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Research Article

NON-INVASIVE METHOD OF BIOSTIMULANT ADMINISTRATION INTO THE EGG: AN ALTERNATIVE TO IN OVO INJECTIONS

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ABSTRACT

This article discusses practical aspects of a non-invasive method to administer biostimulants inside the egg versus an injection method. Considering literature data and our own long-term studies, benefits and disadvantages were identified for each method of biostimulant administration. Given a large number of small poultry farms, the non-invasive method is technologically simple and cost-effective for the poultry industry. The purpose of this research was to determine the effectiveness of certain biostimulants composition for different types of poultry in a non-invasive way of its administration. The studies were conducted on different poultry farms in the Russian Federation. The experiments used eggs of Hybrid Converter turkeys, Japanese quails, grey-speckled guineafowls, and Ross 308 broilers. Biological control indicators of incubation were recorded against hatched eggs by a method generally accepted on the poultry farms. We received positive results that proved the high efficiency of the non-invasive method of delivering biostimulants into eggs, which contributed to the increased hatchability of young birds. Thus, considering all advantages of the presented method, it can be recommended for use on poultry farms.

Keywords: poultry farming, embryogenesis, biostimulants, *in ovo* injection, aerosol method, spraying method.

INTRODUCTION

The embryonal development of birds is a significant stage of ontogenesis, and its formation quality directly affects the viability and productivity of the bird in postembryonal development. Currently, international academic community has proposed several options for biostimulants used in the embryonal development of poultry, namely, by differences in composition and timing of injections into eggs while using the injection method using specially designed installations, insulin syringes or equipment for *in ovo* vaccination of poultry embryos. However, only Russian scientists have been doing research and developments over the years using various substances with the non-invasive administration method (spray or aerosol). For instance, Professor M.S. Naidensky with colleagues actively worked with his students in this direction since the 1990s, and as early as in the beginning of the 2000s, he introduced these developments [1]. Over the years of research, the high effectiveness of hatching egg treatment with various biostimulants has been repeatedly proven, hatching rates of young poultry and egg hatchability have improved, and poultry viability and productivity, as well as their disease resistance, have increased.

We analyzed commercial and scientific literature sources, as well as all the studies of our group of scientists and internal data from poultry farms in the Russian Federation in this subject area from which we compared different methods to introduce biostimulants into eggs (Table 1).

Table 1 shows the main distinguishing parameters in different methods of delivering biostimulants to hatching eggs.

Dose Accuracy. In injection, the dose accuracy is guaranteed and, dispensers are set on vaccination lines to doses on average of 48-52 microliters. In case of an insulin syringe, the gradation allows choosing a dose from 0.025 mL; it is also possible to use the so-

called pens which are electronic or mechanical that allow you to accurately dose a given volume of solution depending on the cartridge volume. In this case, the needles are 4 to 12 mm long and G32 to G29 thick. As with an insulin syringe, the larger the number, the thinner the needle is.

Administration Site. Professor Vincent Racaniello in 2009 marked vaccination sites using the *in ovo* method (Figure 1) [2].

Similarly, to the below Figure, it is possible to introduce not only vaccines but also biostimulants by injection. Sites for biostimulant administration by injection can be the following: chorioallantoic membrane, amniotic fluid, allantoic cavity or yolk sac. The non-invasive treatment method uses biostimulants applied to the eggshell surface.

Table 1: Comparative characteristics of different methods to introduce biostimulants into eggs

Items	Injection	Non-Invasive Method (Spraying or Aerosol Method)
Dose Accuracy	Guaranteed	Not Guaranteed
Administration Site	Chorioallantoic Membrane, Amniotic Fluid, Allantoic Cavity, Yolk Sac	Shell Surface
Complexity	High	Medium
Personnel Training	Required	Required
Technological Effectiveness	High	High
Equipment Price	Expensive	Low Cost
Effectiveness	Medium	High
Outcome Control	Full	Full
Labor Efforts	Low	Low
Risks:	Yes	Yes
Human Factor	Yes	Yes
Malfunction Probability	Medium	Low
Risk Traceability	Monitoring	Monitoring
Egg Pre-Sorting	Required	Not Required
Infection	Due to Infection Transmitted Through a Contaminated Needle	None
Productivity	Up To 60,000 Eggs/Hour	Not Determined
Cost-Effectiveness	Medium	High
Requirements for Egg	Same Size	None
Administration Time	At the beginning, in the Middle, at the End of Incubation	Before Incubation and/or Before Hatching
Administration Frequency	Once, twice	Once, Twice, Three Times

