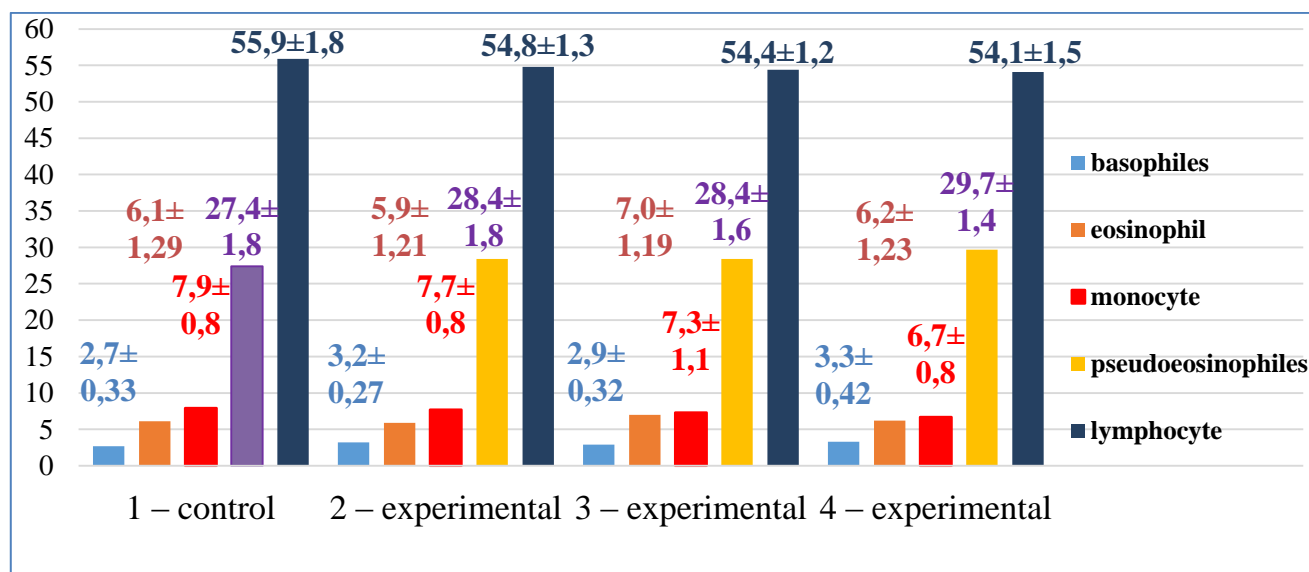
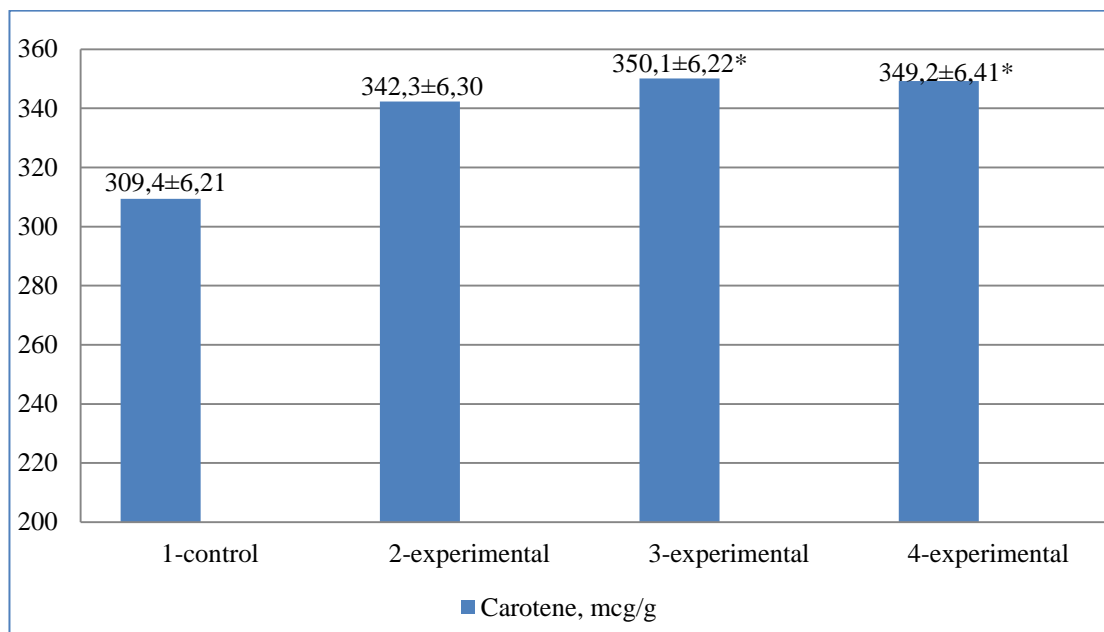


