KnE Life Sciences

AgroSMART 2019
International scientific and practical conference
``AgroSMART - Smart solutions for agriculture''
Volume 2019

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Conference Paper

Ecofriendly Method for Treatment of Hatching Eggs

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Abstract

The aim of this research is to study the influence of ozone on the efficiency of incubating quail eggs. The study had been conducted in the period from 2014 to 2018 under the SIE LLC ``EcoDom''. In the course of the experiment the Estonian quail egg were served as an object of research. To carry out the experiment a household ozonizer "Groza" was used. The Eggs of the first ``control'' group were disinfected with formaldehyde vapors according to the standard method -- 35 ml of 37 % formalin solution + 20 ml of tap water + 20 g of potassium permanganate per 1 cubic metre of volume in a special chamber. The other three groups were ozonized. The eggs of the 2nd experimental group were treated for 10 minutes exposure time, the third -- 20 minutes and the fourth -- 30 minutes, the ozone concentration in all experimental groups was the same and was 10 mg/m^3. After treatment, the eggs were placed for brooding. Biochemical parameters of blood serum were determined by the analyzer "Microlab-300". Ozonation of hatching eggs contributes to increase crude protein in the quail's blood serum by 9.37, 9.65 and 8.57 % compared to the control group. Hatching conditional quails was increased by 43.35 % in comparison with the control group.

Keywords: embryo, incubation, *ozonation*, hatchability, quails, egg productivity.

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Received: 25 October 2019
Accepted: 15 November 2019
Published: 25 November 2019

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the AgroSMART 2019 Conference Committee.

1. Introduction

In recent decades, people around the world have become more active in eating quail eggs and meat. This is due to their high taste, nutritional value and undoubted benefits for the human body [1, 2]. At the same time, the production of quail eggs is much cheaper than chicken eggs.

Under the conditions of modern high-quality poultry farming, incubation plays an important role in increasing the production of meat and eggs [3], heavily determining the success of breeding, breeding new crosses, as well as the mass distribution of

highly productive poultry in the industrial zones of the country. The development of the embryo occurs in conditions created by a man in an incubator, where the main environmental factors are temperature, humidity, air composition, the position of eggs and their movement during the incubation process.

At present, scientific developments are being carried out and new technological methods for preparing eggs, incubation modes, methods of biological control have been introduced into production [4--9].

In the 21st century in the world practice, preventive measures are becoming more and more widespread, contributing to the improvement of the sanitary condition of poultry houses, air and feed disinfection, purification of air, feed, products with the use of the systems of filterless cleaning and disinfecting of air, producing ozone [10, 11].

One of the most important ozone properties is its ability to stimulate bird embryonic development. The analysis of the literature shows that there is a necessity to carry out more research on the stimulating effect of ozone. Ozone penetrates through the shell inside the egg and interacts with its contents, particularly, on the protein soluble phase, and directly on the protein molecule [12].

According to chemical nature, ozone is characterized by a very high activity. In 1934 it has been determined that egg albumin is poorly digested by proteolytic enzymes. Ozone tends to denature proteins, and due to this the speed of their digestion increases. Consequently, the protein digestibility for the embryo increases in the process of its development [13].

The use of ozone in the poultry industry increases the production productivity, enhances the resistance of birds to various diseases.

The aim of this research is to study the effect of ozone on the efficiency of quail eggs incubation.

2. Methods and Results

The research had been conducted in the period from 2014 to 2018, in the MIP LLC ``EcoDom'', located in the Republic of North Ossetia (RNO)-Alania in the city of Vladikavkaz. In the course of the experiment, Estonian quails were the objects of the research. To carry out experiments the household ozonizer ``Groza'' was used. It is a plastic frame with a diffusion stone attachment, two silicone tubes of 100--120 cm. Its capacity of the ozonizer is 30 W, with a capacity of 300 mg per hour. The maximum session time should not exceed 30 minutes.

