Toxic Effects of Magnesium Nitrate on Cardiac Muscle Tissue of Gallus Domesticus Embryos and Chicks

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Eggs from avian hybrid ROSS-308 have undergone incubation process, after previously being controlled, sanitized, measured and weighed. We made three groups of eggs, one control (LM) and two experimental (LE1 and LE2).The 3 groups were homogeneous, both as a weight (59.47 to 62.371 g) and volume (55.417 to 58.177 cm^3) and surface (59.315 to 72.305 cm^2). The eggs of the two experimental groups were injected 0.1 mL of a solution of magnesium nitrate, solution which had a concentration of 0.16%, for LE1 and of 0.61% for LE2. The timing of injection of the solution was at 2, 4, 6 and 8 days after the beginning of the incubation process, for both experimental groups. At the end of incubation period we studied both embryos and the resulting offspring. They were weighed, measured and evaluated in terms of necropsy, and some of them were slaughtered and dissected, extracting from them a series of body organs such as the heart, liver, stomach and intestinal mass. From the heart some histological samples were taken which were processed by paraffin sectioning techniques to yield 20 three-color (HEA) stained histological slides. These samples were studied by optic microscopy and we highlighted the modifications that appeared to the myocardial tissue. Heart weighted between 0.3308 to 0.3843 g, representing 0.807 to 1.062% of the body weight. Myocardial tissue has undergone significant pathological changes such as metaplasia of the vascular wall, perivascular edema, disintegration and degeneration of muscle cells and myocardial steatosis.

Keywords: bird embryos, myocardial tissue, magnesium nitrate

The soil, water and air of our planet suffered a serious pollution process in all areas as a result of irresponsible activity of the species *Homo sapiens sapiens*. Pollution with various chemicals, including that of nitrates and nitrites has reached a very dangerous state, but apparently this is not really acknowledged by those who produced it.

Nitrates and nitrites are found naturally in the soil and in plants, but in very small quantities. From this level they pass in animal and human body [1]. But in large amounts nitrates and nitrites, have very serious and diverse toxic effects, affecting all organs and tissues, especially the liver, heart, brain, adrenal glands, spleen, etc. [2, 3]. They are known teratogenic effects of nitrites, although this issue is still under discussion in the scientific literature [4, 5].

Magnesium nitrite, which is a salt of magnesium with nitric acid, has a white color, it is soluble in water and it is a substance with a very wide application in industries such as cos¸

metics, textile, petrochemical and in agriculture as a fertilizer [4, 6]. It is found in varying proportions in many fertilizers aimed to develop foliage of plants and it is administered for several crops: vegetables, grains, industrial crops and fruit trees [7].

Therefore, given the certain information regarding this pollutant, we addressed in our study, the possible toxic effect of this substance on *Gallus domesticus* embryos, with the spotlight on their weight and development [8] and especially pathological changes of heart suffered by the embryos [9, 10].

Experimental part

Materials and methods

The multitude and diversity of materials used in this research leads us to classify them into two broad categories namely: biological and nonbiological materials. The first category includes chicken eggs, embryos and offspring resulting from them. In the second category we included instruments and devices, laboratory glassware and the equipment used in performing the experiments (analytical balances, pH meters, dissecting kits, Leica DM-750 microscope with camera, Dremmel drill device, semi-automatic microtome, tissue processor, dye room, etc.), which can be added to a list of reagents and dyes.

The methods we used are also very different, namely: methods of preparation and incubation of eggs, methods of inoculation of the magnesium nitrate solutions in eggs, methods of dissection and removal of organs and methods of preparation of the histological slides [11], methods of statistical processing the obtained data.

Thus, we bought 100 eggs from Agricola International Bacau hatchery, eggs produced by 31 weeks old ROSS-308 meat type commercial hybrid hens. These eggs had

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Table 1
RESEARCH SCHEME

Specification:					Parameters measured for eggs, embryos and hatched chicks
Egg lots/ groups	Symbols	$Mg(NO_3)_2$ solution concentrations	Moment of inoculation	Number of eggs/group	
Control	LM	-	-	8	1. *Physical indices of the incubated eggs: weight, large diameter, small diameter, format index, profile index, egg surface, egg volume.* 2. *The weight of embryos and hatched chicks* 3. *Body development of embryos and hatched chicks* 4. *Heart weight of embryos and hatched chicks* 5. *Macroscopic and microscopic pathological changes of the heart and cardiac tissue*
Experimental number 1	LE1A	0.16 % (0.1ml/egg)	after 2 days from the beginning of incubation	5	
	LE1B		after 4 days from the beginning of incubation	5	
	LE1C		after 6 days from the beginning of incubation	5	
	LE1D		after 8 days from the beginning of incubation	5	
Experimental number 2	LE2A	0.61 % (0.1ml/egg)	after 2 days from the beginning of incubation	5	
	LE2B		after 4 days from the beginning of incubation	5	
	LE2C		after 6 days from the beginning of incubation	5	
	LE2D		after 8 days from the beginning of incubation	5	

weights between 55 and 67 g. They were cleaned and disinfected (UV), individualized, measured and weighed, then went through a preincubation phase for 4 days at a temperature of +23 ÷ +37.5 ° C.