Figure 1. Leukogram

Note that the changes of blood biochemical parameters after watering chlorprenol. So, at the end of the experimental period, blood serum of chickens of all experimental groups showed a significant

increase in carotene (by 10.6-13.2 %), and the difference with the control was confirmed statistically from the maximum doses of the drug (Figure 2).



*- $p \leq 0.05$

Figure 2. The content of carotene in the blood serum of broiler chickens after chloroprenol administration

Chlorophenol did not have a significant effect on the content of calcium, phosphorus and protein (Figure 3-4), however, the level of vitamin A

exceeded the control by 34.6-40.8%, however, in none of the cases the difference with the control was not confirmed statistically (Figure 5).

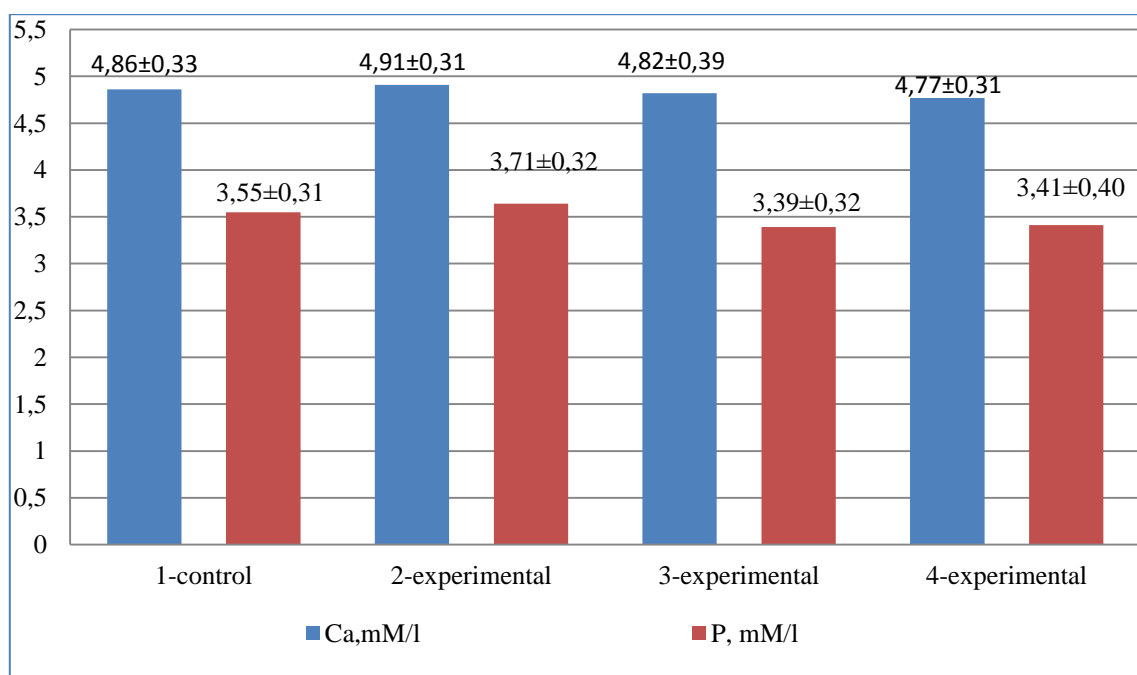


Figure 3. Calcium and phosphorus content in the broiler chicken blood serum after chloroprenol administration

