

Development of A Zinc Asparaginate-Based Disinfectant for Disinfection of Hatching Eggs

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Abstract: Based on the results of the previous studies of bactericidal and fungicidal properties of zinc asparaginate, a new disinfectant for the incubation of eggs has been developed. The use of the zinc asparaginate-based disinfectant for treatment of hatching eggs is recommended in the concentration of 3 %. The dosage for treating hatching eggs is 0.2 ml of the solution, which corresponds to 0.6 mg of zinc per egg. The preparation has passed the experimental storage periods test; the shelf life of this product is 2 years. Two experiments were performed: the proof-of-concept experiment with 3 repetitions, and the production one. During the period of incubation, the research was performed for detecting the growth of bacterial cultures. It has been noted that zinc asparaginate can inhibit bacterial growth for 6 days, which has an advantage to formaldehyde. The yield of the conditioned broiler chickens of the "Cobb-500" cross in the production experiment in the experimental group was 5.6 % higher than that in the reference group, where chickens were treated with a 37 % aqueous solution of formaldehyde.

Index Terms: zinc asparaginate, hatching eggs, hatchability, formaldehyde, disinfection, disinfectant, antimicrobial activity, broiler chickens.

I. INTRODUCTION

Disinfectants differ from one another by the diversity of chemical components. The mechanism of disinfectants action on the components of microbial cells is also specific.

It should be noted that the mechanism of disinfectants' antimicrobial activity has not been studied enough, and raises disputes among scientists. At the same time, a common vision has been formed on numerous important regularities, which are used for guidance in the practice of disinfection.

The active effect of disinfectants is due to the fact that the preparation penetrates the microbial cells and reacts with its various components that are responsible for vital functions (metabolism, respiration, reproduction, etc.). Thus, the mechanism of disinfectants' action on the microbial cells is

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determined not only by its death but also by the nature of changes in the metabolic processes that occur in it. Weak solutions of disinfectants only slow down (bacteriostatic effect) the vital functions of microbial cells (inhibition of growth, ability to reproduce), which over time may partially or fully recover with the appearance of favorable conditions, for example, with the introduction of other chemical compounds or removal of the disinfectant.

For disinfection, use only the disinfectants which result in irreversible damage to the metabolism of microbial cells and cause their complete destruction.

There are many products used for disinfection of hatching eggs, most of which contain toxic substances such as chlorine, formalin, ethanol, which quickly lose their antibacterial properties, due to evaporation of the active substance. Disinfectants containing surfactants that do not decompose in the environment are also widely used; therefore, the use of such products causes irreparable harm to environmental safety [1, 2].

Proceeding from the above, the authors believe that the development of new highly efficient, environmentally safe and economically profitable antibacterial preparations for disinfection of domestic hatching eggs is an important way in today's veterinary medicine.

The purpose of the research:

This work was aimed at developing a zinc asparaginate-based disinfectant for disinfection of hatching eggs.

The tasks were the following ones:

1. Development of a zinc asparaginate-based disinfectant and the scheme of using it for hatching eggs treatment.

2. The use of the disinfectant for disinfection of hatching eggs.

Scientific novelty

A new disinfectant has been developed on the basis of zinc asparaginate for processing hatching eggs.

II. MATERIALS AND METHODS

The zinc asparaginate-based disinfectant was studied and developed at the laboratory at the Department of Microbiology, Biotechnology, and Chemistry of the SSAU n.a. N. I. Vavilov, and the Saratovskaya poultry station. For the experiments, the authors used the following literature: the handbook "Microbiology with the Methods of Microbiological Research" [3] and the original method



of G. A. Kutuzova [4]. The zinc asparaginate-based disinfectant for disinfection of hatching eggs was prepared in accordance with the Federal Law dated 12.04.2010 No. 61-FZ (as amended and supplemented) [5] "On the circulation of medications", regulatory document dated October 17, 1997 No. 13-5-2/1062 "Veterinary products [6]. Quality Indicators. Requirements and standards", directive dated June 2, 2010, P4.2.2643-10 "Methods of laboratory research and testing of disinfectants for assessing their efficacy and safety" [7] as well as using the guidance on proof-of-concept (preclinical) study of new pharmacological substances [8]. To prepare a disinfectant solution based on zinc asparaginate, the following studies were performed:

- Determination of the solubility of aspartic acid-based trace element according to ND No. 13-5-2/1062 "Veterinary medicines. Quality Indicators. Requirements and norms" [6].
- Determination of the shelf life of the aspartic acid-based micro-element in the form of a solution (GF XIII 2015, OFS.1.1.0009.15) [9].