After the first egg candling process, from remaining eggs (96) were made 3 groups, of which one is the control group (LM) and two experimental groups (LE1 and LE2) (table 1). These groups of eggs were sufficiently homogeneous, both in terms of their weight, as well as in terms of surface area and volume (table 2) (fig. 1, 2, 3). Incubation conditions (temperature, humidity and sanitation) were maintained within the limits prescribed by technical rules for this avian species [12].

While the eggs of the control group continued the incubation, the eggs of the experimental groups were inoculated with the magnesium nitrate solution. Thus, in the 20 eggs in the first experimental group we injected 0.1 mL of solution of magnesium nitrate having a concentration of 0.16% (0.16 mg / mL) (0.016 mg / egg). The eggs from the second experimental group (20) were injected with the same amount of magnesium nitrate solution (0.1 mL / egg), but at a concentration of 0.61% (0.65 mg / mL) (0.061 mg / egg).

The times at which the solution inoculation was done were at 2, 4, 6 and 8 days after the start of the incubation, in both experimental groups (table 1). In the 18th day of incubation second mirage (candling) of eggs from all groups was made, by checking the status of embryos development with a candling lamp. Eggs with undeveloped or dead embryos have been removed from the incubator, and the remained embryos were weighed and evaluated in terms of necropsy; the largest were dissected, extracting the heart and other organs [9]. They were weighed, measured, observed and then we took samples for the histopathological study. We also evaluated the stage of development of embryos (HH stages) in the two experimental groups using the Hamburger-Hamilton scale [13].

The tissue samples taken from the heart were processed by paraffin sectioning technique, obtaining 20 trichrome (HEA) stained slides. They were studied with a Leica DM-750 binocular photonic microscope, equipped with a camera [11]. In the microscopic field we studied: the general appearance of the myocardial tissue, the appearance and integrity of the vascular walls, the appearance and integrity of cardiac cells, various allergic reactions, etc. Every abnormal aspect was photographed and the most relevant images were illustrated, demonstrating the toxic effect of magnesium nitrate solutions on cardiac tissue (heart).

The raw data obtained from weighing and measurements was statistically processed and the general statistical estimators calculated were as follows: the mean and standard error of mean; standard deviation; the variance and coefficient of variation. The data were statistically processed and graphically represented and also percentage comparisons were made between the control group and the experimental groups.

Results and discussions

The obtained results can be grouped into three categories namely: those relating to the subject of eggs hatching process; those describing modifications of embryos and hatched chicks; those relating to the pathological aspects found on the heart of embryos and chickens from these eggs.

Thus, the chicken eggs that were used in this experiment were characterized by a good uniformity in terms of weight, size (the longitudinal and transverse diameter), area and volume. This uniformity of the 9 groups of eggs is evidenced by the data in table 2 and figures 1, 2 and 3. It is noted that the differences between these lots are: between 0.002 and 2.79%, for the weight of eggs (fig. 1); between 0.77 and 3.74% for the volume (fig. 3); and between 0.85 and 21.90%, for their surface (fig. 2). If we consider the average/ mean value of nine groups of eggs in terms of their weight, we find that it is of 61.369 g and it can be seen from figure 1, that practically all these lots are very close to this average value.

The same is true regarding the volume of eggs (fig. 3), the mean value of the nine groups is of 57.0268 cm^3. The average value of nine lots for the eggs surface is 65.063 cm^2, but for this character there is a noticeable difference

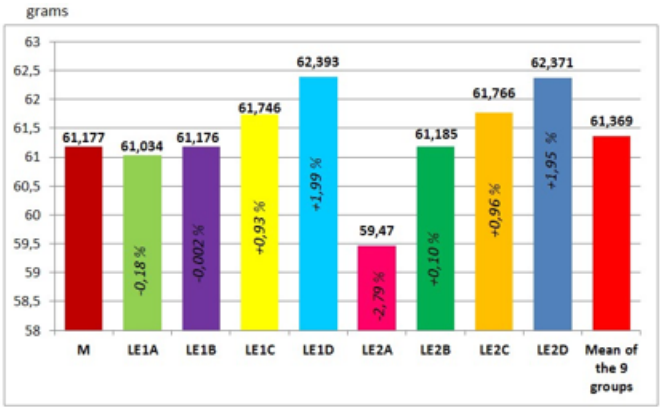

Fig. 1. Egg weight at the beginning of the incubation period for the 9 groups studied

Table 2

STATISTICAL INDICATORS REGARDING CHARACTERIZATION PARAMETERS OF THE STUDIED HEN EGGS (BEFORE INCUBATION)

