Effect of disinfectants on hatchability and safety of ducklings

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Abstract. High contamination of incubation eggs of waterfowl and, as a consequence, penetration of microorganisms through the shell leads to embryonic mortality, poults weakness, high mortality in the first day of life, so it is important to sanitize eggs immediately after their collection. This article presents the results of using 1.6% hydrogen peroxide solution as a disinfectant for treating incubation duck eggs. The results of microbiological research of washes from the surface of incubation duck eggs, setter air, otoscopy, hatchability and safety of day-old growing are also reflected. During the study, it was found that pre-hatching treatment of duck eggs with 1.6% hydrogen peroxide solution reduced the degree of contamination on the shell surface, hatchability of eggs in the experimental group was higher by 3.1% than in the control group, safety - by 3.6%, respectively. Keywords: Duck eggs, contamination, hydrogen peroxide, incubation, conservation, hatchability, disinfectant.

1 Introduction

Poultry farming is the most intensive branch of agriculture. It produces a wide range of products, but the main ones are hatching and food eggs and poultry meat. In the meat market in the Russian Federation, poultry meat takes 48%, and in the world, Russia ranks 4th in egg production and 6th in poultry meat production [1; 10].

In the Russian Federation, meat production by poultry species can be described as follows: broilers - 91.4%, technological cull of chicken egg crosses - 3%, turkey - 5%, ducks - 1% and geese - 0.4% (2017); broilers - 90.2%, technological cull of chicken egg crosses - 4, turkey - 4, ducks - 1.3% and geese - 0.5% (2016) [1; 10].

Hatching eggs are the foundation of poultry production, the success of further production of any product depends on their quality. Quality requirements for hatching eggs are regulated by standards and include a number of indicators that affect the hatchability and safety of young animals.

The quality of hatching eggs is influenced by a wide range of factors, such as the origin of the parent flock, egg production technology, health status and age of egg-laying hens and others [2; 14; 15; 28].

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The cause of embryonic mortality, the birth of weak chicks and their low growth energy, as well as unsatisfactory preservation is most often caused by foreign microorganisms penetrating through the shell of incubated eggs.

A freshly laid egg, if properly obtained, is reliably protected against the ingress of microorganisms, and the shell and under-shell serve as a barrier to undesirable microflora [8; 13; 20; 28].

In waterfowl, egg shells are covered with a layer of fat, and eggs are larger and dirtier. This increases the likelihood of infections on the shell surface. Fat and moisture create a breeding ground for harmful bacteria, so the fresher the duck egg, the more likely it is to produce healthy offspring [9].

The egg can be infected endogenously and exogenously. Endogenous infection occurs during egg formation in the ovary or oviduct of a sick bird; thus, microorganisms penetrate the egg and infection with various pathogens occurs. These pathogens include viruses, bacteria, fungi, pathogens of salmonellosis, tuberculosis. Such an egg is one of the main causes of viral infections and is dangerous not only for poultry, but also for humans [13].

Exogenous contamination is associated with the contamination of the shell with manure, feathers, bedding, poor hygienic condition of nests, as well as contaminated containers for eggs [8; 22].

Temperature, especially its fluctuations, and high humidity increase the rate of penetration of microorganisms into the egg. The older the bird, the more infected eggs - up to 37.5%. This is associated with a decrease in the shell thickness, hence an increase in its permeability [4].

Investigations have established that the egg surface contains from 1 thousand to 25 million bacteria, which are represented by salmonellae, Escherichia coli, Staphylococcus aureus, fungi and spore bacteria [5-7; 16-18].

Exposure to microorganisms contributes to spoilage of eggs, which in turn leads to large economic losses [23; 26].

Hatching house is the most vulnerable place in the poultry farm, it creates favorable conditions not only for hatching chicks, but also for reproduction of pathogenic microflora that can survive the entire period of incubation, penetrate through the shell and cause infection of embryos and their death, thereby reducing the hatchability of eggs [5].

To avoid this, it is recommended to disinfect the eggs before incubation or at least to wash them, even this treatment significantly reduces the contamination of eggs and increases the hatchability. Studies by K. Cantu et al. (2018), found that the contamination of untreated duck eggs was 5.82 log10 CFU and water-washed eggs was 2.27 log10 CFU [21].

