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Improving The Efficiency Of Quail Eggs Incubation.

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ABSTRACT

The research aims to study the ozone effect on the efficiency of quail eggs incubation. Studies were conducted in the period between 2014 and 2016, in the conditions of small innovative enterprise LLC "EcoDom". During the experiment, the research objects were Estonian quails. The household ozonizer "Groza" was used for the experiment. Eggs of the first group, which served as a control one, were disinfected with formaldehyde vapor according to the generally accepted procedure – 35 ml of the 37% formalin solution + 20 ml of tap water + 20 g of potassium permanganate for 1 m^3 of the special chamber volume. Three remaining groups were exposed to ozonization. The treatment exposure for eggs of the second experimental group was 10 minutes, for the third one – 20 minutes and the fourth — 30 minutes, the ozone concentration in all experimental groups was the same — 10 mg/m^3. After treatment the eggs were laid for incubation. Eggs were incubated in the incubator MX-1000 CD using the usual conventional mode. During the incubation process on the 5th, 10th and 15th day, according to the established standards. Biochemical parameters of blood serum were determined by means of the device "Mikrolab-300". Egg production was calculated according to the data of eggs laid daily by groups. Organoleptic and quantitative methods were used for the quality assessement of eggs. Ozonization of incubating eggs increases the total protein in quails' serum by 9,37%, 9,65% and 8,57% compared to the control. The concentration of the total protein positively correlates with the morphological blood parameters in daily quails. Ozone effectively affected the amount of embryonic waste by the number of blood rings, frozen embryos, addled eggs, substandard quails. The output of standard quails increased by 43,35% compared to the control group. Pre-incubation eggs treatment with ozone helps to increase the subsequent hens' egg production by 5,9-10,4% and improve the eggs quality.

Keywords: embryo, incubation, ozonization, hatchability, quails, egg production.

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INTRODUCTION

Using quail eggs and meat as food in our country has become more active in recent decades. This is due to their high taste quality, nutritional value and undoubted benefits for the human body. Quail eggs exceed the chicken ones in vitamins: A (retinol) – by 2-2,5 times, B_1 (thiamine) — 2,8-3 and B_2 (riboflavin) — 2-2,2 times. Five quail eggs, equal in weight to a chicken one, contain 5 times more potassium and phosphorus, and 4,5 times more iron. Quail eggs are much richer than chicken ones in copper, cobalt, essential amino acids and lysozyme [1, 2, 3, 4, 5]. At the same time, the quail eggs production is much cheaper than that of chicken.

In the conditions of modern highly marketable poultry farming, incubation plays an important role in increasing meat and eggs production [6]. Largely determining the success of breeding, breeding new crosses, as well as the mass distribution of highly productive birds in the industrial zones of the country. The embryo development takes place in conditions provided by a man, in the incubator, where the main environmental factors are temperature, humidity, air composition, the eggs position and their motion during incubation.

Nowadays, scientific developments are carried out and new technological methods of egg preparation, incubation regimes, methods of biological control are introduced into production [7].

Preventive measures to improve the sanitary condition of poultry houses, disinfection of air and feed become more and more widespread in the world practice of the 21st century. Purification of air, forages, and the resulting products using systems of filterless cleaning and air disinfection that produce ozone [8, 9, 10, 11].

One of the most important ozone properties is its ability to stimulate the poultry embryonic development. The literature review suggests more research on the ozone stimulatory effects. Ozone penetrates into the egg through the shell and interacts with its content. In particular, the soluble protein phase and directly the protein molecule [12].

By its chemical nature, ozone differs in very high activity. Since 1934 it has been determined that egg albumin is poorly digested by proteolytic enzymes. Ozone has the property to denature proteins that increases the rate of their digestion. Therefore, it increases the protein accessibility for the fetus during its development [13].

The ozone application in poultry farming tends to increase productivity, the birds' resistance to various diseases.

This research aims to study the effect of ozone on the efficiency of quail eggs incubation.