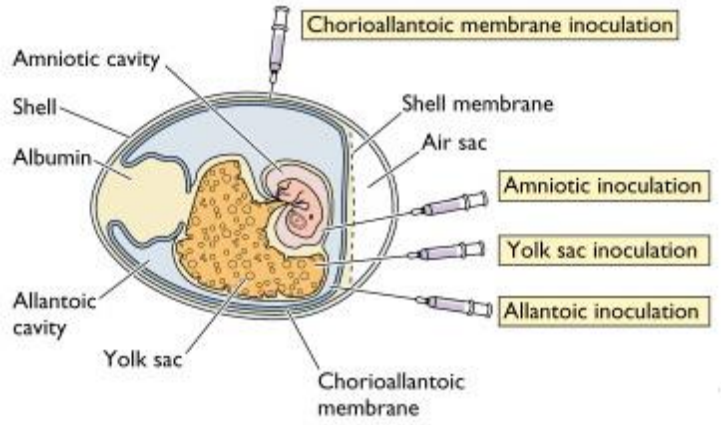

Figure 1: Sites of possible biostimulant administration by injection

Complexity. A normal operation manual for the vaccination line is more than 100 pages. It shows the equipment installation and operation stages. A cold fogger or a sprayer is easier to use, and the operation manual is no more than 5-20 pages. Accordingly, it will be easier for personnel to read a small operation manual since sprayers and cold foggers are much easier to operate than special equipment for injection or than the need to learn how to correctly inject biostimulants with syringes.

Personnel training is necessary for any equipment operation.

Technological Effectiveness. Both methods are technologically easy and can be built into the incubation technique.

Equipment Price. The price of an *in ovo* vaccination line (biostimulants are administered instead of a vaccine) is (at the US Dollar exchange rate in April 2022) more than 40 thousand US Dollars, and the price of a cold fogger or sprayer starts from 150 US Dollars, that is more affordable for small poultry farms. The price of an insulin syringe (for 10 pieces) starts from 30 cents.

According to the literature, the effectiveness of the injection method of biostimulant administration is different.

According to S.K. Bhanja *et al.* [3], glucose injections (50 mg per embryo) on day 18 of the incubation affected the development of the digestive organs and the biochemical profile of the chicken blood.

Other scientists found that the egg hatchability was higher in the group with the arginine- and lysine-treated embryos on day 18 of incubation [4].

After a 3% arginine solution was injected into the air sac of quail embryos, synchrony in chicks hatching improved, and live weight increased on days 7 and 42 of growing; feed conversion improved versus the control group, which indicates the effect of the "aftereffect" [5].

Considering the vitamin C effect on the hatchability of chicken and duck eggs, S. Nowaczewski *et al*. [6] found that its positive effect was only manifested in duck eggs.

It was found that vitamin C injected into chicken eggs showed no improvement in egg hatchability. Duck eggs obtained the best hatching results in the experimental groups regardless of the dose and time of ascorbic acid *in ovo* administration [6]. However, opposite results on chicken eggs were obtained by Y. Zhu et al. [7]: ascorbic acid injected to 11-day-old embryos (3 mg/egg) showed hatchability improvement and an increase in the chicks' growth rate up to 42 days of age.

In some experiments, L-carnitine administered before incubation at doses of 2 to 12 mg/egg did not significantly affect the post-embryonal development of chicks [8]. The increased dose of L-carnitine increased hatching time and reduced hatchability; however, the authors do not provide an explanation of the mechanism that caused the hatchability decrease [8]. In other studies, L-carnitine *in ovo* administration did not reduce hatchability or increase chick hatching time [9]. At the same time, an increase in the absolute and specific weight of chicks at hatching, glycogen content in the liver and pectoral muscle, and the insulin-like growth factor in plasma was observed [9]. Another study found that L-carnitine injection significantly increased hatchability, increased chick growth rate, and improved feed conversion [10], on day 14 of the incubation.

However, a non-invasive method of treating eggs with solutions of biostimulants achieved high results both according to the literature and as a result of our own research. When using a non-invasive L-carnitine administration method, the chicks hatching and hatchability had an advantage over the control by 12% and 9%, respectively (chicks hatching and hatchability, 88.89% and 96.55% in the experimental chicks versus 76.96% and 87.78% in the control) [11].

The outcome control of biostimulants injected *in ovo* is performed using computer technology, and visually when treated by an aerosol or spraying method taking into account the number of eggs, the area and volume of the treated surface.

With proper operation and trained personnel availability, their labor efforts are low and approximately the same.

Both methods have several risks: human factor (errors, insufficient qualifications of personnel, ect.), and technical malfunctions (failure of parts, power surges, etc.). Risk traceability is carried out by monitoring and should take into account technological features of equipment and personnel qualifications.

The method of biostimulant *in ovo* administration requires egg pre-sorting by size to minimize dead embryos due to deep penetration of the needle, while the non-invasive method does not require egg sorting.

Infection of eggs in the *in ovo* injection method is possible by inserting a needle first into an infected egg, then into the next one by contamination and transmission from an infected embryo to a healthy one. In this way, staphylococci, streptococci, Pseudomonas aeruginosa, some viruses, mycoplasmas, etc. can be transmitted. Currently, several manufacturers are already producing vaccination lines that have the function of needle disinfection to solve this problem. The non-invasive method has no contamination risk since biostimulant solutions are applied to the shell with spray or aerosol without violating the egg integrity.

