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Research Article

The Use of Sorbents for Intoxication of Chickens with Imidacloprid

VLADISLAV I. EGOROV, DAMIR V. ALEYEV, ANDREY V. MALANEV, KADRIA F. KHALIKOVA, GULNARA G. GALYAUTDINOVA, GUZALIYA R. YAMALOVA, ANNA M. TREMASOVA, ILNUR R. KADIKOV, ALEXANDER M. SAIFUTDINOV, EDUARD I. SEMENOV, EDUARD A. SHURALEV, SERGEY YU. SMOLENTSEV

Federal Center for Toxicological, Radiation and Biological Safety, Nauchnyi Gorodok-2, Kazan city, 420075, Russia

Kazan (Volga Region) Federal University, Kremlevskaya street 18, Kazan, 420008, Russia

Mari State University, Lenin Square 1, Yoshkar-Ola city, 424000, Russia

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ABSTRACT

Providing population with high-quality food is one of the important tasks in the modern world. Various factors prevent from achieving this goal, one of which is the use of chemicals in agriculture. On the one hand,the use of various chemicals increases the yield of crops, which is very important from an economic point of view, but on the other hand,chemicals cause various diseases of humans, animals becausetheir residuesenter the body.The aim of this work was to study the effectiveness of the use of a combination of sorbents to reduce the impact of imidacloprid on broiler chickensthat received it constantly with feed. Sorbents of different origin and structure, zeolite and shungite, were usedin the experiment. Zeolite is a mineral formed from sedimentary volcanic rocks which contains a large number of microelements, shungite is a carbon containing rock with sorption ability to various organic and inorganic substances. The identification and determination of imidacloprid was carried out by chromatographic methods with high performance liquid chromatography. The addition of imidacloprid to the diet of broilers has a negative effect on the body, inhibits physical health, reduces productive indicators; the addition of a combination of mineral sorbents has a positive effect on growth rate, improves metabolism and all other indicators. For the objectivity of the research results, there is a need to conduct experiments on other neonicotinoids and other bird species. The practical significance of the study lies in the fact that these sorbents reduce the toxic effect of imidacloprid due to their sorption properties which positively affects the health of broiler chickens.

Keywords: broiler chickens, neonicotinoids, imidacloprid, sorbents, zeolite, shungite.

INTRODUCTION

Providing population with high-quality food is one of the important tasks of agriculture. Poultry farming, as one of the most dynamically developing branches of agriculture, is capable of fulfilling this task to a large extent, as it is characterized by fast poultry population reproduction rates, high productivity and viability, low direct labourcosts and material resourcecosts per unit of output. The quality of products depends on healthy poultry feed, housing conditions and other factors. Poultry farms are located on the outskirts of large settlements, that are the places of product sales and consumption (Muktyaz et al., 2017).

Hens are most widely used in industrial poultry farmingof all poultry. The physiological state of chickens depends on many factors, nutrition should be balanced, natural and of high-quality, grain feeds and products of their processing, oilcakes, fodder of animal originshould be used in feeding. Compound feeds contain herbal flour, dried pulp. The use of feed additives that can increase productivity and improve meat quality is also an important aspect of modern poultry farming. Premixes, vitamins, enzyme preparations, fodder yeast, chalk and lime, travertine, marl, eggshell, bone and fish flour, wood ash are usedfor good metabolic processes in poultry. Gravel and shell are used asgastrolith. In recent years, the use of enterosorbents has become of paramount importance.

Contamination of feed with various toxicants in natural conditions is possible while processing crops from weeds and parasites, during harvestingand also while storing and processing feed material (Soudamini et al., 2019). Agricultural products are intensely contaminated when pesticides are used improperly, crop processing times are not met, preparations are overused, processing ratio increases (Bridi et al., 2018). In the case ofimproper storage of pesticide-treated seeds, their mixing with food and coarse grains may occur, and thus they may be used for food and feeding (Wang et al., 2018).

When poultry feed containsmore pesticides than it is permitted, the organism's resistance decreases (Ehsan et al., 2019; Chao et al., 2019), and infectious diseases appear. Dysbacteriosis of intestinal microflora also appears (Martínez et al., 2019; Chiaki et al., 2014), the growth and development of poultrybecome slower, their productivity and lifeabilitydecrease (Madhuri et al., 2018). One of the ways to eliminate the negative effects of toxicants is to add feed additives with high sorption properties to the diet (Tanka et al., 2017). Representatives of such additives are sorbents. Sorbents are substances that selectively absorb various substances. Activated carbon, silica gel, alumina, silicon dioxide, various ion-exchange resins, dibutyl phthalate, zeolite, shungite and others are widely used (Yu et al, 2019).