Specification:		MU	n	Statistical indicators			Variation limits	
Groups	Studied parameters			$\bar{x}\pm s_{\bar{x}}$	s	V (%)	min.	max.
LM	Eggs weight	g	8	61.177±1.084	3.0666	5.01	57.007	66.918
	Longitudinal diameter of eggs	mm	8	54.106±0.589	1.6651	3.08	51.10	56.30
	Transverse diameter of eggs	mm	8	44.381±0.283	0.7996	1.80	43.35	45.85
	Format index	x/1	8	1.219±0.011	0.0301	2.47	1.171/1	1.276/1
	Egg surface	cm^2	8	59.315±0.759	2.1459	3.62	55.991	63.038
	Egg volume	cm^3	8	56.08±1.15	3.2533	5.801	51.322	61.920
LE1A 0.16%	Eggs weight	g	5	61.034±1.174	3.3204	5.44	56.412	64.615
	Longitudinal diameter of eggs	mm	5	55.70±0.672	1.9010	3.41	52.90	58.20
	Transverse diameter of eggs	mm	5	43.88±0.35	0.7837	1.79	43.225	45.125
	Format index	x/1	5	1.270±0.019	0.0427	3.36	1.224/1	1.320/1
	Egg surface	cm^2	5	59.82±0.901	2.0143	3.37	56.828	61.963
	Egg volume	cm^3	5	56.514±1.354	3.0267	5.36	52.176	59.573
LE1B 0.16%	Eggs weight	g	5	61.176±1.581	3.5349	5.78	57.922	67.090
	Longitudinal diameter of eggs	mm	5	55.59±0.731	1.6357	2.94	54.10	57.60
	Transverse diameter of eggs	mm	5	44.28±0.37	0.8268	1.87	43.05	45.15
	Format index	x/1	5	1.245±0.012	0.0278	2.24	1.213/1	1.276/1
	Egg surface	cm^2	5	60.291±0.725	1.6213	2.69	58.811	63.028
	Egg volume	cm^3	5	57.339±1.134	2.5354	4.42	55.108	61.578
LE1C 0.16%	Eggs weight	g	5	61.746±1.347	3.0111	4.81	56.608	64.226
	Longitudinal diameter of eggs	mm	5	55.7±0.65	1.4526	2.61	54.00	57.70
	Transverse diameter of eggs	mm	5	44.26±0.388	0.8669	1.96	43.00	45.425
	Format index	x/1	5	1.259±0.016	0.03626	2.88	1.211/1	1.308/1
	Egg surface	cm^2	5	60.353±0.787	1.7608	2.92	57.355	61.604
	Egg volume	cm^3	5	57.418±1.224	2.737	4.77	52.75	59.461
LE1D 0.16%	Eggs weight	g	5	62.393±1.558	3.4832	5.58	58.102	66.962
	Longitudinal diameter of eggs	mm	5	56.05±0.537	1.2005	2.14	54.65	57.80
	Transverse diameter of eggs	mm	5	44.425±0.241	0.5397	1.21	43.85	45.00
	Format index	x/1	5	1.261±0.009	0.01923	1.53	1.236/1	1.284/1
	Egg surface	cm^2	5	60.851±0.685	1.5311	2.52	59.454	62.962
	Egg volume	cm^3	5	58.177±1.061	2.3732	4.08	55.962	61.414
LE2A 0.61%	Eggs weight	g	5	59.47±1.525	3.4110	5.74	55.083	63.794
	Longitudinal diameter of eggs	mm	5	55.02±0.56	1.2473	2.27	53.20	56.45
	Transverse diameter of eggs	mm	5	43.70±0.488	1.0923	2.50	42.475	45.075
	Format index	x/1	5	1.259±0.014	0.0318	2.52	1.224/1	1.305/1
	Egg surface	cm^2	5	69.961±1.238	2.7695	3.96	65.981	73.353
	Egg volume	cm^3	5	55.417±1.473	3.2932	5.94	50.797	59.524
LE2B 0.61%	Eggs weight	g	5	61.185±1.684	3.7665	6.16	57.957	67.601
	Longitudinal diameter of eggs	mm	5	54.52±0.785	1.7552	3.22	52.45	57.00
	Transverse diameter of eggs	mm	5	44.44±0.509	1.1386	2.56	43.20	46.15
	Format index	x/1	5	1.227±0.017	0.03856	3.14	1.175/1	1.271/1
	Egg surface	cm^2	5	70.869±1.475	3.2987	4.66	68.151	76.563
	Egg volume	cm^3	5	56.655±1.76	3.9367	6.95	53.303	63.447
LE2C 0.61%	Eggs weight	g	5	61.766±0.853	1.9065	3.09	59.864	64.207
	Longitudinal diameter of eggs	mm	5	56.09±0.373	0.8347	1.49	55.20	57.20
	Transverse diameter of eggs	mm	5	44.145±0.146	0.3271	0.74	43.750	44.475
	Format index	x/1	5	1.271±0.009	0.01914	1.51	1.247/1	1.293/1
	Egg surface	cm^2	5	71.801±0.497	1.1112	1.55	70.544	73.338
	Egg volume	cm^3	5	57.526±0.577	1.2904	2.24	56.063	59.288
LE2D 0.61%	Eggs weight	g	5	62.371±0.951	2.1268	3.41	59.384	65.060
	Longitudinal diameter of eggs	mm	5	56.53±1.00	2.2418	3.97	54.40	60.00
	Transverse diameter of eggs	mm	5	44.21±0.368	0.8226	1.86	43.00	45.15
	Format index	x/1	5	1.279±0.03	0.0661	5.17	1.241/1	1.395/1
	Egg surface	cm^2	5	72.305±0.895	2.0024	2.77	69.641	75.038
	Egg volume	cm^3	5	58.115±1.029	2.3006	3.96	55.101	61.418

(from 17.95 to 21.90%) between the second experimental group groups (LE2A - LE2D) and the first (control and experimental 1 - LE1).