OBJECTS AND METHODS

The studies were conducted in the period between 2014 and 2016, in the conditions of small innovative enterprise LLC "EcoDom", located in RNO-Alania, the city of Vladikavkaz. During the experiment, the research objects were Estonian quails. The household ozonizer "Groza", which is a plastic frame, with the attached to it diffusion stone nozzle, two 100-120 cm silicone tubes was used for the experiment. Its power is 30 W, capacity - 300 mg/h. The maximum session time must not exceed 30 minutes.

To do research, eggs were collected from laying hens at 60 days old that meet the existing parameters: regular in shape, with distinct sharp and blunt ends, without irregularities, roughness and limescale on the shell. In addition, the eggs speratura was performed. It also helped to determine the eggs suitability for incubation: the position and integrity of the yolk, which should be in the center of the egg, were examined. Also the location of the air chamber, which should be at the blunt end of the egg was determined. Eggs that meet these requirements were selected for the experiments. Eggs weighed 13-14 g. The colour and pigmentation of the shell met the parameters typical for the Estonian breed. Before eggs treatment and laying for incubation morphological analysis was carried out. Quality indicators of hatching eggs used in the experiment are given in table 1.

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Table 1: Average morphological parameters of hatching eggs

Egg weight, g	Egg size, cm^3	Shell weight	Shape index	Protein index	Shell thickness, mm	Yolk contains, mkg/kg	
						vitamin A	carotenoids
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

Table 1 shows that the given morphological parameters of eggs meet the existing standards.

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

Table 2: Biochemical parameters of quail eggs

Protein refractive index	Yolk refractive index	Protein pH, units	Yolk pH, units	Cholesterol, mmol/l	Triglycerides, 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 refractive index gives an idea of the optical properties of protein and yolk, the relationship between water and solids in them. The refractive indices of protein and eggs yolk were within the physiological values. The same is for cholesterol and triglycerides.

Eggs of the first group, which served as a control one, were disinfected with formaldehyde vapor according to the generally accepted procedure – 35 ml of the 37% formalin solution + 20 ml of tap water + 20 g of potassium permanganate for 1 m^3 of the special chamber volume. The remaining three groups were exposed to ozonization. The treatment exposure for eggs of the second experimental group was 10 minutes, for the third one – 20 minutes and the fourth — 30 minutes, the ozone concentration in all experimental groups was the same — 10 mg/m^3.

After treatment the eggs were laid for incubation. Eggs were incubated in the incubator MX-1000 CD using the usual conventional mode. All existing requirements to the incubation parameters were met. During the incubation process on the 5th, 10th and 15th day, according to the established standards. After the end of incubation, the number of young birds incubated from each sitting of hatching eggs was determined and the incubation losses were calculated. Biochemical parameters of blood serum were determined in the running water by means of the device “Mikrolab-300” using spectrophotometric method with the further computer processing of the results.

Egg production was calculated according to the data of eggs laid daily by groups. Organoleptic and quantitative methods that intend to measure eggs quality indicators were used for the quality assessement of eggs. The shell thickness was measured by a micrometer with pointed rods to an accuracy of 1 µm.

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

RESULTS AND DISCUSSION

The bird embryo, developing under a sitting hen has the electric air mode other than in the incubator. The ozone formation under the sitting hen appears to occur due to the action of statistical electricity on the bird’s plumage. The more the air is ionized, the more ozone it contains. Ozone penetrates into the egg through the shell and interacts with its content. In particular, the soluble protein phase and directly the protein molecule.

By its chemical nature, ozone differs in very high activity. Since 1934 it has been determined that egg albumin is poorly digested by proteolytic enzymes. Ozone has the property to denature proteins that increases the rate of their digestion. Therefore, it increases the protein accessibility for the fetus during its development.

It was also noted that the action of ozone reduces the activity of lysozyme, up to 40%, which appears to reduce its enzymatic effect on protein carbohydrates, and this leads to long-term eggs storage.

Ozone improves the ability of the embryo to assimilate vitamins in the egg, which are often difficult of access for the embryo. As mentioned above, ozone, penetrating into the egg, is able to change the protein state.