Zeolite is a widespread useful mineral related to sedimentary volcanic rocks formed as a result of the interaction of lava products and rocks. Due to its properties, it helps to maintain mineral homeostasis in a living organism, enriches it with trace elements and improves metabolism. This sorbent contains oxides of such elements as potassium, calcium, magnesium, manganese, aluminum, sodium, iron, silicon, titanium, and others (Rathi et al., 2019).

Shungite is a unique natural mineral, carbon containing rock, formed from organic bottom sediments - sapropel. These organic sedimentswere covered with new layers gradually compacting, dehydrated and sank into the depths of the earth. Under the influence of compression and high temperature, a metamorphization process took place, as a result, amorphous carbon in the form of characteristic globules dispersed in the mineral matrix was formed. Shungite has high mechanical strength, low abrasion, filtering ability, as well as the ability to sorb both organic and mineral substances. Preparations based on it have pronounced bactericidal properties, relieve itching and have an analgesic effect (Wang et al., 2018).

The aim of our study was to study the effectiveness of the use of various combinations of sorbents - zeolite and shungite to reduce the effects of imidacloprid which has been absorbed into the organism of a broiler chickenfor a long time.

MATERIALS AND METHODS

The Cobb-500 broiler chickens of the age of two weeks were used for the experiment. The chickens were kept under the same conditions in compliance with all requirements. The layout of the experiment is presented in Table 1.

Table 1: The layout of the experiment

Group	Feeding habits
1 Control	Basic diet (BD)
2 Experimental	BD + imidacloprid (IK)
3 Experimental	BD + IK + zeolite (Z) 0.5% ofthe diet
4 Experimental	BD + IK + shungite (Sh) 0.5% ofthe diet
5 Experimental	BD + IK + Z/Sh (70:30) 0.25% ofthe diet
6 Experimental	BD + IK + Z/Sh (70:30) 0.5% ofthe diet
7 Experimental	BD + IK + Z/Sh (70:30) 1.0% ofthe diet

For feeding poultry, complete compound feed of domestic production was used (elevator in Naberezhnye Chelny City, Russia).

The experiment was carried out on 35 broiler chickens divided into 7 groups with five chickens in each. The first group was control and received onlycomplete compound feed. Other groups were experimental and received compound feed with imidacloprid at a dose of 1/4 LD_{50}.

The second experimental group received compound feed only with imidacloprid. Other groups received compound feedwith various combinations of sorbentsin addition to imidacloprid,thus, the third experimental group received zeolite in the quantity of 0.5% of the diet, the fourth - shungitein the quantity of 0.5% of the diet. The fifth, sixth and seventh groups received both zeolite and shungiteat a ratio of 70 to 30 in the quantityof 0.25%, 0.5% and 1.0% of the diet. The experiment lasted 23 days.

As additives, shungite from the Zazhoginsky field in Karelia, Russia and zeolite of the Shatrashansky field in Tatarstan, Russia were used. The addition of imidacloprid to the feed was carried out by uniform distribution of a 20% aqueous solution of neonicotinoid (1 ml of solution per 1 kg of feed) with a manual spray gun,then it was dried. Sorbents were added to the pesticide-treated compound feed immediately before feeding, the clinical state of the poultry was monitored during the experiment. Productivity indicators were assessed by the poultry growth rate and feed consumption per unit of production. The quantity of pesticide in the muscle tissue of broiler chickens was determined by chromatographic method, based on (García et al., 2006). Sample preparation was done by the M7 methodology from (Souza et al., 2016). Muscle tissue samples were taken from the lower leg. The samples of muscle tissue of 10 g were minced and homogenized with 10 ml of water, extracted with 30 ml of ethyl acetate, and then shaken for 1 min. After that, 8 g of anhydrous $MgSO_4$ and 3 g of NaCl were added, the mixture was shaken for 3 min and centrifuged for 5 min at 4000 r.p.m. The cleanup procedure was done as follows. A freeze-out step of the extract was performed overnight at −20 °C. Then the concentration of the resulting solution up to about 8 ml was done on a rotary evaporator at 30 °C. In the dispersive SPE step 120 mg of C-18, 200 mg of Al_2O_3, and 600 mg of $MgSO_4$ were used. The extract was shaken in a vortex intensively for 30 s and centrifuged at 4000 r.p.m for 5 min. Finally, the extract was dried under a nitrogen stream to dryness, re-dissolved in 1 mL of MeCN, and filtered through a 0.45 µmAcrodisk PVDF Membrane for HPLC-UV analysis.