To do research, eggs were selected from 60-days egg-laying birds, corresponding to the existing parameters: the regular shape, with a pronounced sharp and blunt end, without irregularities, roughness and lime scale on the shell. In addition, eggs candling was performed. It helped to established the suitability of eggs for incubation: they examined the position and integrity of the yolk, which should be in the center of the egg. We determined the location of the air chamber, which should be at the blunt end of the egg. We selected those eggs for the experiments that met the specified requirements. The mass of eggs was 13--14 g. The color and pigmentation of the shell corresponded to those typical of the Estonian breed. Before processing and laying on incubation, a morphological analysis of eggs was performed. The quality indicators of hatching eggs, used in the experiment, are presented in the table 1 (table 1).

Table 1: Average morphologic indicators of hatching eggs.

Egg mass, g	Egg volume, sm^3	Shell mass	Shape index	Protein index	Shell thickness, mm	Yolk content, mkg/kg	
						Vitamine A	каротиноидоВ
13,25±1,11	14,24±3,7	1,8±0,1	76,6±3,4	0,07	0,23±0,002	9,5	19,6

According to the table 1, the given morphological parameters of eggs corresponded to the existing standards.

In addition, some basic biochemical parameters were also identified (table 2).

Table 2: Biochemical parameters of quail eggs.

Protein refraction index	Yolk refraction index	White pH, ea.	Yolk pH, ea.	Cholesterol, mmol/l	Triglyceride, mmol/l
1,3581±0,0004	1,3990±0,0297	8,50±0,00	7,00±0,00	12,80±1,40	6,6±1,10

The refraction index or refractive index gives an idea of the optical properties of egg white and yolk, the ratio of water and solids in them. The refractive coefficients of egg white and yolk were within physiological values. The same was with the indices of cholesterol and triglycerides.

Eggs of the first group, selected as a control group, were disinfected with formaldehyde vapors according to the standard method: 35 ml of 37 % formalin solution + 20 ml of tap water + 20 g of potassium carbonate. The other three groups were ozonized. For eggs of the 2nd experimental group, an exposure treatment of 10 minutes was applied, for the third -- 20 minutes and for the fourth -- 30 minutes, the ozone concentration in all experimental groups was the same -- 10 mg/m^3.

After processing, the eggs were placed for incubation to the incubator ``MX--1000 CD'' using the common standard mode. All existing requirements for incubation parameters were followed in the process of incubation on the 5th, 10th and 15th day of incubation, according to the established standards. At the end of the incubation, the number of hatched young was determined from each batch of incubated eggs, and the incubation waste was taken into account.

Blood serum biochemical parameters were determined by the analyzer ``Microlab-300'' using a spectrophotometric method in a flow cell processing of the results with computer.

Egg production was calculated according to the daily records of eggs laid in groups. To assess the quality of eggs, organoleptic and quantitative methods were used to measure the quality parameters of eggs. The thickness of the shell was measured with a micrometer with pointed rods with an accuracy of 1 μm.

The data, obtained in the experiment, were processed by the method of variation statistics using the program Microsoft Excel Office 2007).

3. Results and Discussions

The bird embryo, developing under the hen, has a different electro-mode of air than in the incubator. We suppose that ozone under the hen is formed due to the effect of statistical electricity on the feather of birds. The stronger the air is ionized, the greater the ozone content. Ozone penetrates through the shell inside the egg and interacts with its contents, in particular, on the soluble phase of the protein, and directly on the protein molecule.

By chemical nature, ozone is characterized by a very high activity. In 1934 it was determined that egg albumin is poorly digested by proteolytic enzymes. Ozone tends to denature proteins, and due to this the speed of their digestion increases. Consequently, the digestibility of the protein for the embryo increases in the process of its development.

It is also noted that lysozyme activity decreases up to 40 % on the exposure of ozone, which, apparently, reduces its enzymatic effect on albumen carbohydrates, and this leads to long-term egg preservation.