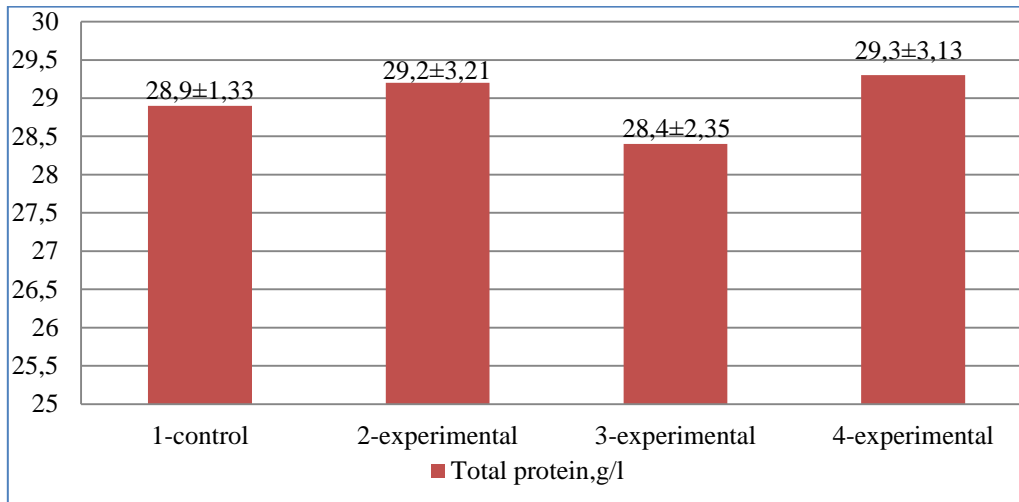


Figure 4. Total protein content in the broiler chicken blood serum of after chloroprenol administration

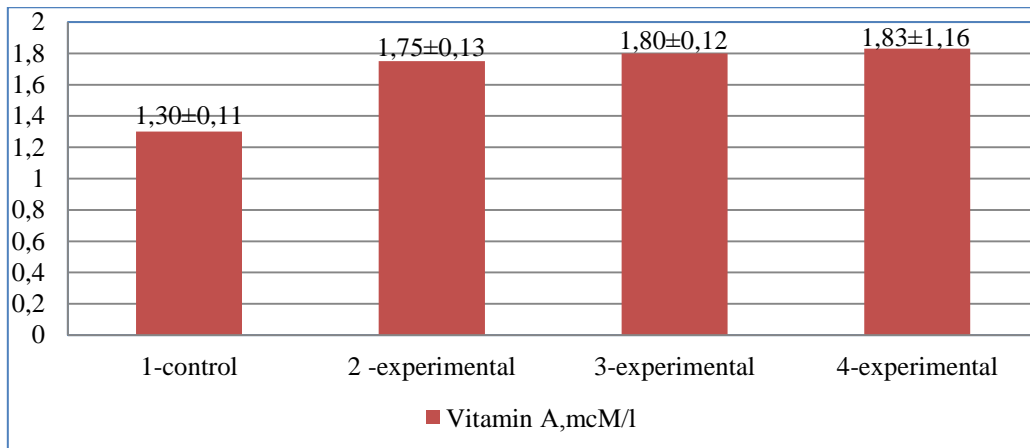


Figure 5. The content of vitamin A in serum of broiler chickens after chloroprenol administration

In the second, third and fourth experimental groups transaminase activity in the blood serum of chickens (Figure 6) did not differ from control,

indicating the absence of the negative impact of chlorophenol on liver function.