The identification and determination of imidacloprid were carried out on a liquid chromatograph Waters 2690 Alliance HPLC (Waters Corp., Milford, MA, USA) system, including a binary pump solvent management system, an online vacuum degasser, and an autosampler, equipped with a Waters 2996 PDA detector. The analytical column used is the Hypersil ODS (250 × 4.6 mm, 5 µm (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Imidacloprid in the analyzed samples was calculated as the average of two parallel determinations. Chromatograms were collected and analyzed with Empower 2 software. An ACN:water gradient mobile phase program was as follows: initially 6 min isocratic program with ACN:water 10:90 (v/v) at a flow rate of 1mL min−1, then 6 min linear gradient to ACN:water 30:70 (v/v) at a flow rate of 1.4mLmin−1 followed by an additional period of 1 min linear gradient to the initial conditions.

Statistical analysis. The 95% confidence intervals (CI) were calculated and compared for the weight parameters. Data were tested for normality using the Shapiro-Wilk test, and then Levene's test used to assess the equality of variances. Data were analyzed with a one-way ANOVA, followed by a Tukey HSD test to compare means. The level of significance for all tests was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The quantity of feed consumedduring the whole experiment was calculated. Taking into accountthe live weight of a chicken, daily feed intake per head was calculated. From the 1st to the 7th days of the experiment the daily feed intakewas 100 g per head; from the 8th to the 12th days - 125 g; from the 13th to the 17th days - 147 g and fromthe 18th to the 23d days - 160 g. 105.7 kg of feed was consumed by the broiler chickens in all the groups for 23 days of the experiment.

The gain in live weight was determined by the difference in weight at the end and the beginning of the experiment, the average daily gain was calculated by dividing the gain by the number of days of the experiment. Feed consumption per head was determined by dividing the quantity of the consumed feed in the group by the number of chickens in the group, and feed consumptionper kilogram of gain - by dividing feed consumption per head bygain in live weight.

The results of comprehensive assessment of the productive indicators of broiler chickens chronically intoxicated with imidacloprid with simultaneous use of sorbents are given in Table 2.

Table 2: Productive indicators of broiler chickens

Group	N	Mean (g)	Std. deviation	Coef. Var., %	Lower 95% CI	Upper 95% CI	Shapiro-Wilk test	Levene's test
Live weight at the beginning of the experiment								
1	5	685.6	22.6	3.30	657.5	713.7	W=0.886; p=0.4207	F=0.955; df1=6; df2=28; p=0.473
2	5	693.4	19.7	2.84	668.9	717.9	W=0.865; p=0.3031	
3	5	656.8	21.8	3.32	629.7	683.9	W=0.977; p=0.9966	
4	5	690.2	22.5	3.26	662.3	718.1	W=0.791;	

							p=0.0936	
5	5	656.2	15.5	2.36	637.0	675.4	W=0.783; p=0.0818	
6	5	634.4	39.9	6.29	584.8	684.0	W=0.799; p=0.1048	
7	5	642.0	31.6	4.92	602.8	681.2	W=0.893; p=0.4633	
Live weight at the end of the experiment								
1	5	2518.8	164.6	6.53	2314.3	2723.3	W=0.837; p=0.1919	
2	5	2158.4	180.6	8.37	1934.1	2382.7	W=0.929; p=0.7427	
3	5	2198.0	193.8	8.82	1957.4	2438.6	W=0.907; p=0.5607	
4	5	2219.6	139.0	6.26	2047.0	2392.2	W=0.818; p=0.1418	F=0.661; df1=6; df2=28; p=0.682
5	5	2217.8	168.1	7.58	2009.1	2426.5	W=0.843; p=0.2137	
6	5	2288.2	147.5	6.45	2105.1	2471.3	W=0.948; p=0.8926	
7	5	2297.8	131.2	5.71	2134.9	2460.7	W=0.999; p=1.000	
Total gain								
1	5	1833.2	143.7	7.84	1654.7	2011.7	W=0.813; p=0.1314	
2	5	1465.0	161.6	11.03	1264.4	1665.6	W=0.922; p=0.6892	
3	5	1540.8	173.3	11.25	1325.6	1756.0	W=0.884; p=0.4042	
4	5	1529.4	123.6	8.08	1376.0	1682.8	W=0.765; p=0.0619	F=1.230; df1=6; df2=28; p=0.321
5	5	1561.8	157.2	10.07	1366.6	1757.0	W=0.830; p=0.1718	
6	5	1653.6	112.0	6.77	1514.5	1792.7	W=0.931; p=0.7598	
7	5	1656.0	101.6	6.14	1529.9	1782.1	W=0.992; p=1.000	