Also, it can be seen that the values for the coefficients of variation for all the characters studied, for the nine groups of eggs, are very low (0.74 to 6.95%) (table 2), confirming their homogeneity.

Regarding the weight of embryos and chicks hatched from these eggs, the data presented in Table 3 highlights the toxic and destructive effects of magnesium nitrate solution. Thus, if for the control group, the hatched chicks had body weights between 39.407 and 50.147 g , with an average statistical value of 43.321 ± 1.305 g (v = 8.52%), in all experimental groups from the first series (LE1) the body weight was reduced (table 3). The reduction was less pronounced at LE1A (-14.7%), LE1B (-16.36%) and the group LE1D (-50.53%) and very dramatic at LE1C (-95.63%), (table 6).

Regarding the situation in the experimental groups in the second series (LE2A - LE2D), the embryos and chicks body weights were much lower compared to the control group and even to the experimental groups in the first series. Thus, the difference was (for this indicator) of -53.16% for LE2A; of -51.97% for LE2D; of -99.18% and - 99.19% for LE2B and LE2C (tables 3 and 6). Based on the weight of eggs from which they originated, the embryos and offspring weight is (on average): 70.765 ± 1.39% for the control group LM; 60.724 ± 5.762% for LE1A; 60.608 ± 2.864% for LE1B; 3.065% for LE1Cand 34.352% for LE1D (table 4).

For the experimental groups in the second series, the embryos and chicks weight represented 33.362% - 34.121% (at LE2D and LE2A) and only 0.574 to 0.578% (at LE2B and

Table 3

STATISTICAL INDICATORS REGARDING THE WEIGHT OF EMBRYOS AND OF HATCHED CHICKS FROM THE STUDIED GROUPS AT THE END OF THE INCUBATION PERIOD

Specification:		MU	n	Statistical indicators			Variation limits	
Studied character	Groups			$\bar{x}\pm s_{\bar{x}}$	s	V (%)	Min.	Max.
Weight of embryos and hatched chicks	LM	g.	8	43.321±1.305	3.691	8.52	39.407	50.147
	LE1A	g.	5	36.953±3.481	7.784	21.06	24.446	44.880
	LE1B	g.	3	36.233±1.267	2.194	6.05	33.842	38.153
	LE1C	g.	3	1.8925±0.790	1.369	72.34	0.9908	3.4678
	LE1D	g.	2	1.4101	*	*	1.3402	1.4800
		g.	2	41.4553			39.9833	42.9272
	LE2A	g.	2	0.0393	*	*	0.0328	0.0458
		g.	2	40.5437			39.9274	41.160
	LE2B	g.	3	0.564±0.174	0.30065	53.31	0.3436	0.9066
		g.	2	0.0323	*	*	0.0318	0.0328
	LE2C	g.	4	0.357±0.068	0.1362	38.14	0.1564	0.4591
	LE2D	g.	2	9.959	*	*	7.9345	11.9835
		g.	2	31.6562			25.9635	37.349

Table 4

STATISTICAL INDICATORS REGARDING THE PERCENTAGE RATIO OF THE EMBRYOS/CHICKS WEIGHT FROM THE WEIGHT OF THE EGGS FROM WHICH THEY HATCHED FROM

Specification:		MU	n	Statistical indicators			Variation limits	
Studied character	Groups			$\bar{x}\pm s_{\bar{x}}$	s	V (%)	Min.	Max.
Embryos and chicks weight percentage from the egg weight	LM	%	8	70.765±1.390	3.9321	5.56	65.905	77.140
	LE1A	%	5	60.724±5.762	12.885	21.22	38.302	69.4575
	LE1B	%	3	60.608±2.864	4.9613	8.19	56.0141	65.8692
	LE1C	%	3	3.066±1.207	2.0906	68.19	1.587	5.4578
	LE1D	%	2	2.2236*	*	*	2.1504	2.2969
		%	2	70.182*			66.4817	73.8822
	LE2A	%	2	70.010*	*	*	65.2977	74.723
		%	2	0.0675			0.055	0.080
	LE2B	%	3	0.951±0.284	0.4927	51.81	0.580	1.510
		%	2	0.05025*	*	*	0.0485	0.0520
	LE2C	%	4	0.574±0.111	0.2212	38.53	0.2543	0.7642
	LE2D	%	2	51.40*	*	*	39.9073	62.8936
		%	2	16.012*			12.9265	19.0975