The protein acidity increases from 8,99 to 9,10. Changes in the egg content had a great impact on the metabolic processes of the embryo. Embryos developed better, growth and metabolism increased, as well as the protein and yolk application.

Table 3: Indicators of eggs incubation

Group	Unfertilized, pcs	Blood rings, pcs	Frozen, pcs	Addledeggs, pcs	Substandard chickens, heads	Standard chickens
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, influenced by ozone new decay products, which actively affect the embryo appear in the egg. These substances cause the nerve endings irritation in the circulatory system, and in its turn, this leads to metabolic changes in the organ tissues.

Studies have shown (table 3) that the incubation losses by the number ofblood rings decreased compared to the control group: in the second and fourth groups by 46,1%; in the third – by 48,7% (P≤0,001). The number of frozen embryos decreased significantly (P≤0,001) compared to the control: in the second and fourth – by 21,6%; in the third group – by 27,0%.

The number of addled eggs in the control group was 2,9 pcs, which is 31,0% more in the second group, 62,1% – in the third group and 34,5% – in the fourth group (P≤0,001). Comparative results analysis of the hatchability of viable, standard quails showed that compared to the control group, the number of laid eggs produced standard quails in the second group by 5,6%, in the third – by 9,4% and in the fourth – by 8,0% more than in the control group. There were no substandard quails in the third and fourth experimental groups. Their number in the second group was 2,0 heads, which is 31,0% less than in the control group, the differences are significant at P≤0,001. We also analyzed the dependence of the weight of hatching eggs and daily quails, and ozone effect on the hatchability.

One of the factors influencing the eggs hatchability is the incubation period. The generally known fact is that in the hatchery the embryo can be influenced by various microorganisms penetrating the shell. To control microorganisms many different preparations are used but most of them are not effective in controlling the pathogenic microflora, unsafe for humans and can have a negative impact on the embryo. Ozone, due to its powerful oxidizing action, destroys the microbial cell, in particular, affects phospholipids and lipoproteins. Viruses are also not ozone-resistant, as its action destroys the integrity of the protective virus envelope, in this connection, adherence to the membranes of a healthy cell is impossible. Unlike other antiseptics, ozone does not destroy or irritate body tissues, as cells have antioxidant protection. Moreover, in this research ozone is used as a stimulator of embryonic development, due to the oxygen-enriched embryo cells.

Metabolism in the poultry body is associated with the intensity of the transport functions of circulating blood. The normal metabolic processes depends on the morphological and biochemical composition of blood in birds. Many factors have a significant impact on the blood state. So influenced by ozone embryos absorb more than 40% oxygen, increase hematopoiesis and erythropoiesis. Based on this, the morphological parameters of blood in the experimental birds influenced by different duration of hatching eggs ozonization have been studied. In the course of calculating morphological parameters, the positive influence of ozone on the quails' blood parameters was determined (table 4).

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Table 4: Changes in blood corpuscles

Age	Groups	Leukocytes 10^9/l	Hemoglobing/l	Erythrocytes, 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 day	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 day	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 day	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 day	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 natural increase in erythrocytes is found in quails of all experimental groups. As mentioned above, when exposed to ozone, oxygen uptake by embryos increases, accordingly, the erythrocytes number in the experimental groups increases significantly. Thus, by 14 days old the erythrocytes number was $4,8*10^{12}$/l in quails of the control group, in the second, third, and fourth groups: $5,8*10^{12}$/l; $6,3*10^{12}$/l; $7,0*10^{12}$/l, respectively.

At 56 days old, the erythrocyte content in the second experimental group was $6,0*10^{12}$/l; in the third - $6,5*10^{12}$/l; in the fourth - $7,8*10^{12}$/l, which exceeded the control by 13,20%; 22,64%; 47,16%, respectively.

In a day old quails the erythrocytes content in the control group was $35,44*10^9$/l; in the second - $36,09*10^9$/l; in the third - $36,37*10^9$/l. At 42 days old there was a stable increase in leukocytes, and is within $40,75*10^9$/l - $40,20*10^9$/l.