As shown in Table 2 the difference between the average live weight of broiler chickens at the beginning of the experiment between the control and experimental groups was insignificant (in each group Mean was 634.4-693.4 g, and coefficient of variance (CV) within the groups were <10%), which confirms the correct formation of the groups.

At the end of the experiment, the live weight of chickens in the groups was different. In the control group, the live weight at the end of the experiment was 2518.8±164.6 g, in the experimental groups the increase in live weight was slower on average by 8.8%-14.3% and ranged from 2158.4±180.6 g to 2297.8±131.2 g. In the second experimental group that received feed with imidacloprid, the increase in live weight at the end of the experiment was lower by 14.3% compared to control group. In the third group that received zeolite with imidacloprid, the live weight at the end of the experiment was lower by 12.7% than in the control group, in the fourth group – by 11.9%. In the fifth, sixth and seventh groups that received combined sorbents with imidacloprid, the increase in live weight at the end of the experiment was lower in comparison with the control group by 11.9%, 9.1% and 8.8%, respectively.

Clinical signs of poisoning were not observed in the experimental chickens; in all the groups, the complete liveability of the livestock was observed.

Feed consumption in the experimental groups varied from 2.834 kg to 2.952 kg, which is lower by 2.2% to 6.1% than the control figures, in the control group feed consumption was 3.02 kg. So, in the second groupthat received imidacloprid in its pure form, the reduction in feed consumption was lower by 4.6% than in the control group, in the third group that received zeolite -by 2.4% and in the fourth group that received shungite- by 4.1 %, respectively. In other groups that received combined sorbents zeolite and shungite, feed consumptionwas lower than in the control group by 6.1%, 4.4% and 2.2%, respectively. The reduction in feed consumption in the experimental groups occurred from the 15th day of the experiment that wasthe result of the decreased appetite of chickensbecause of imidacloprid contained in compound feed.

Gainin live weight and feed consumption in the experimental groups that received sorbents were higher than in the groups that did not receive them in the diet. Overall, the best results were in the control group of chickens. The values of the average daily gain in live weight of experimental chickens are presented in Fig. 1, Table 3.