Table 5

STATISTICAL INDICATORS REGARDING THE HEART WEIGHT AND ITS PROPORTION FROM THE LIVE BODY WEIGHT OF THE HATCHED CHICKS

Specification:		MU	n	Statistical indicators			Variation limits	
Studied character	Groups			$\bar{x}\pm s_{\bar{x}}$	s	V (%)	Min.	Max.
Heart weight and proportion	LM	g.	8	0.3476±0.018	0.05164	14.86	0.2391	0.396
		% from BW*	8	0.8072±0.049	0.13888	17.21	0.5406	1.005
	LE1A	g.	5	0.3308±0.0274	0.06136	18.55	0.2476	0.4186
		% from BW*	5	0.9335±0.129	0.28934	30.995	0.6404	1.4019
	LE1B	g.	3	0.3612±0.0034	0.0059	1.64	0.3584	0.3698
		% from BW*	3	1.0045±0.032	0.0561	5.581	0.947	1.059
	LE1C	g.	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
		% from BW*	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
	LE1D	g.	2	0.38425	***	***	0.3790	0.3895
		% from BW*	2	0.92855	***	***	0.8829	0.9742
	LE2A	g.	2	0.34675	***	***	0.3392	0.3543
		% from BW*	2	0.85575	***	***	0.8241	0.8874
	LE2B	g.	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
		% from BW*	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
	LE2C	g.	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
		% from BW*	nbd**	nbd**	nbd**	nbd**	nbd**	nbd**
	LE2D	g.	1	0.3398**	***	***	***	***
		% from BW*	1	1.0623**	***	***	***	***

LE2C). If we calculate an average value for their weight, for all four experimental groups of the first series, it is 24.128 g, representing (as an average value) 39.297% from weight of the eggs from which they originated.

Compared to the control group, the difference is of -44.3% and -31.52 percentage points (table 6). Regarding the weight of the embryos in the second experimental series, in which the inoculated solution of magnesium nitrate had a concentration of 0.61%, it has been greatly reduced, with an average of 10.452 g and being with 78.87% smaller than that of the chicks/embryos of the control group and with 56.68% lower than that of chickens/embryos from the first test series (table 6). Based on the

Table 6

COMPARISONS BETWEEN THE GROUPS OF EGGS AND OF CHICKS OR EMBRYOS REGARDING THEIR BODY WEIGHT AT THE BEGINNING OF INCUBATION AND THEIR HEART WEIGHT

Compared groups		Studied parameters				
		Egg weight (grams)	Embryos/chicks weight (grams)	Chicks/embryos weight proportion from egg weight (%)	Heart weight (grams)	Heart weight proportion from the body weight of embryos/chicks (%)
LM	absolute values (grams)	61.177	43.321	-	0.3476	-
	relative values (%)	100.00	100.00	70.813	100.00	0.8072
LE1A	absolute values (grams)	61.034	36.953	-	0.3308	-
	relative values (%)	99.82	85.30	60.545	95.167	0.9335
	± (%) (pp*)	-0.18	-14.70	-10.268	-4.833	+0.1263
LE1B	absolute values (grams)	61.176	36.233	-	0.3612	-
	relative values (%)	99.998	83.638	59.227	103.913	1.0045
	± (%) (pp*)	-0.002	-16.362	-11.586	+3.913	+0.1973
LE1C	absolute values (grams)	61.746	1.8925	-	LD	-
	relative values (%)	100.93	4.369	3.065	LD	LD
	± (%) (pp*)	+0.93	-95.631	-67.748	LD	LD
LE1D	absolute values (grams)	62.393	21.433	-	0.38425	-
	relative values (%)	101.99	49.475	34.352	110.544	0.92855
	± (%) (pp*)	+1.99	-50.525	-36.461	+10.544	+0.12135
LE2A	absolute values (grams)	59.470	20.292	-	0.34675	-
	relative values (%)	97.210	46.841	34.121	99.755	0.85575
	± (%) (pp*)	-2.79	-53.159	-36.692	-0.245	+0.04855
LE2B	absolute values (grams)	61.185	0.3514	-	LD	LD
	relative values (%)	100.01	0.8112	0.574	LD	LD
	± (%) (pp*)	+0.01	-99.189	-70.239	LD	LD
LE2C	absolute values (grams)	61.766	0.357	-	LD	LD
	relative values (%)	100.96	0.824	0.578	LD	LD
	± (%) (pp*)	+0.96	-99.176	-70.235	LD	LD
LE2D	absolute values (grams)	62.371	20.808	-	0.3398	-
	relative values (%)	101.95	48.032	33.362	97.756	1.0623
	± (%) (pp*)	+1.95	-51.968	-37.451	-2.244	+0.2551

*pp = procentual points; LD = no data

weight of eggs from which they hatched, the average weight of these embryos (LE2A - LE2D) represents 17.159%, which is by 53.56 percentage points lower than that of the control group (table 6).

Regarding the status of development of embryos, status appreciated by the HH scale, it has been very variable, depending on the lot, the dose of magnesium nitrate solution and the time of inoculation.