To determine the solubility of trace elements, a weighed amount of 1 g of the substance was prepared, which was then dissolved in distilled water. The composition was stirred until complete dissolution and obtaining a transparent solution.

To determine the shelf life, the stability of the solution was tested by the method of "accelerated aging". The method consists in testing at elevated temperatures. "Accelerated aging" consists in exposing the tested product to elevated temperatures and humidity.

The quality of the medication in the process of "accelerated aging" is determined at time intervals of 6 months of storage under storage conditions specified in the normative documents.

The disinfectant solution was prepared in accordance with the indicators of zinc asparaginate microbiological activity, and with regard to the embryotoxic action of the substance.

The research with the use of the disinfectant for treatment of hatching eggs in the proof-of-concept experiment was performed at the permanent study area No. 3 of the Saratov State Agricultural University n.a. N. I. Vavilov in accordance with the incubation handbook [10]. Three automatic hatchers BL-2 Nesushka with a capacity of 104 eggs were used for the experiment. Hatching eggs of laying hens of the Kuchinsky Anniversary breed were used in the experiments.

Disinfection of hatching eggs with a 3 % solution of zinc asparaginate was tested in 3 repetitions. Each hatcher was loaded with 104 eggs. The number of the hatcher corresponded to the number of the experimental groups of eggs. The hatching modes were identical for all groups. In the reference batch, hatching eggs before loading were disinfected with formaldehyde vapors using the conventional method. The eggs in the experimental groups were treated with a 3 % solution of zinc asparaginate in the dosage of 0.2 – 0.3 ml per egg. Disinfection was performed according to the following scheme: the eggs in the first reference group were disinfected by formaldehyde vapors according to the conventional method – before loading, 6 hours after loading, on the 6th, 12th, and 18th day of hatching; the eggs in the

second experimental group were treated immediately after loading into the incubator, then on the 6th, 12th, and 18th day of hatching; sanitation of the eggs in the third experimental group was performed immediately after loading them into the hatcher with the follow-up processing performed on day 12 of hatching.

For monitoring bacterization of the eggshells, wash-offs were taken. The bacterization was studied in accordance with GOST 31659-2012 [11], GOST 31747-2012 [12] for the presence of bacteria of genus Salmonella, and bacteria of a group of coliforms with the use of simple and differential diagnostic nutrient media on the 6th, 12th, and 18th day of hatching, before each treatment.

Development of the embryos was studied throughout the hatching process. The hatching rate was determined with an indication of the reasons for their death. The preservation rate of hatched out chickens was studied in the first 10 days of growing, and the change in the live weight of the chickens was considered at the age of 1 day and 7 days.

III. RESULTS AND DISCUSSION

Zinc asparaginate is included in the composition of the organic mineral complex (OMC) developed by the innovative technologies of JSC Bioamid, Saratov. Its physicochemical properties are shown in Table 1.

Table 1. Indicators of zinc asparaginate quality

Indicator name	Characteristic and norm
Appearance and color	White powder
Odor	Odorless
Mass fraction of sodium sulfate, %	Not more than 30.0 and not less than 25.0
Mass fraction of zinc, %	Not more than 13.8 and not less than 11.8
Mass fraction of moisture (105), %	Not more than 6.0
Solubility	Soluble in water

Zinc asparaginate is a white odorless powder that is soluble in water, with the zinc mass fraction of not more than 13.8% and not less than 11.8%.

A. Bactericidal and fungicidal indicators of zinc asparaginate

The bactericidal properties of zinc asparaginate were studied in accordance with the "Methodical instructions about the procedure of testing new disinfectants in veterinary practice" [13], and the methodical textbook "Microbiology with the Methods of Microbiological Research" [3] at the Department of Microbiology, Virology and Biotechnology of the Saratov State Agricultural University n.a. N. I. Vavilov.

The antibacterial and antifungal activity of the new organic substance — zinc asparaginate — has been determined. For this study, the authors used 9 strains of microorganisms:



Staphylococcus aureus, Escherichia coli, Bacillus cereus, Serratia mercenscens, Diplococcus septicus, Klebsiella pneumonia, Salmonella typhimurium, Candida albicans, and Aspergillus niger. The choice of museum strains of bacteria and fungal cultures is due to the fact that they all belong to conditionally pathogenic ones, and may cause purulent-septic diseases. The influence of zinc asparaginate on pure cultures of microorganisms was studied using 2 methods: by placing a zinc asparaginate solution in pits in the meat-extract broth, followed by seeding on dense media. The action of the substance on the strains of bacteria and fungal cultures was considered in 3 different concentrations of 77, 15.4, and 7.7 mg/ml, as well as the delayed growth of microorganisms under the influence of the zinc asparaginate solution. The experiment was repeated 3 times.

