The Effect of Negative Air Ionization Exposure on Ontogenetic Development of Chicken

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Abstract

Most of the benefic effects of negative air ions (NAI) quoted in the literature until the end of the 20th century were obtained with high doses of NAI, but at these doses a phased action was noted: favorable at the beginning, then unfavorable on extended exposures. In Romania, experimental studies on animal or human subjects were made mostly with moderate doses of air ions, close to those in the nature, and the duration of ionization was limited. In order to clear out some methodological issues regarding the air ionization use, we proposed to make a stepped set of investigations, on the hen egg submitted to incubation.

The first goal of our study follows to evaluate the role of NAI on the development of the chicken embryo, in average concentration, but with extended exposure. The second goal is to detect the effects of negative air ionization in high doses on the incubated eggs, as well as to accentuate the periods of chicken eggs' ontogenetic development periods, when air ionization acts stronger, or with more benefits.

In the first experiment, the eggs were submitted to moderate air ionization day and night (in continuous application), during all the incubation period (21

days). In the second experiment the eggs were ionized with high doses of negative air ions, in different period of ontogenetic development.

Continuous ionization (day and night) with moderate doses of NAI, during entire period of eggs incubation (21 days), supports the idea of phased action of air ions in moderate doses: favorable at the beginning and unfavorable later, if the exposure to air ions is extended. The application of higher doses of air ions appeared to be positive in the eggs development and hatching, but only if the exposure was made in the second half of incubation, after the chicken development was finished.

Keywords

Negative air ions; Egg; Chicken.

Introduction

Ions are small particle that take on an electrical charge (positive or negative) [1]. They can occur naturally or can be produce by using some devices called ionizers (ion generators). While positive ionization is known to have detrimental effects on health [2, 3] (reduction of the rate of ciliary's beat, sometime progressing to complete stoppage, and a parallel reduction of the rate of mucus flow; contraction of the membranous posterior wall of the trachea; drying of mucosal surface; induction in the cilia of a state of vulnerability to trauma), the negative ions have proved to have as positive effects (antidepressants [4], improvement erythrocyte deformability [5], could reduce the rectal temperature and increase the immune system [6]). More researches must to be conducted to determine the effective minimum and maximum doses of negative ions (i.e. number of ions per cm^3 of air are in a given period). No other element of the physical environment has caused so much confusion as ionized air [7, 8], partially because certain models of negative ions generators are being sold without assurance that ions are being produced at all.

The previously reported studies on the negative air ions effects used high [3, 9, 10] or moderate doses that are close to those in the nature [11-13].

The issue of ionic density (concentration) suited to each application still remains open. Most of the benefic effects quoted in the literature until the end of the 20^{th} century were

obtained with high doses of NAI, but at these doses a phased action was noted: favorable at the beginning, then unfavorable on extended exposures [14][Palmer 1993]. Much work remains to be done before effective minimum and maximum dosage (i.e. number of ions per c. of air are in a given period of time, are finally determined).

The hen egg and the chicken embryo have been in experimental medicine for more than two centuries [15-17]. The chicken embryo has been used in embryology [18], virology [19], microbiology [20], tissue transplant [21], and teratology [22].

A chicken embryo was used as an experimental model in Pediatric Surgery [23], in studies of esophageal atresia [24], Hirschsprung's disease [25], meconial peritonitis [26], and meningomyelocele [27], as a models for the study of gastroschisis in Holland, Spain and Turkey [28-31].

The aim of our study was to demonstrate that continuously application of moderate concentration of negative air ions might be detrimental to health. In addition, the effects of high doses of negative air ions on different ontogenetic periods of hen egg development were studied in order to identify the most sensitive period.

Material and Method

The study has two main objectives: (1) the assessment of the NAI on the development of the chicken embryo, in average concentration and with extended exposure, and (2) to analyze the effects of NAI in high doses on the incubated eggs.