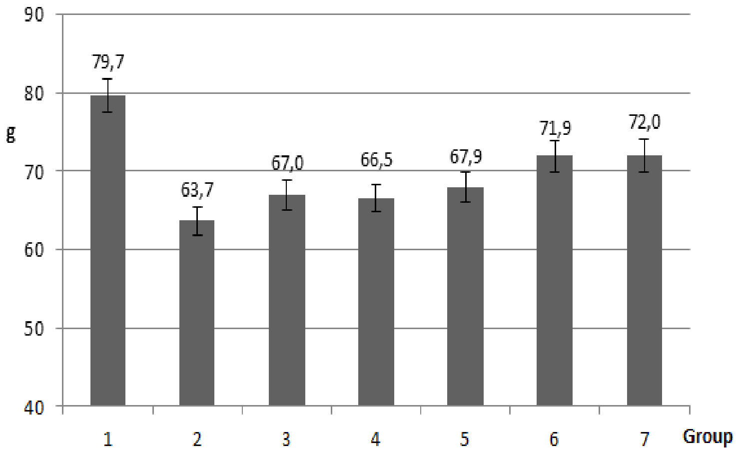

Fig.1: Average daily increase in broiler chickens

Table 3: Average daily increase in broiler chickens

Group	M±SD	Shapiro-Wilk test	Levene's test	One Way ANOVA test
1	79.80±6.30	W=0.803; p=0.1129	F=1.446; df1=6; df2=28; p=0.233	$F_{6,28}$=3.872; p=0.0061 (<0.05)
2	63.60±6.95**	W= 0.914; p=0.6190		
3	66.80±7.40*	W=0.890; p=0.4401		
4	66.60±5.41*	W=0.767; p=0.0643		
5	67.80±6.69	W=0.800; p=0.1069		
6	71.80±4.66	W=0.933; p=0.7780		
7	71.80±4.15	W=0.995; p=1.000		

* - p<0.05; ** - p<0.01 (Tukey HSD test: compared to Group 1).

The average daily increase in chickens in the control group was 79.80±6.30 g (Fig. 1, Table 3). In the group of chickens that received feed with imidacloprid without sorbents, the average daily increase was lower than in the control group by 20.1% (p≤0.01) and was 63.60±6.95 g. The use of zeolite in combination with toxic feed led to a decrease in the average daily gain in comparison with the control group by 15.9%, the addition of shungite to compound feed reduced the gain by 16.6%. In the group that received toxic feed with combined sorbents in the quantity of 0.25% of the diet, the average daily increase was lower than in the control group by 14.8%, in the sixth group, the increase was lower by 9.8% and in the seventh group received combined sorbents (1% of the diet) was lower by 9.7%.

Feed consumption per kilogram of gain is presented in Fig. 2 and Table 4.

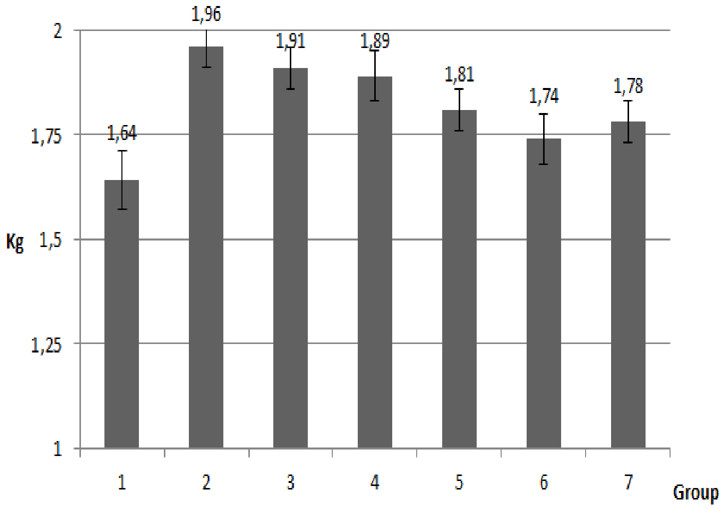

Fig.2: Feed consumption per kilogram of gain

Table 4: Feed consumption per kilogram of gain

Group	M±SD	Shapiro-Wilk test	Levene's test	One Way ANOVA test
1	1.644±0.066	W=0.886; p=0.4137	F=0.777; df1=6; df2=28; p=0.595	$F_{6,28}$=11.860; p=0.00000133 (<0.001)
2	1.965±0.104[1***]	W=0.986; p=0.9999		
3	1.921±0.103[1***]	W=0.869; p=0.3216		
4	1.895±0.053[1***]	W=0.945; p=0.8722		
5	1.817±0.050[1*,2*]	W=0.950; p=0.9049		
6	1.743±0.060[2***,3**,4*]	W=0.937; p=0.8124		
7	1.782±0.046[2**]	W=0.972; p=0.9901		

[1*] - p<0.05; [1**] - p<0.01; [1***] - p<0.001 (Tukey HSD test: compared to Group 1); [2*] - p<0.05; [2**] - p<0.01; [2***] - p<0.001 (Tukey HSD test: compared to Group 2); [3*] - p<0.05; [3**] - p<0.01; [3***] - p<0.001 (Tukey HSD test: compared to Group 3); [4*] - p<0.05; [4**] - p<0.01; [4***] - p<0.001 (Tukey HSD test: compared to Group 4).