The studies have shown that zinc asparaginate can delay the growth of conditionally pathogenic microflora in both the maximum and the minimum concentrations. The substance has bactericidal activity against strains of microorganisms S. aureus, E. coli, S. mercenscens, D. septicus, K. pneumonia at the concentrations of the solution of 77 and 15.4 mg/ml; zinc asparaginate has fungicidal action against C. albicans, A. niger at the concentrations of the solution of 77, 15.4, and 7.7 mg/ml. At high concentrations, solutions of the substance can maintain bactericidal and fungicidal properties for up to 72 h [14].

B. Obtaining and preparing the zinc asparaginate-based disinfectant, its physical and chemical properties

The disinfectant solution was prepared in accordance with the indicators of zinc asparaginate microbiological activity, and with regard to the embryotoxic action of the substance. The physicochemical properties are shown in Table 2.

Table 2. Physicochemical properties of the developed disinfectant

Name of the preparation	Appearance, color	Degree of solubility
Solutions of zinc with aspartic acid	A clear solution	Easily soluble in water

During the experiment, the organoleptic characteristics and stability of disinfectant were studied at the temperature of 30°C; the experiment lasted for 47 days. After the experiment, it has been noted that the appearance and color of the solution did not change, all solutions remained transparent. The preparation withstood testing of the time of experimental storage by the method of "accelerated aging" for 47 days, i.e. the expiry term for the medications in the form of a solution is 2 years.

The disinfectant was obtained and prepared as follows: to prepare the solution, a weighed amount of zinc asparaginate in the amount of 23.1 g was taken, which corresponded to 3 g of zinc, which was the active substance. The preparation was dissolved in 1 liter of distilled water. Thus, a 3 % solution of zinc asparaginate was obtained. The recommended dosage for treating hatching eggs is 0.2 ml of the solution, which corresponds to 0.6 mg of zinc per egg.

C. The use of the disinfectant for disinfection of hatching eggs in the proof-of-concept experiment.

416 hatching eggs of laying hens of the Kuchinsky Anniversary breed were used in the experiments. The eggs were divided into 4 groups: 1 — reference group (the eggs of this group were not treated); 2 — the group of eggs treated with a solution of formaldehyde, 3 and 4 — the groups of eggs were treated with a 3 % solution of zinc asparaginate with a different number of repetitions. The scheme of the experiment is shown in the "Materials and methods" section.

During the period of incubation, the research was performed for detecting the growth of bacterial cultures. The method of determining the presence of the bacteria of the genus Salmonella consists in the fact that they can be present in the material in small quantities together with an overwhelming number of other bacteria or other families. It is, therefore, necessary to perform preliminary enrichment of the material for detecting the small number of bacteria of genus Salmonella, or sublethally damaged bacteria of genus Salmonella.

For this purpose, a wash-off was introduced into the buffered peptone water in the amount of 10 cm³ and incubated at the temperature of (37 ± 1) °C for (18 + 2) hours. Then the RVS broth and one of 2 media: Muller-Kaufman tetrathionate broth (MKT broth) were inoculated by the culture obtained from the buffered peptone water. After seeding, the RVS broth was incubated at (41.5 ± 1.0) °C for (24 + 3) h, and the MKT broth — at (37 ± 1) °C for (24 + 3) h. The obtained cultures were resown on agar-agar selective medium: xylose-lysine-desoxycholate agar (XLD agar), and again subjected to incubation at (37 + 1) °C for (24 ± 3) h. The colonies probably related to bacteria of genus Salmonella obtained in cups with XLD agar were identified using the biochemical tests.

For the identification, from each cup (2 medium-sized cups or 1 large-sized cup) with each selective medium, one typical or not quite typical colony was taken first, and then 4 colonies were taken if the first one turned out to be negative. The selected colonies were transferred onto the surface of dried meat-extract agar in Petri dishes, and the dishes with inoculations were incubated at (37 ± 1) °C for (24 + 3) h. The colonies selected for biochemical identification were used for preparing smears to be Gram stained and microscopied.

The method of detecting coagulase-positive staphylococci and Staphylococcus aureus is based on sowing dilutions of weighed amounts of the material in a liquid selective medium, followed by resowing the culture broth on the surface of agar selective and diagnostic medium, and biochemical interpretation of the results.