Ozone improves the absorption of vitamins in the egg by the germ, which often becomes difficult to reach for the embryo. Penetrating into the egg, ozone tends to change the state of the albumen, as it has been indicated earlier. The acidity of the albumen increases from 8.99 to 9.10. The change in the eggs content had a great influence on the metabolic processes of the embryo. Embryos were developed better,

grew faster and their metabolism was intensified, as well as the use of albumen and yolk.

Table 3: Indicators of egg incubation.

Group	Unfertile, pcs	Blood ring, pcs	Addled egg, pcs	Late dead, pcs	Cull chicks, unit	Good chicks, unit
Control -- 1	6,8±0,17	3,9±0,02	3,7±0,40	2,9±0,04	2,9±0,04	79,8±0,21
Experiment -- 2	6,7±0,02	2,1±0,12	2,9±0,08	2,0±0,06	2,0±0,08	84,3±0,42
Experiment -- 3	6,9±0,04	2,0±0,02	2,7±0,21	1,1±0,10	--	87,3±0,12
Experiment -- 4	6,9±0,04	2,1±0,08	2,9±0,04	1,9±0,08	--	86,2±0,17

As a result, under the influence of ozone new decomposition products appear in the egg, which actively influence the embryo. These substances cause irritation of the nerve endings in the blood-vascular system, and in the tissues of the organs, this in turn leads to metabolism changes.

Studies have shown (Table 3) that the incubation waste decreased by the number of blood rings in comparison with the control group: in the second and the fourth groups it decreased by 46.1 %; in the third -- by 48.7 % ($P \leq 0.001$). The number of addled embryos in comparison with the control group, significantly ($P \leq 0.001$) decreased: in groups 2 and 4 -- by 21.6 %; in 3 -- by 27.0 %.

The number of the late deads in the control group was 2.9, which is 31.0 % more than in the second group, 62.1 % in group 3 and 34.5 % in group 4 ($P \leq 0.001$). A comparative analysis of hatchability results of viable, conditional quails showed that, compared to the control group, we received the conditional quails from the number of laid eggs in group 2 by 5.6 %, 3 -- 9.4 % and 4 -- 8.0 % more than in control. There were no cull quails in the 3rd and 4th experimental groups. Their number of cull quails in the 2nd group was 2.0 goals, which is 31.0 % less than in the control group, the differences are significant at $P \leq 0.001$. We also analyzed the dependence of the mass of hatching eggs and 1 day quail, and the effect of ozone on the hatchability.

One of the factors affecting the hatchability of eggs is the incubation period. It is a well-known fact that in the hatchery the embryo can be exposed to the influence of various microorganisms penetrating into the shell. A lot of different drugs are used to fight microorganisms, but most of them do not fight effectively against pathogenic microflora, are unsafe for humans and can have a negative effect on the embryo. Ozone, due to its powerful oxidative action, destroys the microbial cell, in particular, acts on phospholipids and lipoproteins. Viruses are also unstable to ozone, since its effect disrupts the integrity of the protective membrane of the virus. Therefore, it is impossible to attach to the

membranes of a healthy cell. Unlike other antiseptics, ozone does not destroy and does not irritate the tissues of the body, as the cells have antioxidant protection. Moreover, we used ozone in this study as a stimulator for embryonic development to enrich cells of the embryo with oxygen.

Metabolism in the body of poultry is associated with the intensity of the transport functions of circulating blood. The normal course of metabolic processes depends on the morphological and biochemical composition of the birds' blood. The state of the blood is significantly affected by many factors. Thus, under the influence of ozone embryos absorb oxygen more than 40 %, hematopoiesis and erythropoiesis are increased. Based on this, we have studied the blood morphological parameters of a test bird influenced by the different duration of ozonation of hatching eggs. Over the course of the morphological parameters determination, we have established the positive effect of ozone on the quails' blood indicators (Table 4).

Table 4: Changes in blood corpuscles.