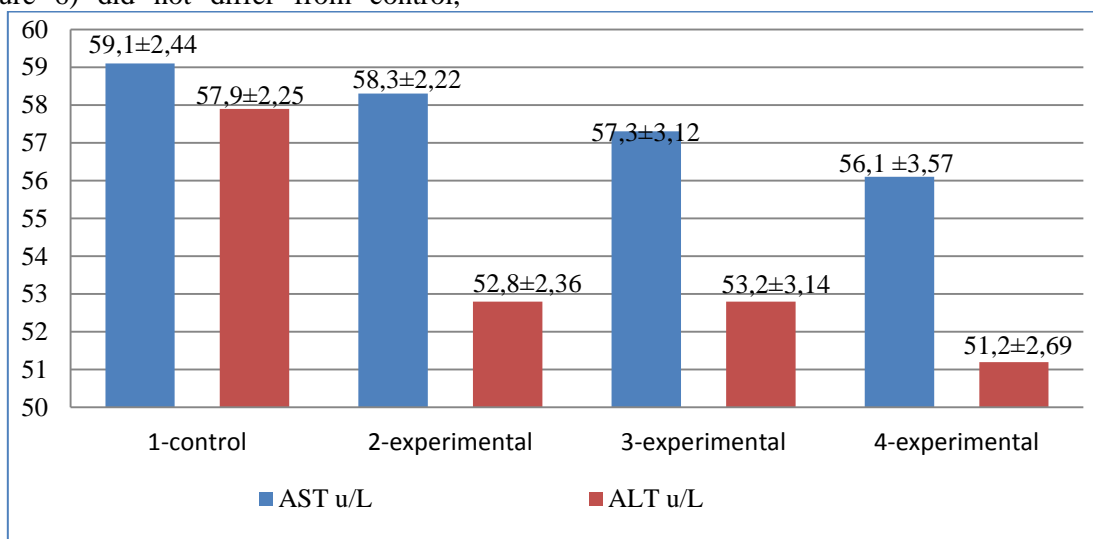


Figure 6. The transamination enzyme activities in the serum of broiler chickens after chloroprenol administration

Thus, conducted studies have shown that chlorophenol in the studied doses did not cause significant deviations in the natural course of metabolic processes in the chicken body.

At the end of the experience, the slaughter of chickens was done and vitamin A concentration is determined in their liver (Table 5).

A significant increase of vitamin A in the liver birds was also from the maximum doses of the drug. At the end of the experimental period its value exceeded the indicators of control in the third experimental group by 20.5, in the fourth – by 18.3% (in all cases $p < 0.01$).

Table 5

The concentration of vitamin A in the liver of broiler chickens

Parameters	Groups			
	1 control	2 experimental	3 experimental	4 experimental
Vitamin A mcg/g	94.83±3.78	96.21±3.26	11.25±3.66**	12.21±3.80**

** – $p < 0.01$

Thus, the most effective doses of chlorophenol for chickens should be considered 5.0 and 10.0 ml/l of water in which there is high bioavailability of vitamin A from the drug. However, the optimal as more economically beneficial, may be considered to be a dose of 5.0 ml/l.

In the next step, the natural resistance of broiler chickens was studied (Table 6).

The presented data show that the preparation administration caused the increasing of phagocytic activity of pseudoeosinophiles in all experimental groups, but only in the third and fourth groups there was statistically significant difference with the control in the third and fourth groups of doses 5.0 and 10.0 ml/l (by 23.6 and 26.1 %, respectively, $p < 0.05$).

At the end of the experimental period, other factors of natural resistance were also increased by all used doses of preparation: bactericidal activity increased by 1.7-4.9; lysozyme activity – by 6.3-19.0; the level of immunoglobulins increased by 2.3-5.0. However, in none of the cases the difference was not statistically confirmed with control that it could be considered as the tendency.