To detect other bacterial cultures, the authors used generally adopted nutrient media — beef-extract broth and Saburo. Wash-offs from hatching eggs were introduced into test tubes with beef-extract broth and Saburo in the amount of 0.1 ml. The media were incubated in a thermostat at the optimal temperature of (37 ± 1) °C for (24 + 3) h.



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After that, inoculations were made from the resulting media onto respective MPA and Saburo agars. Petri dishes with inoculations were placed on cultivation at a temperature

of $(37 + 1) ^\circ\text{C}$ during $(24 + 3)$ h. The results of the tests are shown in Table 3.

Table 3. Presence of bacterial cultures' growth, depending on the disinfectant and its multiplicity

Duration of incubation	Bacteria	Group 1 (reference)	Group 2 (processed with formalin)	Group 3 (multiplicity 2)	Group 4 (multiplicity 3)
Day 1	Bacteria of genus Salmonella	+	+	+	+
	Other bacterial cells	+	+	+	+
Day 6	Bacteria of genus Salmonella	+	-	-	-
	Other bacterial cells	+	-	-	-
Day 12	Bacteria of genus Salmonella	+	-	-	-
	Other bacterial cells	+	-	+	-
Day 18	Bacteria of genus Salmonella	+	-	-	-
	Other bacterial cells	+	-	-	-

Table 3 shows that bacterial infection of eggs was detected before the first disinfection. After that, cleanliness of wash-offs from the processed eggs was maximum on days 6, 12 and 18 of hatching, except for the eggs processed twice, as single colonies of the bacterial cultures had formed in the inoculations. From the data obtained it follows that zinc

asparaginate can inhibit bacterial growth for 6 days, which has an advantage over formaldehyde.

The effect of zinc asparaginate on chickens' hatchability was considered at the end of the incubation period. The results are shown in Table 4.

Table 4. Chickens' hatchability depending on the method of disinfection

Groups	Total eggs loaded	Unfertilized, pcs	Addle eggs, pcs.	Blood rings, pcs	Yield, chickens
Group 1 Reference	104	16	32	12	44
Group 2 Reference (processed with formalin)	104	15	12	9	68
Group 3 experimental (multiplicity 2)	104	21	11	8	64
Group 4 experimental (multiplicity 3)	104	14	14	7	69

The chickens of the Kuchinskaya anniversary breed hatched on day 20 – 21. The yield of young stock in the reference group was 45.8 % of the total number of eggs, and 50 % of the fertilized eggs. The eggs treated with vapors of formaldehyde showed the best hatchability, compared to the reference, which was 65.4 % of the total number of the eggs loaded into the hatcher, and 76.4 % of the fertilized eggs. Hatchability of chickens in the third group was 61.5 % of the total number of eggs, 77.1 % of the fertilized eggs, which was 0.7 % more than in the eggs treated with formaldehyde. Disinfection of the eggs with a solution of zinc asparaginate with 3 repetitions contributed to the hatching of 66.3 % of the total number of the eggs, and for the fertilized eggs, this indicator was 76.7 %. From these data, it follows that the hatchability of the eggs in experimental groups 3 – 4 differed from the hatchability in the reference group by 26.7 – 27.1 %.

Based on the above, the solution of zinc asparaginate should be used for the disinfection of eggs by the method of atomized irrigation in 3 repetitions to avoid recontamination of the eggs by the pathogenic microflora.

IV. RESULTS APPROVAL

The production approval was performed at the Saratov city poultry hatching station. A domestic industrial hatcher IV-18-STI with the capacity of over 16,000 eggs was used for hatching with the temperatures of 36 – 39 °C.

16,128 eggs of cross Cobb-500 broilers received from the Krasnokutsk poultry factory were used in the experiment. The hatching eggs were



divided into 2 groups – experimental and reference, 8,064 eggs in each group.

The experimental batch of hatching eggs was treated with a 3 % solution of zinc asparaginate on the trays before loading them into the hatcher using the method of atomization irrigation of the eggshells. Then the trays were

placed into the hatcher preheated to 37 °C. Secondary disinfection was performed directly in the hatcher using a cold fog generator on day 6 and 12 of hatching. The eggs in the reference group were disinfected with a 37 % aqueous solution of formaldehyde.

Table 5. Hatchability of eggs of the COBB-500 cross on days 20 – 21 of hatching

Experimental solution	Total eggs loaded, pcs.	Unfertilized, %	Addle eggs, %	The yield of conditioned broilers, %
37% aqueous formaldehyde solution	8,064	1,105 (13.7%)	873 (10.8%)	6,086 (75.5%)
3 % solution of zinc asparaginate	8,064	928 (11.5%)	601 (7.4%)	6,535 (81.1%)

Thus, from Table 5 it follows that hatchability of the conditioned broilers of the Cobb-500 cross in the experimental group (treatment with a 3 % solution of zinc asparaginate) was by 5.6 % higher, compared to the reference group (treatment with a 37 % aqueous solution of formaldehyde).