Age	Group	Leucocytes 10^9/l	Haemoglobin g/l	Erythrocyte, 10^{12}/l
1 day	Control -- 1	35,44±0,7	102,1±1,2	8,5±0,1
	Experimental -- 2	36,37 ±0,9	92,1±1,6	6,0±0,6
	Experimental -- 3	35,44±0,7	150,8±0,6	9,1±0,1
	Experimental -- 4	37,19±0,5	158,5±0,4	8,8±0,2
7 days	Control -- 1	37,69±0,3	107,1±3,0	7,9±0,4
	Experimental -- 2	37,52±0,3	104,4±4,3	6,9±0,3
	Experimental -- 3	37,89±0,5	128,1±5,7	8.1±0,3
	Experimental -- 4	38,12±0,3	137,5±5,2	8,1±0,3
14 days	Control -- 1	37,85±0,5	105,8±3,0	4,8 ±0,6
	Experimental -- 2	37,58±0,8	124,4±5,5	5,8 ±0,4
	Experimental -- 3	38,30±0,4	142,1±4,5	6,3±0,2
	Experimental -- 4	38,04±0,5	143,4±2,2	7,0±0,2
42 days	Control -- 1	40,53±0,3	90,7±3,1	4,5 ±0,4
	Experimental -- 2	40,20±0,6	131,7±6,1	5,9 ±0,6
	Experimental -- 3	40,75±0,2	138,7±4,0	5,7 ±0,5
	Experimental -- 4	40,48±0,3	137,1±3,0	6,1±0,4
56 days	Control -- 1	40,43±0,6	120,7±3,6	5,3±0,4
	Experimental -- 2	41,28±0,2	135,8±2,8	6,0±0,3
	Experimental -- 3	40,98±0,3	145,1±1,8	6,5±1,4
	Experimental -- 4	41,52±0,1	146,1±0,4	7,8±0,3

A regular increase in erythrocytes has been observed in quails of all experimental groups. As it was mentioned earlier, when ozone affects the increase in absorption of oxygen by the embryo, consequently, the number of erythrocytes in the experimental groups increases significantly. Thus, by the 14-days of age, the number of erythrocytes in quails of the control group was $4{,}8 \cdot 10^{12}$/l, in quails of 2, 3, 4 groups: $5{,}8 \cdot 10^{12}$/l; $6{,}3 \cdot 10^{12}$/l; $7{,}0 \cdot 10^{12}$/l, respectively.

At the age of 56 days, the number of erythrocytes in the 2nd experimental group was $6.0 \cdot 10^{12}$/l; 3rd -- $6.5 \cdot 10^{12}$/l; 4th -- $7.8 \cdot 10^{12}$/l, which exceeded the control by 13.20, 22.64 and 47.16 %, respectively.

The number of leucocytes at the 1 day of age in the control group was -- $35{,}44 \cdot 10^{9}$/l; 2nd -- $36.0^{9} \cdot 10^{9}$/l; 3rd -- $36.37 \cdot 10^{9}$/l. At the age of 42 days there is a steady increase in leucocytes, and it is in the range of $40.75 \cdot 10^{9}$/l -- $40.20 \cdot 10^{9}$/l.

By the end of the experiment at the 56 days of age, the analysis of the number of leucocytes showed that the highest values were observed in quails of the 4th experimental group, $41.52 \cdot 10^{9}$/l, although there were no significant differences. Therefore, it should be noted that ozone has a positive effect on the performance of protective and adaptive reactions.

Analysis of the age dynamics of haemoglobin content has significant differences between the control and experimental groups. So at the age of 1 day, the highest haemoglobin values were observed in groups 3 and 4, with a result of 102.1 g/l and 158.5 g/l, which exceeded the control group by 47.69 and 5.40 % respectively. A significant increase of haemoglobin ($P \leq 0{,}001$) was also observed by the age of 14 days. By the end of the experiment, haemoglobin indices were: control group -- 120.7 g/l, experimental 2 -- 135.8 g/l; experimental 3 -- 145.1 g/l; experimental 4 -- 146.1 g/l. The highest rates were observed in the group 4, which exceeds the control group by 20.21 %, the reliability is $P \leq 0{,}001$.