Thus, if in the control group, the chicks have hatched at the right time and had a good body development without morphological defects, with subsequent normal growth specific for ROSS-308 hybrid, in the case of the two experimental series, the embryos stopped developing and even they died in an early stage (HH = 26, 27, 30, 33, 36, 37) and very early (HH = 14, 18, 20, 21). There have been some cases when some of the embryos have reached advanced developing stages (HH = 40, 43, 45), but they were not able to hatch.

Regarding the weight of the chickens' heart, it was different depending on the lot, but not too much. Thus, for the control group, the heart of the eight chicks weighed between 0.2391 and 0.396 g, the statistical mean value for this character being of 0.3476 ± 0.018 g (v = 14.86%). Relative to the body weight of these chicks, heart weight has an average value of 0.8072 ± 0.049% (v=17.21%).

For LE1A experimental group, the heart of the five embryos had a statistical average weight of 0.3308 ± 0.0274 g (v = 18.55%) and this represented 0.9335 ± 0.129% of their body weight. For LE1B group, the heart had an average weight of 0.3612 ± 0.0034 g (v = 1.64%), this being 1.0045 ± 0.032% of live weight (v=5.58%) (table 5) (fig. 4 and 5). In LE1C group the embryos were insufficiently developed so we could not harvest and weigh the heart, and in group LE1D, the heart had an average weight of

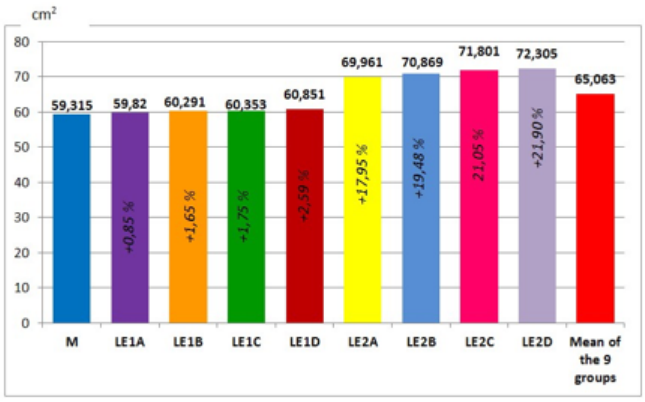

Fig. 2. The surface of the studied eggs

0.38425 g, representing 0.929% of the live body weight (table 5).

For the 4 groups of the second experimental series the heart weight was of 0.34675 g for LE2A and of 0.3398 grams for LE2D and for the other two groups (LE2B and LE2D) embryos being too small, we could not harvest the heart, so that there are no such data. From frigures 6 and 7 it can be observed that the heart of the embryo has an average weight of 0.35173 g and represents 0.932% of the live weight of the body.

It can also be observed that the chicks/embryos in the first experimental series had the heart weight increased by 3.21%, whereas in the second test series it decreased to 1.244% (fig. 4 and 5).

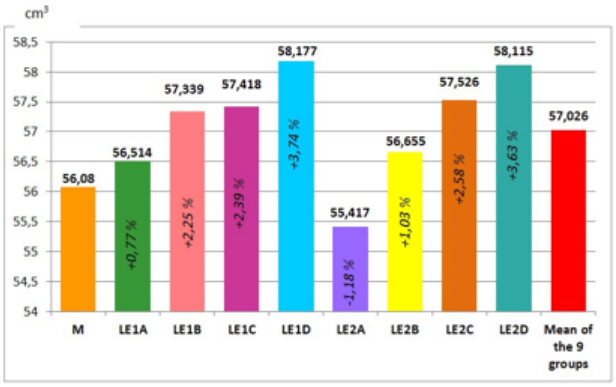

Fig. 3. The volume of the studied eggs

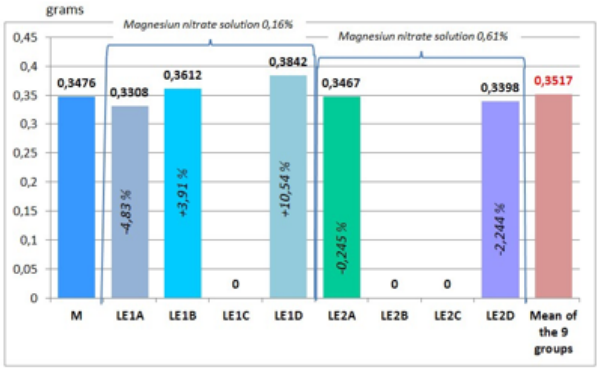

Fig. 4. Heart weight of the embryos and chicks hatched from the studied egg groupps