Disinfectants must be safe for humans and environment, destroy pathogens, and not adversely affect embryos [3; 5; 11; 12]. Ease of use and cost are also important.

2 Purpose of the study

To determine the effect of hydrogen peroxide (H2O2) disinfectant on the hatchability and safety of ducklings. The objectives were:

1. to determine the contamination degree of duck hatching eggs;
2. to determine the hatchability of ducklings;
3. to determine the safety of ducklings.

3 Material and research methods

There is a large number of studies on the effect of various disinfectants on the incubation quality of chicken eggs. Waterfowl eggs, including duck eggs, have a much higher concentration of microorganisms, which means that the use of disinfectants during incubation is not less relevant than in the incubation of chicken eggs.

The shells of duck eggs are highly porous, so their treatment should be carried out no longer than 30 minutes. In this way the detergents will not penetrate inside. Subsequently, it is desirable to dry without wiping the shell to preserve the natural coating [9].

To find out the expediency of disinfectant application during incubation of duck eggs, the experiment on influence of pre-hatching treatment of duck eggs with hydrogen peroxide (H_2O_2) on hatchability and safety of young animals was carried out. For the experiment, 130 duck eggs were selected and divided into two groups of 65 eggs each by random sampling; the first group was a control, and the second one was an experimental group. Fifteen eggs from each group were selected for microbiological studies, all other eggs (50 eggs) were incubated. Hatching eggs of the Bashkirian parent flock of ducks used in the experiment were in full compliance with the requirements for hatching eggs.

Eggs of the first (control) group were washed with clean water at +34 ... +36 0C before incubation. Eggs of the second (experimental) group were treated before incubation with 1.6% hydrogen peroxide solution by dipping for 25-30 seconds before laying in the incubator.

Hatchery duck eggs of the control and experimental groups were subjected to sanitary and microbiological control, while monitoring of compliance with technological and sanitary regimes of incubation, sedimentation method. Sanitary and microbiological examination of the incubation eggs consisted in the simultaneous study of microflora on the shell surface and the study of microbiological pattern of air in the incubation cabinets.

Hatching duck eggs from different laying sites were selected for analysis by random sampling of 30 eggs, 15 eggs from each group. The selected samples were packed in clean containers, excluding their damage and secondary contamination (infection).

For microbiological examination of the shell surface of the hatching eggs, washes were made by rinsing, for this purpose, 10 ml of sterile liquid (saline) was poured into a sterile dish, in which the hatching eggs of the experimental group were immersed and the mixture was shaken for 5 minutes. The resulting wash was examined.

The total bacterial contamination of the egg surface (i.e. the amount of MAFAM (mesophilic aerobic and facultative anaerobic microorganisms) was determined by conventional methods by seeding 1 ml of the flush into two Petri dishes, which were filled with 15 ml of melted and cooled to 500C meat-and-peptone agar, cultured at 300C in a thermostat for 48-72 h.

All the colonies that grew in the depth and on the surface of dense nutrient medium were counted, the arithmetic mean number of colonies was determined for two cups of the same dilution, multiplied by the dilution value and divided by the surface area of the eggshell. The result was the number of microorganisms (CFU/cm^2) for 1 cm^2 of an eggshell.

Mathematically, the surface of the egg was calculated using the formula:

QMAFAM = n 10m/S, CFU/cm^2 (1),

where n is the arithmetic mean number of the colonies grown on Petri dishes; m is the number of tenfold dilutions; S is the surface area of the eggs (cm2), determined by the formula:

S = 3.14 BP/2 (2),

where B is the width of the egg - 4.6 cm; P is the length of the circle equal to 6.05 cm.

To study the sanitary condition of the hatching house, the sedimentation method (Koch's method) was used; it is usually used to determine the composition of microflora in closed rooms (in this case in the hatching house). Petri dishes with the medium (in the quantity of two) were placed in different places of the incubator (exposure time 10-30 min).

Then the Petri dishes were incubated (in the thermostat at +300C for 72 hours). After incubation, all colonies grown in the depth and on the surface of dense nutrient medium were counted, and the arithmetic mean number of colonies in two dishes was determined. Total microbial count (TMC) was determined. The grown colonies were examined according to general criteria, evaluating their structures (size, shape, coloration, relief, surface).