There were no pathologies in leucogram indices. According to the table 5, all elements are within the physiological norm.

The observed aftereffect on the processes of embryonic development of eggs treated with ozone is expressed in a more intensive growth of the embryo, and also affects the growth of muscle mass in the further life of birds. This is caused by changes in the condition of the protein. The content of protein and its fractions is presented in the table 6.

Thus, in the course of the experiment, the best physiological effect on the protein metabolism of quails was revealed in the experimental groups. Compared with the

DOI 10.18502/kls.v4i14.5651

Table 5: Leucogram.

Age	Group	Basophile	Eosinophil	Neutrophils				Lymphocytes	Monocytes
				Juvenile	Myelocytes	Stab	Segmented		
1 day	Control -- 1	--	1	--	--	2	7	88	2
	Experimental -- 2	2	9	--	--	2	14	69	4
	Experimental -- 3	1	2	--	--	1	4	85	7
	Experimental -- 4	2	2	--	--	3	6	83	4
7 days	Control -- 1	2	1	--	--	4	2	86	5
	Experimental -- 2	1	3	--	--	5	1	86	4
	Experimental -- 3	1	8	--	--	5	2	81	3
	Experimental -- 4	1	3	--	--	4	1	87	4
14 days	Control -- 1	--	--	--	--	4	15	79	2
	Experimental -- 2	--	1	--	--	5	7	84	3
	Experimental -- 3	2	--	--	--	3	6	84	5
	Experimental -- 4	1	2	--	--	2	4	87	2
42 days	Control -- 1	2	1	--	--	1	3	89	4
	Experimental -- 2	1	2	--	--	4	8	77	8
	Experimental -- 3	2	--	--	--	5	10	76	7
	Experimental -- 4	2	3	--	--	3	15	74	3
56 days	Control -- 1	--	2	--	--	5	2	85	6
	Experimental -- 2	2	1	--	--	5	7	81	4
	Experimental -- 3	1	1	--	--	4	3	88	3
	Experimental -- 4	--	--	--	--	3	4	89	4

control group the level of the total protein was higher by 9.37, 9.65 and 8.57 %, respectively.

The content of albumin in the 4th experimental group (P ≤0,001) also significantly increases in experimental groups and it is 32.47 g/l that exceeds the control group by 1.79 times.

Table 6: The content of total protein and its functions in the blood serum of experimental birds.

Index	Group			
	Control -- 1	Experimental -- 2	Experimental -- 3	Experimental -- 4
Total protein, g/l	31,37±0,5	34,31±0,9	34,40±0,7	34,06±0,8
Fractions, % Albumin	30,68±0,7	32,79±0,9	32,75±0,9	32,47±0,01
Globulin:	58,52±3,5	66,02±1,9	68,02±0,6	68,69±1,2
α-globulin	8,13±0,5	9,97±0,5	9,72±0,6	10,0±0,5
γ-globulin	36,01±2,6	40,15±0,9	41,51±1,1	42,28±0,1
β-globulin	14,38±0,3	15,89±0,8	16,72±0,4	16,37±0,4

The results of studying the content of total protein and its fractions in the quails' blood serum under ozonation permit to draw the following conclusions:

1. the concentration of total protein in the blood serum of quails is a sustainable value. However, the indicator ``ozone effect'' increases by 9.37, 9.65 and 8.57 % compared to the control group;

2. the concentration of total protein is positively correlated with the morphological indices of the blood of 1-day quails.

4. Conclusions

The impact of ozone has effectively reflected on the number of embryonic waste by the number of blood rings, addled embryos, late died embryos, cull quails. The output of good (qualified) quails has increased by 43.35 % in comparison with the control group.

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