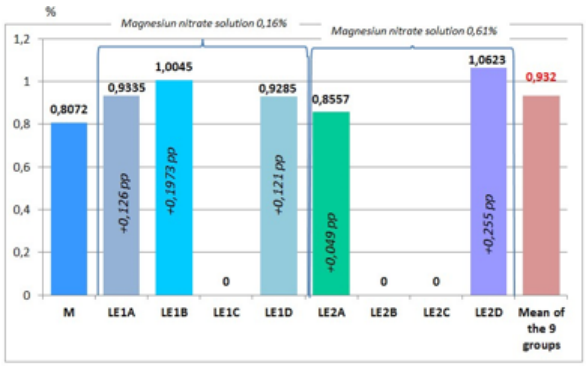

Fig. 5.Heart weight of the embryos and chicks hatched reported to their body weight

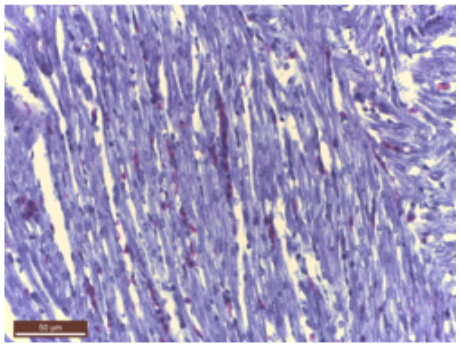

Fig. 6. Natural aspect of cardiac heart muscle tissue taken from chickens in the control group(microscope view)

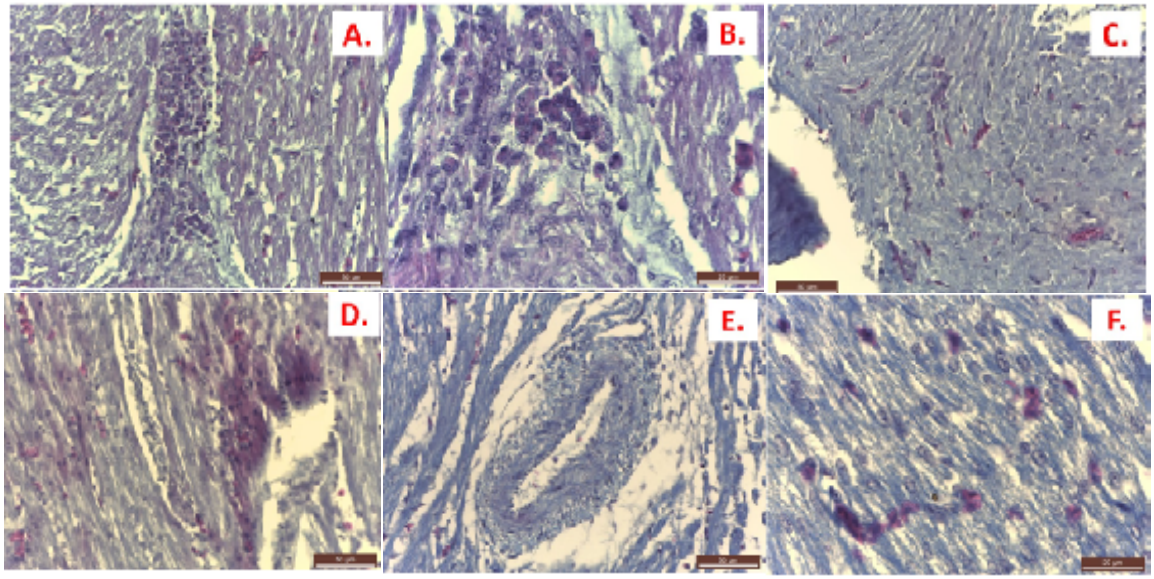

Fig. 7. Abnormal aspects observed on the heart muscle tissue following the action of magnesium nitrate solutions (microscope view): A and B- agglutinated eosinophils in perivascular spaces of cardiac tissue; C-eosinophils infiltrates in-between fibers space; D-hyalinosis of cardiac muscle fibers; E-perivascular edema and infarction; F-severe degeneration of cardiac muscle fibers

From a macroscopic point of view, the hearts of the chickens in the second experimental series had a number of pathological changes that were caused, most likely, by the experimental factor studied, namely magnesium nitrate solution. Thus, we have found area of necrosis of the myocardial tissue, heart *brittle* appearance, the presence of blood clots in the pericardial sac, bleeding in the chest cavity as a result of these changes, the development process has stopped in early (HH = 11, 20) or average (HH = 20, 30) embryonic stages.

From the histopathological point of view we highlighted, on slides prepared from heart muscle tissue, a number of issues of interest in the context of this research.

Thus, if the chicks from the control group had a cardiac tissue that appears normal during microscopic evaluation, with normal developed cardiac muscle cells (fig. 6) and blood capillaries also normally developed, then for the chickens and embryos from the experimental groups, the condition was altered.