By the end of the experiment at 56 days old, the analysis of the leukocytes number showed that the highest values were found in quails of the fourth experimental group, $41,52*10^9$/l, although there were no significant differences. Thus, it should be noted that ozone has a positive effect on the performance of protective and adaptive reactions.

The analysis of age dynamics in hemoglobin content has significant differences between the control and experimental groups. So, at a day old the highest rates of hemoglobin were found in the third and fourth groups, resulted in 102,1 g/l and 158,5 g/l, which exceeded the control by 47,69% and 5,40% respectively. By 14 days old, there was also a significant increase in hemoglobin ($P \leq 0,001$). By the end of the experiment, hemoglobin level was: in the control group – 120,7 g/l, in the second experimental – 135,8 g/l; in the third experimental – 145,1 g/l; in the fourth experimental – 146,1 g/l. The highest level was found in the fourth group, which exceeds the control by 20,21%, the significance is $P \leq 0,001$.

There were no pathologies in leucogram indices. Based on table 5, all elements are within the physiological range.

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Table 5: Leucogram

Age	Group	Basophils	Eosinophils	Neutrophils				Lymphocytes	Monocytes
				metamyelocyte	myelocytes	band	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 day	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 day	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 day	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 day	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

The observed aftereffect on the processes of embryonic development of eggs treated with ozone appears in a more intense embryo growth and also affects the growth of birds' muscles in their subsequent life, this is caused by changes in the protein state. The protein content and its fractions are shown in table 6.

Table 6: Level of total protein and its fractions 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, % albumins	30,68±0,7	32,79±0,9	32,75±0,9	32,47±0,01
globulins:	58,52±3,5	66,02±1,9	68,02±0,6	68,69±1,2
α-globulins	8,13±0,5	9,97±0,5	9,72±0,6	10,0±0,5
γ-globulins	36,01±2,6	40,15±0,9	41,51±1,1	42,28±0,1
β-globulins	14,38±0,3	15,89±0,8	16,72±0,4	16,37±0,4

Thus, during the experiment the best physiological effect on quails' protein metabolism was found in the experimental groups. Compared to the control, the level of the total protein was higher by 9,37%, 9,65% and 8,57%, respectively.

The content of albumins in the fourth experimental group ($p \leq 0,001$) was also significantly increased and was 32,47 g/l, which is 1,79 times higher than in the control group.

The results of studying the total protein content and its fractions in the blood serum of quails during ozonization give grounds to conclude the following:

the total protein concentration in blood serum of quails is a stable value, whereas the ozone effect increases this index by 9,37%, 9,65% and 8,57% compared to the control;

- the total protein concentration is positively correlated with the morphological parameters in a day old quails' blood.

Egg production is the most important productive quality of birds that reflects their physiological state and the reproductive system activity. Quails' egg production according to their physiological characteristics at the beginning of oviposition increased rapidly, then remained at a high level for a few months, and by the end of oviposition, due to the birds age, gradually decreased. Throughout this study, we have recorded the egg production for a year. In birds produced from eggs exposed to ozonization, there was an increase in egg production within the physiological range (up to 300 eggs per year). So the data of table 7 show that pre-incubation treatment of eggs with ozone contributes to the subsequent laying hens' egg productivity by 5,9-10,4%. The maximum egg production was found in the third experimental group of birds produced from eggs with treatment exposure – 30 minutes. Lower rates of egg production had laying hens in the control group. The difference between them was 10,4% (P≤0,001). However, it should be noted that the differences between the results in the control and the first experimental groups were not significant.

Table 7: Quail egg production for 48 weeks

Groups	Egg production, pcs./48 weeks	Difference, %
Control	253,8±5,8	-
Experimental 1	268,8±2,1	+5,9
Experimental 2	276,2±3,4	+8,8
Experimental 3	280,2±3,2	+10,4

Also throughout the experiment, the egg quality of quails was estimated (table 8). As it is known, the eggs quality is mainly influenced by: bird breeds, the conditions of feeding and housing. In our experiment, the weight of quail eggs as well as egg production were positively influenced by pre-incubation eggs treatment with ozone. Thus, the weight of quail eggs in the experimental groups was higher than in the control group by 6,0%, 9,6%, and 11,40%, respectively, although the difference was significant only between the control group and the second and third experimental groups (P≤0,05).