The average feed consumption per 1 kg of gain of the chickens in the control group was 1.64 kg (Fig. 2, Table 4). In the group of broilers that received a toxic diet without sorbents, this indicator was higher than in the control group by 19.5% (p≤0.001). In the groups that received zeolite and shungite separately with toxic feed, the feed consumption per 1 kg of weight gain was higher than in the control group by 16.4% (p≤0.001) and 15.2% (p≤0.001). In the groups of broiler chickens that received combined sorbents with toxic feed in the quantity of 0.25%; 0.5% and 1.0% of the diet, feed consumption per 1 kg of weight gain was higher than control figures by 10.3%, 6.1% and 8.5%, respectively. The efficiency of combined sorbents at 0.5% of the diet (group 6) was observed (1.743±0.060 kg) compared to groups 2 (p≤0.001), 3 (p≤0.01) and 4 (p≤0.05).

The appearance of the muscles met requirements at the moment oftaking muscle tissue samples, the muscles in the section were pale pink, the texture was elastic, dense, no visible changes were observed. Pesticide residues in muscle tissue were determined by chromatographic method on a liquid chromatograph. The quantity of imidacloprid residues in the muscle tissue of chickens are shown in Fig. 3 and Table 5.

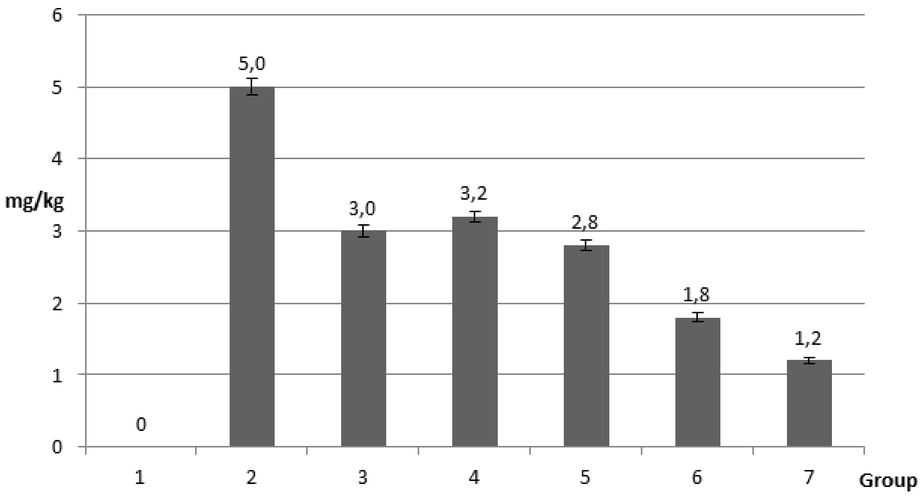

Fig.3: The quantity of imidacloprid residues in the muscle tissue of chickens

Table 5: The quantity of imidacloprid residues in the muscle tissue of chickens

Group	M±SD	Shapiro-Wilk test	Levene's test	One Way ANOVA test
2	5.00±0.43	W=0.980; p=0.9987	F=0.387; df1=5; df2=24; p=0.853	$F_{5,24}$=70.630; p=1.46e^{-13} (<0.001)
3	3.00±0.34[2***]	W=0.925; p=0.7114		
4	3.20±0.34[2***]	W=0.977; p=0.9971		
5	2.80±0.25[2***]	W=0.943; p=0.8585		
6	1.80±0.41[2***,3***,4***,5**]	W=0.934; p=0.7873		
7	1.20±0.29[2***,3***,4***,5***]	W=0.9281; p=0.7365		

[2***] - p<0.001 (Tukey HSD test: compared to Group 2); [3***] - p<0.001 (Tukey HSD test: compared to Group 3); [4***] - p<0.001 (Tukey HSD test: compared to Group 4); [5**] - p<0.01; [5***] - p<0.001 (Tukey HSD test: compared to Group 5).

The imidacloprid was not found in the muscle tissue of chickens in the control group, that indicates the absence of the studied pesticide in the feed given to this group (Fig. 3, Table 5). The largest quantity of imidacloprid was observed in the muscles of the broiler chickens in the second group, where they received the pesticide without sorbents. In the groups that received zeolite and shungite separately with toxic feed, the quantity of imidacloprid in muscle tissue was lower than in the second group by 40.0% (p≤0.001) and 36.0% (p≤0.001), respectively. In the groups of broiler chickens that received combined sorbents with toxic feed in the quantity of 0.25%; 0.5% and 1.0% of the diet, the level of imidacloprid in the muscles was lower than in the group of chickens that received the pesticide without sorbents by 44.0% (p≤0.001), 64.0% (p≤0.001) and 76.0% (p≤0.001), respectively. The best effect of the combined sorbents was observed in groups 6 and 7 with a concentration of imidacloprid 1.80±0.41 and 1.20±0.29 mg/kg, respectively. These values were significantly lower, than in all other groups (p≤0.001).