A total of 269 Leghorn hen (*Gallus domesticus*) eggs were used. The eggs selected for the study were kept in the incubator at a temperature of 37.5°C in an environment with 80% humidity and were rotated manually 1 or 2 times a day. Turning the eggs prevents the yolks from touching the shell and injuring the embryo. The eggs were stored with small ends down and slanted at an angle of 30 to 45 degrees and turned by elevating alternate ends.

Assessment of NAI in average concentration with extended exposure on the chicken embryo

The eggs were submitted to moderate air ionization in continuous application (day and night) during the whole incubation period (21 days).

Two batches of hen eggs were incubated: a witness batch consisting of 100 non-ionized eggs, and a batch consisting of 75 eggs. The incubation was done in a regular incubator (type IO 100) using a mirror type air ions generator, emitting at a distance of 20 cm from the eggs a moderate amount of NAI (11000 pairs of small negative ions per 1 cm^3 of air). The negative air ions generator operated day and night, for all the incubation period (21 days). In the 6^{th} day of incubation, the eggs looming or candling (examination of the eggs in the ovoscope) was performed (candling is the examination of the contents of the eggs using a shielded light in a darkened area or candler). The eggs with embryos were left in the incubator and submitted to continuous aero-ionization, and the eggs without embryos were removed. At the end of the incubation period, the normally/abnormally hatched eggs were noted, as well as the non-hatched, knocked/non knocked eggs.

Normally, when eggs fail to hatch properly, the reason is often hard to determine. The possible causes could be in the management of the breeder flock, the incubation procedures, or any step between the breeder flock and final hatch.

Analysis of the effects of NAI in high doses on the incubated eggs

The second objective of the study was to detect the effects of negative air ionization in high doses on the incubated eggs, as well as to accentuate the periods of chicken eggs' ontogenetic development periods, when air ionization acts stronger, or with more benefits.

In this experiment, the ionic concentration was increased, in order to find out the most appropriate periods of exposure to air ionization. Ninety-four eggs were submitted to incubation, split into batches of 23-25 eggs:

÷ A witness batch of non-ionized eggs.
÷ A batch of ionized eggs in the embryo-genesis period (days 1-6 of incubation).
÷ A batch of ionized eggs in the organ-genesis period (days 13-18 of incubation).
÷ A batch ionized in both critical phases (both in the embryo-genesis and organ-genesis period).

The ionization was made with a "Genion" aero-ions generator (manufactured by the "Electrocontact" of Botoşani, Romania). The emission of a high amount of ions (180000 pairs of small negative ions per 1 cm^3 of air) was set at 50 cm from the eggs. The ionization was applied 10 minutes per day, from the exterior of the incubator, in the specific periods according to the batches (see the information above). Higher doses of NAI (180000 pairs of

small negative ions/ cm^3 air) were used due to their possible negative effects on eggs' metabolism.

The interpretation of the results was performed according to the type of data at a significance level of 5%. Statistical software was used for comparison between proportions. The confidence interval at a significance level of 5% associated to the relative frequencies were computed based on a binomial distribution method [32, 33]).

Results

Assessment of NAI in average concentration with extended exposure on the chicken embryo

The results obtained by continuous exposure to moderate doses of NAI of a batch of eggs, compared to a non-ionized batch of eggs were presented in Table 1.

Table 1. Influence of the continuous exposure to moderate ionization of a batch of eggs submitted to incubation

BATCH	No. of eggs	Eggs with embryos 6 days after the incubation		From the eggs with embryos:							
				Normally hatched eggs		Abnormally hatched eggs (with pathological phenomena)**		Non-hatched, knocked eggs		Non-hatched, non-knocked eggs	
		f_a	% [95%CI]	f_a	% [CI95%]	f_a	% [CI95%]	f_a.	% [CI95%]	f_a	% [CI95%]
Witness (non-ionized)	100	70	70 [60-79]	65	93 [84-97]	3	4 [1-11]	0	0 [n.a.]	2	3 [0-10]
Continuously ionized (21 days)	75	60	80 [52-66]	19	32 [20-45]	7	12 [5-23]	8	13 [5-25]	26	43 [30-57]
p-values*		p = 0.1360		p < 0.01		p = 0.0470		p < 0.0003		p < 0.01	

f_a = absolute frequency;
n.a. = not applicable;