One of the main egg quality indicators are the yolk and protein indices. Protein index is the relationship between the dense protein height and the average diameter of its spreading. Yolk index is the percentage of the yolk height poured on a horizontal surface.

Thus, the yolk index in the experimental groups exceeded the control group by 1,73%, 1,73% and 3,1%, respectively. The differences were significant between the control and the third experimental groups (P≤0,01).

The protein index in the control group was 5,36%, in the first experimental group – 5,93%; in the second experimental group – 5,97%; in the third experimental group – 5,91%.

Table 8: Eggs quality indicators

Indicators	Groups			
	Control	Experimental 1	Experimental 2	Experimental 3
Eggs weight, g	11,14±0,43	11,81±0,21	12,21±0,21	12,41±0,21
Shell thickness, g	0,21±0,006	0,23±0,006	0,22±0,002	0,22±0,002

Yolk weight, g	3,34±0,11	3,58±0,002	3,56±0,02	3,59±0,002
Protein weight, g	6,60±0,09	6,81±0,06	6,77±0,006	6,68±0,03
Shell weight, g	0,77±0,02	0,80,±0,004	0,79±0,002	0,79±0,004
Protein index, %	5,36±0,17	5,93±0,03	5,97±0,002	5,91±0,004
Yolk index, %	28,9±0,10	29,4±0,02	29,1±0,49	29,8±0,17

The chemical composition of eggs is mainly affected by the diet. But since ozone acts as a biological activator of processes occurring in the poultry body, in particular laying hens, the proportion of vitamins and nutrients in the egg increases. By protein content in the egg groups had approximately the same indexes. The amount of fat was within range, had no significant differences, ranged from 12,99 to 13,58 g. Carbohydrates content in the quail eggs of the experimental groups was 16,16% higher than that in the control group (P≤0,1).

Table 9: Chemical composition of eggs, per 100 g

Indexes, per 100 g	Groups			
	control 1	experimental 2	experimental 3	experimental 4
Proteins	11,11±0,23	11,15±0,17	11,31±0,15	11,91±0,22
Fats	12,99±0,36	13,37±0,15	13,35±0,01	13,58±0,12
Carbohydrates	0,6±0,04	0,7±0,02	0,7±0,02	0,7±0,02
Water	72,71±0,21	73,54±0,23	73,73±0,13	73,53±0,10
Ash	1,20±0,03	1,24±0,002	1,23±0,004	1,24±0,004
Vitamins				
A	0,42±0,01	0,47 ±0,002	0,47±0,002	0,48±0,002
PP	0,23±0,006	0,26±0,002	0,37±0,004	0,28±0,002
B_1	0,10±0,002	0,11±0,002	0,11±0,004	0,11±0,004
B_2	0,61±0,01	0,65±0,004	0,65±0,006	0,65±0,006
Mineral substances				
Calcium, Ca	54,90±1,5	55,56±1,1	57,23±0,28	57,38±1,1
Phosphorus ,P	214,69±1,5	217,11±0,69	216,69±0,42	217,46±0,42
Iron, Fe	140,52±1,9	142,09±2,2	144,03±0,34	143,98±0,34

It is found that pre-incubation eggs treatment with ozone promotes improving the chemical composition indices of the produced laying hens. So the content of vitamin A in the experimental groups increased by 11,90-14,28%; vitamin PP – by 13,04-27,73% and B_2 – by 6,55% (P≤0,5). The content of mineral substances was within the physiological range and there were no significant changes.

CONCLUSIONS

1. The impact of ozone effectively affected the amount of embryonic losses by the number of blood rings, frozen embryos, addled eggs, substandard quails. The output of standard quails increased by 43,35% compared to the control group.

2. Pre-incubation eggs treatment with ozone contributes to increase the subsequent egg production of the produced laying hens by 5,9-10,4% and improve the eggs quality.

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