The problem of the impact of various chemicals on the organism of animals and poultry attracts great attention of scientists from many countries of the world (Walderdorff et al., 2019). The study of this problem is urgent due to both widespread chemicalization in agriculture to increase productivity and the use of new preparations to bring weeds and parasites under control. Chemicalization in agriculture influences human health as chemical residues enter the human body in chain order "plant → animal → human", and thus four main approaches can be identified to solve this problem (Wilke et al., 2019).

The first approach combines the research that deal with the negative effects of imidacloprid on the organism of animals and birds. The discovery of neonicotinoids as a means of controlling weeds led to the widespread use of these preparations. There are cases of animal and bird imidacloprid poisoning. In studies on the Leghorn chicken embryos (Muktyaz et al., 2016), experiments were carried out on the possible effect of various concentrations of the insecticide; as a result, its direct negative effect on the development of embryos was established. The same results are shown in other studies (Khandiaet al., 2019). There is evidence of the effect of these compounds on other bird species. There are data on the harmful effects of imidacloprid on South American Eared Dove (Addy-Orduna et al., 2019), Rock Dove (Ehsan et al., 2019), Japanese Quail (Madhuri et al., 2018), American Goldfinch (Rogers et al ., 2019), hummingbirds (Bishop et al., 2018), partridges (Bonneriset al., 2019), etc. There are studies on the effect of imidacloprid on various laboratory animals (Khalil et al., 2017).

Neonicotinoidresidues can be found in the treatment area for a long time that leads to chronic effects on the organism. There is more evidence that the chronic effect of neonicotinoids can have a direct effect on the body's immune response leading to poor livestock and poultry performance.

The second approach combines studies on the effects of imidacloprid on the nervous system through the blockade of acetylcholine receptors. Some years ago, the effect of imidacloprid on the human, animal and bird organism was considered non-toxic, destructive properties were manifested only for insects. However, some studies (Chenet al., 2019; Shao et al., 2020) describe casesof severe gastrointestinal toxicity, respiratory distress and neuropsychiatric problems in humans after accidental inhalation exposure of imidacloprid. Patients recovered from poisoning only after supportive and symptomatic treatment. The first case of human acute inhalation intoxication with imidacloprid was described in India.

The third approach is aimed atstudyingthe effect of various sorbents on the reductionof the influence of toxicants on the organism. Thus, in some scientific works, there is information about the adsorption of 19-22% of imidacloprid by bentonites (Yu et al., 2019). The addition of 1.5% zeolite sorbent to the diet of poultry contributes to the sorption of 70% of toxicants (Zavala-Franco et al, 2018), (Rathi et al., 2019), the effectiveness of various sorbents andbiosorbents is thatthey remove intoxicants from the organism. A desorption study confirms hysteresis and concentration dependence. This study shows that organobentonite can be a good sorbent for removing imidacloprid from various samples (Yu et al., 2019).

Scientific works on the effect of neonicotinoids on different animal and bird species are currently relevant, but research on the Cobb 500 broiler chickenshave not been performed yet. Research within this approach study the use of sorbents for intoxication with imidacloprid and areaimed at reducing economic and other losses. (Tanka et al., 2017).

CONCLUSION

After carrying out the research, it was found that the addition of imidacloprid at a dose of 1/4 $LD_{50}$to the diet of broiler chickens had a negative effect on the productivity of poultry. Separate addition of shungite and zeolite to toxic feed had a positive effect on the studied productivity indicators. The best results were obtained when combined zeolite and shungite in the quantity of 0.5% and 1.0% of the diet were used. These combined mineral sorbents with bioactive properties had a positive effect on metabolism, influenced the growth rate and development of chickens, as well as feed conversion. Due to the sorption properties, they reduced the toxic effect of imidacloprid, thereby prevented from getting the pesticide into the muscle tissue and had a positive effect on the organism of the chickens in general.

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