After examining the structure of the colonies grown on all Petri dishes, the tinctorial properties (ratio of microorganisms to dyes) were examined. For this purpose, Gram's method, a method of staining microorganisms that allows differentiating bacteria by biochemical properties of their cell wall (microscopic analysis), was used.

Differential diagnostic nutrient medium bismuth-sulfite agar (E. coli, Proteus, Sallmonela and yolk-salt agar to detect Staphylococcus aureus) was used to determine the species belonging of microorganisms.

Hatchability and safety of young birds were calculated according to the conventional method.

4 Results

Hydrogen peroxide is the simplest representative of peroxides, an unstable compound that can be easily decomposed. Photolysis of the peroxide bond in H_2O_2 yields hydroxyl radicals, the unpaired electron of which interacts with vital cellular components, such as lipids, proteins, DNA, and carbohydrates, and ultimately causes cell death [24; 25; 27].

The results of microbiological examination of washes from the surface of incubation duck eggs of the control and experimental groups and the QMAFAM index of the air in the incubator are presented in Tables 1, 2.

Table 1. Indicators of microbiological examination of washes from the surface of hatching eggs.

Indicator name	Groups	
	Reference	Experimental
Microscopic analysis	Multiple microbial bodies are observed ≥ 6.0 x10 G (-)	Single number of microbial bodies in the view field ≤ 3.0 x10 G (-)
Colonies E. coli	Growth on bismuth-sulfite agar medium was found to be multiple round, greenish-brown colonies; Medium-sized, moist, shiny, transparent, round, convex-edged convex colonies on MPA	Growth on bismuth-sulfite agar medium was detected as single round, greenish-brown colonies (4); On MPA, single medium-sized, moist, shiny, transparent, round, convex-edged colonies (2)
Colonies Staphylococcus aureus	Not detected	Not detected
Colonies Proteus	The bismuth-sulfite agar medium was found to have a	Not detected

	dirty brown color, dark brown under the colonies; On MPA medium - vialeoboise plaque	
Colonies Sallmonela	Not detected	Not detected
QMAFAM, CFU/cm^3 (not more than 1 x 105)	1.5 x 105 Moderate insemination	3.8 x 103 Low level of insemination

Table 1 shows that the surface contamination of the control and experimental duck eggs is different, so microscopic analysis of wipes showed the presence of multiple microbial cells in the control sample and single cells in the experimental sample. Bacteriological analysis showed growth of multiple E. coli and Proteus colonies in the control sample on bismuth-sulfite agar medium. In the experimental sample a single growth on bismuth sulfite agar medium of E. Coli colonies, Proteus colonies were not detected. The QMAFAM value characterizes moderate infestation in the control samples and weak infestation in the experimental sample. The growth of Staphylococcus aureus colonies and Sallmonela colonies was not observed in both groups. Based on the above-mentioned, we can say that the use of preincubation treatment of duck eggs with 1.6% hydrogen peroxide solution significantly reduces the contamination degree on the shell surface, which in turn will contribute to a greater hatching of ducklings.

The results obtained differ from the data obtained by K. Cantu et al. (2018), who studied the effects of H_2O_2/UV AOP treatment of hatching duck eggs. His study showed that there was no statistically significant difference in surface contamination of duck eggs washed with water and treated with H_2O_2/UV AOP, the microbial load of egg surface was 2.27 and 2.31 log10 CFU/egg, respectively [21].

R. C. Baker et al. found that the contamination of the shells of duck eggs washed with water was no more than 9 x 101, while the microbial load on the surface of dirty eggs was more than 9 x 106 [19].

Table 2 shows the results of microbiological examination of the setter air.

Table 2. Indicators of microbiological examination of setter air.