Thus, the use of chlorophenol had a positive influence on the organism of broiler chickens. The obtained results can be explained by the presence in the drug of chlorophyll, carotene, vitamin E, polyphenols and other biologically active substances.

Table 6

Natural resistance parameters of the broiler chickens

Parameters	Groups			
	1 control	2 experimental	3 experimental	4 experimental
Initial data				
bactericidal activity, %	26.20±1.33	25.94±1.77	25.38±1.52	24.80±1.38
lysozyme activity, %	17.82±1.16	17.54±1.38	18.11±1.65	17.44±1.33
phagocytic activity, %	34.57±1.87	33.88±2.12	35.39±2.21	32.56±2.11
immunoglobulins, units.	2.22±0.43	2.21±0.27	2.16±0.39	2.18±0.35
After administration of drug				
bactericidal activity, %	26.87±1.39	27.33±1.25	28.21±1.30	28.10±1.26
lysozyme activity, %	18.12±2.05	19.26±2.33	21.16±2.14	21.57±2.35
phagocytic activity, %	36.57±2.13	43.14±2.19	45.21±2.20*	46.13±2.87*
immunoglobulins, units.	2.98±0.57	3.12±0.46	3.05±0.40	3.13±0.42

* – $p < 0.05$.

Part of chlorophenol vitamin E prevents prevents the formation of free radical processes in the body and their pathological influence on the tissues and organs and it stimulates the immune system.

Phytoncides, contained in the drug, have detrimental effect on microorganisms and pathogens.

The drug polyphenols stimulate the immune system, increasing the phagocytic activity of macrophages. And, in our opinion, the most basic is

the presence in the formulation of chlorophyll, which plays a leading role in the body's absorption of carotene, which is present in chlorophenol and in feed of plant origin.

An unquestionable advantage of chlorophenol is the fact that it mixes well with water. The solubility achieved by the introduction into its composition of food emulsifiers, which improve the use not only of beta-carotene, but also of the feed.

The increase in the factors of nonspecific protection of the body of birds can be explained by the presence in the preparation of carotene, chlorophyll and vitamins that boost natural resistance.

Thus, according to some authors, carotene exhibits antioxidant activity and has a positive effect on the mobility of neutrophils [20]. At the same time, according to A.I. Svezhentsova with et al. [21] carotene increases the organism resistance of newborn animals to nutritional diseases due to the in the permeability of the lining of the digestive tract for toxic substances. According to L.M Corwin [22] and A. Franchini [23] vitamin E is involved in the biosynthesis of gamma-globulins.

Polyprenols are not less important components of chloroprenol and chlorophyll-carotene paste involved in vital processes, both plant and animal organisms. Polyprenols of plants are the most important group of biologically active substances, which is interested of their allocation, and use, including natural complexes.

The modern theory of immunity suggests that entered plant polyprenols into the body may be important for the correction of the immune response. The study of the effect of polyprenol on the immune system have shown that they are immunomodulatory compounds selectively acting on humoral immune response and nonspecific phagocytic activity of macrophage.

Conclusions.

Thus, the conducted studies have shown that chloroprenol has high bioavailability. It increases the phagocytic activity of pseudoeosinophiles and, as a consequence, increases the average daily gains and safety of broiler chickens. Consequently, optimum dose is 5.0 ml/l of water from all of studied because the higher dose (10ml/l) does not give significant weight gain of poultry and improve the immune status, and a low dose (2.5 ml/l) less effective. Therefore, the optimal should be considered as 5.0 ml/l in water.

Recommendation.

Chlorophenol recommended for broiler chickens with drinking water for the prevention of hypovitaminosis and productivity, at the rate of 5.0 mL / L for 20 days Chloroprenol is recommended for broiler chickens with drinking water for the prevention of A-vitamin deficiencies and increase productivity at the rate of 5.0 ml/l for 20 days.

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