V. CONCLUSION

Currently, as a result of the current epizootic and epidemic situations, veterinary specialists, like never before, consider urgent the problem of preventing and removing infectious diseases of animals and birds, including the anthroozoonotic ones. Therefore, in the modern production environments, the veterinary-sanitary and disinfection measures are becoming increasingly important [15].

As noted by scientists from the field of sanitation, disinfection takes a special place in the system of measures that ensure the welfare of animals and birds, increase their productivity, sanitary qualities of the products, as well as raw materials of animal origin [16–19].

Currently, there is a wide range of disinfectants in Russia for disinfection of the objects of veterinary supervision. However, creating new efficient and environmentally friendly disinfectants is one of the most important tasks of the veterinary science and practice [20].

Analysis of the literature sources in the recent years shows that in foreign countries, as well as in Russia, agents based on aldehydes, surfactants, quaternary ammonium compounds (QAS), and electrochemical activated sodium chloride solutions, highly multiple foams, UV radiation, etc. are used to disinfect veterinary objects [17], [21], [22]. In addition, many authors prefer composite products containing several active substances. Due to the synergy of the active ingredients in these preparations, their antimicrobial and virucidal activity increases [18], [23], [24]. Analyzing provision of efficient disinfectants for the veterinary medicine, taking into account the modern requirements to them, the authors came to the conclusion that there was a need to continue developing new competitive products that would help replenish the range of disinfectants for the veterinary purpose.

According to the research, zinc asparaginate has

antibacterial action against strains of *Staphylococcus aureus*, *Escherichia coli*, *Serratia mercrescens*, *Diplococcus septicus*, *Klebsiella pneumonia* and *Salmonella typhimurium* at the concentrations of the solution equal to 77 mg/ml and 15.4 mg/ml.

The concentrations of the substance solutions of 77 mg/ml and 15.4 mg/ml can maintain bactericidal and fungicidal properties for up to 72 hours.

It has been found that zinc asparaginate affects the *Candida albicans*, *Aspergillus niger* fungi in the concentrations of the solution of 77 mg/ml, 15.4 mg/ml, and 7.7 mg/ml.

The use of a zinc asparaginate solution for treatment of the hatching eggs contributed to improving chickens' preservation rate, hatchability, and viability. The preparation is recommended for use in the form of an aqueous 3 % solution (23.1 g/l) 3 times with an interval of 6 days by the method of atomized irrigation in the dosages of 0.2 – 0.3 ml per egg.

Monitoring bacterization of the hatching eggs showed that in the treated eggs, the cleanliness of wash-offs was maximum on days 6, 12, and 18 of hatching, except for the eggs processed twice, as single colonies of the bacterial cultures had formed in the inoculations. From the data obtained it follows that zinc asparaginate can inhibit bacterial growth for 6 days, which has an advantage over formaldehyde.

The production tests showed that hatchability of broiler chickens of the Cobb-500 cross disinfected with the zinc asparaginate solution was 81.1 % of the total number of the eggs loaded into the group, and 91.57 % of the number of the fertilized eggs. Disinfection with a 37% formaldehyde solution provided hatchability of 75.5 % of the total number of eggs, which was 87.45 % of the number of the fertilized eggs.

With the average price of the Cobb-500 cross broiler chicken equal to 70 rubles per chicken, the cost-efficiency of using zinc asparaginate is 31,430 rubles, compared to the reference.

1. A new disinfecting preparation has been developed that is based on zinc asparaginate and recommended for disinfecting hatching eggs in the concentration of 3 %. The recommended dosage for treating hatching eggs is 0.2 ml of the solution, which corresponds to 0.6 mg of zinc per egg.

2. Hatchability of conditioned broiler chickens of the "Cobb-500" cross after disinfection with a zinc asparaginate solution is 5.6 % higher, compared to the number of young chickens hatched from the group that used 37 % aqueous solution of formaldehyde.

3. The economic efficiency of the preparation based on zinc asparaginate is 31,430 rubles with the average cost of a chicken equal to 70 rubles.

In the conditions of industrial poultry farming, the use of the new zinc asparaginate-based disinfectant is recommended for treating hatching eggs in the concentration of 3 %. The recommended dosage for treating hatching eggs is 0.2 ml of the solution, which corresponds to 0.6 mg of zinc per egg.

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