Indicator name	**Setter**
Microscopic analysis	Single number of microbial bodies in the field of view ≤ 3.0 x10 G (-)
Colonies E. coli	Growth on bismuth-sulfite agar medium was found - single round, greenish-brown colonies (2); Single medium-sized, moist, shiny, transparent, round, convex-edged convex colonies on MPA (2)
Colonies Staphylococcus aureus	Not detected
Colonies Proteus	Not detected
Colonies Sallmonela	Not detected
QMAFAM, CFU/cm^3 (not more than 1 x 105)	1.5 x 105 Moderate infestation

The study of the hatching house sanitary condition revealed the presence of single microbial cells in the field of view, a single colony growth of E. Coli on differential diagnostic nutrient media, QMAFAM indicator value characterizes a moderate degree of infestation (Table 2). Based on the data obtained, we can say that the sanitary condition of

the hatching house may be one of the factors of additional contamination of the incubated eggs surface.

During the critical periods of incubation, embryonic mortality is the highest; contamination of egg surface may be one of its causes. The results of duck eggs' candling are in Table 3.

Table 3. Candling results of duck eggs.

Group	Set up for incubation, pcs.	Infertile eggs		Blood ring		Dead-in-shell		Addled egg	
		pcs.	%	pcs.	%	pcs.	%	pcs.	%
Reference	50	11	22.0	-	-	9	18.0	2	4.0
Experimental	50	10	20.0	-	-	7	14.0	2	4.0

The results of candling showed that preincubation treatment of duck eggs with 1.6% hydrogen peroxide solution had a positive effect on the results of incubation, the dead-in-shell embryos in the reference group were 4% more than in the experimental group. Infertile eggs in the reference group were also more than in the experimental group, but this fact is associated not with the processing of duck eggs before incubation, but with the reproductive qualities of the parent flock of ducks.

The studies of O. Yezhova, A. Senko, K. Cantu confirm that the use of disinfectants positively affects the results of incubation. Thus, when using the drug Monoclavit-1, the number of eggs with blood ring decreased by 1.7%, addled eggs by 3.75%, dead-in-shell by 2.5% [5].

Application of the complex treatment of duck eggs with H2O2/UV AOP reduced the embryonic mortality by 11.12% compared with untreated eggs and by 7.31% compared with water-washed eggs. At the same time, the number of addled eggs in the experimental group was lower by 8.07% and 4.14%, respectively ($P < 0.05$) [21], which is consistent with the data obtained in our study.

The hatchability of the young birds is presented in Table 4.

Table 4. Indicators of hatchability of eggs and hatchability of young stock.

Group	Hatching the youngsters		Egg hatchability, %
	birds	%	
Reference	30	60.0	76.9
Experimental	32	64.0	80.0

Table 4 shows that in the experimental group, the hatch of young birds was 2 heads or 4% higher than in the reference group, and the hatchability was higher by 3.1%. These results are slightly lower than those obtained by K. Cantu [21]. It can be concluded that the use of hydrogen peroxide as a disinfectant increases the hatchability of duck eggs.

Pre-incubation treatment of duck eggs with 1.6% hydrogen peroxide solution had a positive effect on the quality and safety of the young birds (Table 5).

Table 5. Preservation of day-old young stock.

Indicators	Group	
	reference	experimental
Ducklings hatched, birds.	30	32
Number of conditioned young animals, birds	28	31
Number of substandard young animals, birds	2	1

including weak young animals, birds	2	-
cripples, birds	-	1
Safety at one day of age, %	93.3	96.9
Mortality, birds	2	1

The experimental group obtained conditioned young birds by 3 ducks more than the reference group. The safety of ducklings at one day of age in the experimental group was higher by 3.6%, and there were no weak young animals in the experimental group, which indicates that the use of hydrogen peroxide as a disinfectant in the treatment of duck eggs before incubation has a positive effect on the safety and quality of the young birds obtained.

5 Conclusions

The results of the research allowed us to draw the following conclusions:

1. Preincubation treatment of duck eggs with 1.6% hydrogen peroxide solution reduces the contamination degree on the shell surface by several times compared to washed eggs. The sanitary condition of the hatching house may be one of the factors of additional contamination of the incubated eggs surface.

2. The hatchability of eggs in the experimental group was higher by 3.1% compared to the control group. The number of dead embryos was 4% less and the number of stiffened embryos was the same.

3. The use of hydrogen peroxide as a disinfectant improved the quality of the young and increased the safety at one day of age by 3.6% compared to that of washed eggs.

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