* comparison of relative frequency between witness and continuously ionized;

** = Chicks fully formed, but dead without pipping; embryos sticking or adhering to shell; crippled and malformed chicks; abnormal, weak, or small chicks; large, soft-bodied mushy chicks, paralysis
95%CI = 95% confidence interval

Analysis of the effects of NAI in high doses on the incubated eggs

The results obtained on eggs after the use of big doses of negative air ions are summarized in Table 2.

Table 2. Exposure of the eggs to high doses of negative air ions in different periods of incubation

BATCH	No. eggs	Eggs with embryos 6 days after the incubation		From the eggs with embryos:							
				Normally hatched eggs		Abnormally hatched eggs*		Non-hatched knocked eggs		Non-hatched non-knocked eggs	
		f_a	% [95%CI]	f_a	% [95%CI]	f_a	% [95%CI]	f_a	% [95%CI]	f_a	% [95%CI]
Witness (non-ionized)	23	14	61 [39-82]	6	43 [15-71]	3	21 [8-49]	0	0 n.a.	5	36 [15-64]
Ionized in the first 6 days of incubation	23	7	30 [13-52]	1	14 [2-55]	1	14 [2-55]	1	14 [2-55]	4	58 [16-84]
Ionized in the days 13-18 of incubation	23	15	65 [44-82]	9	60 [34-86]	0	0 n.a.	2	13 [1-40]	4	27 [7-53]
Ionized in the days 1-6 and 13-18 of incubation	25	9	36 [16-56]	5	56 [23-88]	1	11 [1-54]	0	0 n.a.	3	33 [12-65]

* with pathological phenomena: Chicks fully formed, but dead without pipping; embryos sticking or adhering to shell; crippled and malformed chicks; abnormal, weak, or small chicks; large, soft-bodied mushy chicks, paralysis.
f_a = absolute frequency;
n.a. = not applicable.

The probability matrix associated to the comparison of relative frequencies between different batches and incubations on the results of high doses of negative air ions on the investigated eggs are presented in Table 3.

Table 3. The probability matrix: comparison of results presented in Table 2

	Eggs with embryos 6 days after the incubation	Normally hatched eggs	Abnormally hatched eggs	Non-hatched knocked eggs	Non-hatched non-knocked eggs
W-I(1-6)	0.0405	0.1996	0.7023	n.a.	0.3496
W-I(13-18)	0.7810	0.3680	n.a.	n.a.	0.6059
W-I(1-6)&(13-18)	0.0900	0.5491	0.5407	n.a.	0.8842
I(1-6)-I(13-18)	0.0219	0.0571	n.a.	0.9494	0.1757
I(1-6)-I(1-6)&(13-18)	0.6611	0.1074	0.8588	n.a.	0.3345
I(13-18) -I(1-6)&(13-18)	0.0506	0.8491	n.a.	n.a.	0.7574

W = Witness (non-ionized);
I(1-6) = Ionized in the first 6 days of incubation
I(13-18) = Ionized in the days 13-18 of incubation
I(1-6)&(13-18) = Ionized in the days 1-6 and 13-18 of incubation;
n.a. = not applicable

Discussions

Despite the various critiques, the chicken embryo keeps its utility, with a series of advantages in practice: experiments are relatively cheap, the incubation period is short, sizes are small, the embryological development is known, and the accessibility to the embryo is easy [34, 35].

The main limit of the study is the small number of eggs per batch, for each experiment. This is only the first stage (a pilot stage) of a multiple study targeting to determine the proper dose of negative air ions and the proper periods of application.

In our study, it appears that by continuous ionization in moderate concentration, the percentage of the eggs with embryos until the 6^{th} day of incubation was bigger than the non-ionized (witness) batch, but the statistical difference between the two percentages is not significant.