In the experimental groups, the cardiac tissue presents several degeneration changes including: myocardial and perivascular edema; cardiac muscle cells and capillaries vessel wall metaplasia; the disaggregating, degeneration and hyper hydration of the muscle cells; the presence of agglutinated eosinophils in the perivascular spaces etc. (fig. 7). We also observed the presence of lipid granules and vacuoles in the cardiac muscle cells cytoplasm and severe degeneration of sarcoplasm and sarcolemma, including the existence of a phenomenon of myocardial

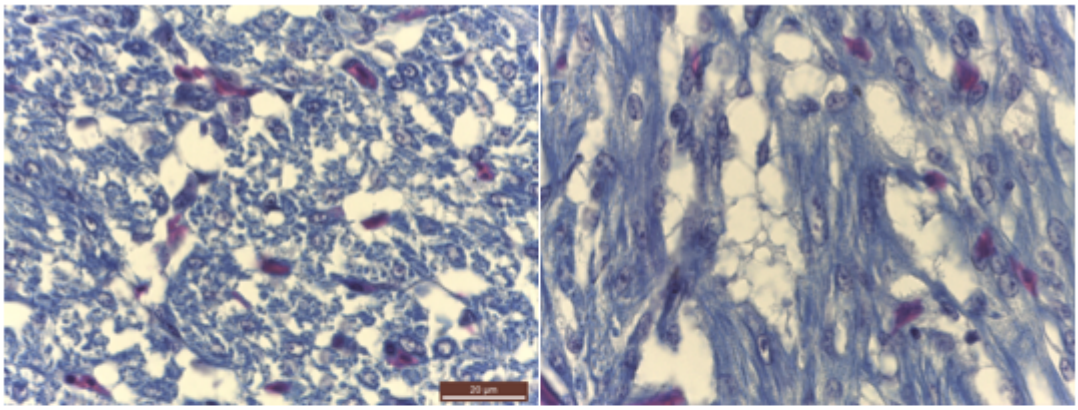

Fig. 8. Abnormal cardiac muscle tissue aspects: severe degeneration of cardiac fibers and vacuolated sarcolemma ipid granules) and stromal lipid infiltration (microscope view)

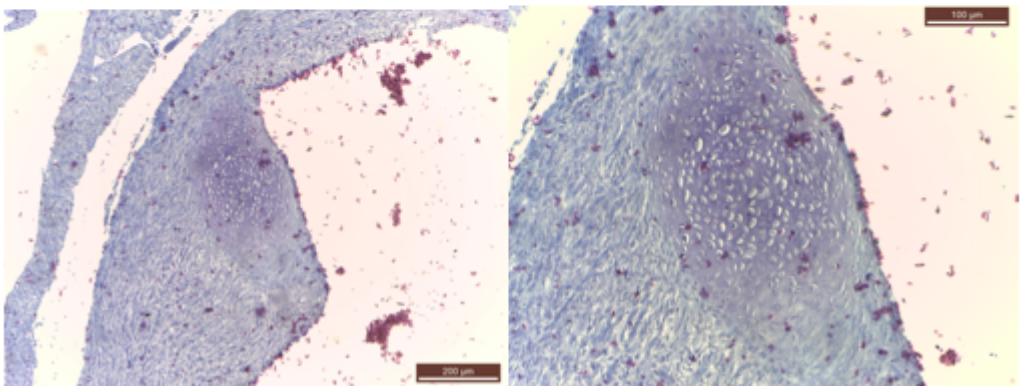

Fig. 9. Abnormal phenomenon of metaplasia of the coronary arteries (microscope view)

steatosis (fig. 8), accompanied by allergic symptoms and even metaplasia of the coronary arteries (fig. 9).

Conclusions

Although they hatched from normal eggs in terms of weight, size, surface area and volume, those embryos and chicks developed differently at least in terms of their body weight and growth.

If in the control group, the resulting chicks had an average weight of 43.321 g, representing 70.813% of the weight of the eggs from which they hatched, the chicks obtained from the first test series (LE1A-LE1D) had a weight reduced by 44.3%, depending on the time of injection of magnesium nitrate solution (0.16%).

The embryos and the chicks in the second experimental series (LE1A-LE2D) reduced their body weight compared with the control group with 75.87%, depending also on the time of injection of nitrate magnesium solution (0.61%).

The most serious and profound negative effects that the magnesium nitrate solutions had on the embryonic development, on body weight and viability of chicken embryos, occurred when the injecting of the solutions was made at 4 and 6 days after beginning of the incubation process (LE1B, LE1C, LE2B, LE2C).

When the solutions of magnesium nitrate were injected at 2 and 8 days after the start of incubation (LE1A, LE1D, LE2A, LE2D) toxic effects were also observed, but had a reduced intensity and severity.

The increased concentration (0.61% - 3.8 times) of magnesium nitrate solution, applied to the eggs in the second experimental series, amplified its destructive effects on cardiac tissue, but also in the other tissues (hepatic and nervous) and in the entire avian body, leading to the death of embryos and chicks.

The heart weight in the embryos and chickens we studied ranged from: 0.3476 g in the control group; 0.35875 g in the groups of the first experimental series; 0.343275 grams in the groups of the second experimental series, the differences being of +3.21% and respectively -1.244%.

Regarding the heart and the myocardial tissue, the toxic effect of magnesium nitrate resulted in: degeneration and destruction of cardiac muscle cells, in heart and perivascular edema, through cardiac muscle cell and vascular walls (capillaries, coronary arteries) metaplasia, in myocardial steatosis and cardiac allergic processes.

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