In the batch of eggs with embryos, by continuous ionization with moderate doses, unfavorable phenomena occurred. At the end of the 21 days incubation period, a smaller percentage of normally hatched eggs than the witness batch ($p < 0.01$) was identified. The percentage of hatched eggs with pathological phenomena (progressive paralysis from the legs level) was three times higher at the continuously ionized batch than the witness batch. It is worth-mentioning that the paralysis of the legs occurred some time after the hatching (up to 6 weeks), although the physical development did not present deficiencies. Moreover, at the batch submitted to continuous negative air ionization, at the end of the incubation period, knocked but not hatched eggs, and not-knocked and non-hatched eggs appeared, with the embryos dead in different evolution stages ($p < 0.0003$ and $p < 0.01$ respectively). The phenomena mentioned above support the idea of phased action of air ions in moderate doses: favorable at the beginning and unfavorable later, if the exposure to air ions is extended [14].

In the second experiment using high doses of NAI, the smallest number of eggs with embryo was recorded in the ionized batch in the first 6 days of incubation (embryo-genesis period). The highest percentage of eggs with embryos at 6 days of incubation was recorded in the ionized batch only in the organ-genesis period (days 13-18), quite equal with the non-ionized witness batch (witch is normal, since none of the batch was ionized the first 6 days).

From the eggs with embryos still kept in the incubator, the highest percentage of normally hatched eggs was noted still in the batch ionized in the days 13-18 of development.

The batch ionized in the first 6 days presented a very small percentage of normally hatched eggs, much under the value recorded in the witness batch, and especially under the batched ionized in the phase of organ-genesis.

The ionization in higher doses of the eggs in both periods of development leaded to smaller percentage of abnormally hatched eggs (with pathological phenomena) compared to the witness batch, non-ionized. In the batched ionized only in the second period, no abnormally hatched egg was detected.

Another favorable phenomenon occurred in the batch ionized in the days 13-18 is the smaller percentage of non-knocked and non-hatched eggs with chicken.

Hence, the conclusion is that ionization with high doses of NAI of eggs seems to be favorable in the second part of incubation (period of consolidation of the organs).

Ionization in high doses in the second period (of organ-genesis), as expected, did not influence the percentage of eggs with embryos at the looming test, which was slightly bigger than in the case of the witness batch. It is to be noted that the application of negative air ions, even in higher concentration, during the organs consolidation period, when the embryo-genesis is finished, has some favorable effects, such as: the higher percentage of eggs normally hatched, and the lower percentage of non-hatched eggs with chickens; no abnormal hatching was noted on this batch.

Negative ionization in higher doses, in both stages of ontogenetic period of development lead to better aspects than the batch ionized only in the first period, suggesting that ionization in the second stage would have been a protective factor in the development of the chicken.

Upon the basis of the described results, a proposal of practical application of the negative air ions on the incubated eggs could be made. The use of moderate doses of ions is only recommended for a limited period of time (first 6 days of incubation), when the effects are favorable.

Conclusions

Studying the few methodological aspects regarding the use of negative air ionization with different doses, or equal doses, but in different phases of a development of the hen

chicken leads to the following conclusions:

1. Continuous ionization (day and night), in the incubator, with moderate doses of NAI, during all the period of eggs incubation (21 days), increased the percentage of eggs with embryos to 6 days of incubation (in looming), as compared to the witness batch. The extension of the duration with negative air ions therapy ended with a much smaller percentage of the normally hatched eggs and higher percentages of abnormally hatched eggs (with pathological phenomena) or non-hatched eggs (knocked or not).
2. The ionization of eggs with high doses, in the period of embryo-genesis (first 6 days of incubation) has negative effects, decreasing the percentage of eggs with embryos. The negative effects in premature application of air ions in big concentration are maintained, that is from the eggs with embryos only a small percentage hatched normally, most of them being knocked and not hatched.
3. The application of higher doses of air ions appeared to be positive in the eggs development and hatching, but only if the exposure was made in the second half of incubation, after the chicken development was finished.

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