As for the productivity of vaccination lines, the maximum output is about 60,000 eggs per hour. With a sprayer or a cold fogger, the productivity will depend on the type of equipment, and the aeration room output and volume. Depending on the number of eggs in the spraying method or the output of an egg treatment room (using a cold fogger), it is possible to select appropriate equipment with the required capacity.

The biostimulant *in ovo* injection will be cost-effective when the number of eggs is more than 200,000 eggs; in a non-invasive technique, the appropriate sprayer or fogger can be chosen depending on the output.

The main requirements for an egg when biostimulants are administered by the *in ovo* method are the same size of eggs; the non-invasive method has no requirements for a hatching egg, except for generally accepted ones.

The biostimulant administration time in the non-invasive method is most often before incubation or before transfer to hatchers; in the *in ovo* injection method, on different days of incubation (studies are known for biostimulant administration at days 0, 7, 11, 12, 14, 16, 17, 17, 5, 18, and 21) [3-10, 12-16].

The frequency of biostimulant administration by *in ovo* injection is usually one, or rarely, 2 times; in the non-invasive administration method, one-, two-, and sometimes even three-time treatment of hatching eggs is possible.

In the non-invasive method of biostimulant administration, active substance concentrations are most often tested in the range of 0.001% to 5%, then the optimal concentration is established that has the best effect on the bird embryo and improves the enterprise's performance.

Thus, the non-invasive method of biostimulant administration inside hatching eggs is an interesting method for stimulating the embryonal and postembryonal development of the poultry.

The research purpose is to determine the effectiveness of the obtained composition of biostimulants that consists of monoethanolamine, serine, succinic acid and pyridoxine hydrochloride for different types of poultry in a non-invasive method of administration.

MATERIALS AND METHODS

As an example of the high efficiency of the non-invasive administration method for several biostimulants, we offer the results of our own research on various poultry farms in the Russian Federation.

Hybrid Converter turkey eggs were obtained from one parent stock and sorted 208 pieces in each batch by observing the similarity of weight, laying time and shelf life. An aqueous solution of colamine, succinic acid, serine and pyridoxine hydrochloride was applied to the shell surface 3-4 hours before

incubation by a spraying method in concentrations of 0.1%, 0.1%, 0.2%, and 0.5%, respectively; they are previously dissolved and mixed in distilled water at 18-22°C.

Japanese quail eggs were obtained from one parent stock, and sorted 270 pieces in each batch, and an aqueous solution of colamine, succinic acid, serine and pyridoxine hydrochloride was applied to the shell surface 3-4 hours before incubation by the spraying method in concentrations of 0.1%, 0.1%, 0.2%, and 0.001%, respectively.

Grey-speckled guineafowl eggs were obtained from one parent stock, and sorted 1008 pieces in each batch, and an aqueous solution of colamine, succinic acid, serine and pyridoxine hydrochloride was applied to the shell surface 6-8 hours before incubation by the aerosol method in concentrations of 0.1%, 0.1%, 0.2%, and 0.01%, respectively, subject to the similarity of weight, laying time and shelf life.

Ross 308 chicken eggs were obtained from one parent stock and sorted 324 pieces in each batch. An aqueous solution of colamine, succinic acid, serine and pyridoxine hydrochloride at concentrations of 0.1%, 0.1%, 0.2%, 2.5%, respectively, was applied to the shell surface 3-4 hours before incubation by the spraying method.

It should be noted that such biostimulant concentrations were optimal and were identified by us in previous studies.

RESULTS

The developed composition that consists of monoethanolamine (colamine), succinic acid, serine and pyridoxine hydrochloride (vitamin B_6) was applied to hatching eggs of chickens, quails, guineafowls and turkeys to establish the effect on hatching rates of young poultry (Figure 2).

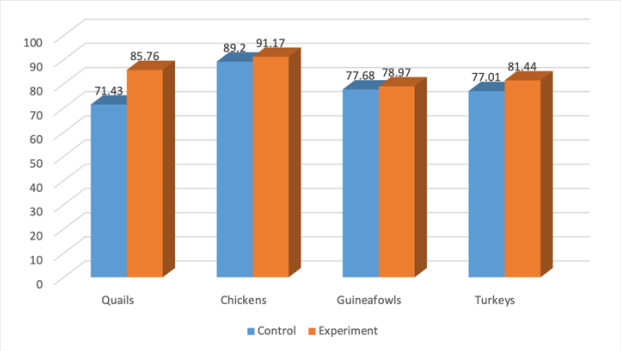

Figure 2: Hatching rates of quails, chickens, guineafowls and turkeys, %

As can be seen from the diagram, a composition of biostimulants in the non-invasive administration method had a positive effect on hatching rates of young poultry. The experimental group showed hatchability increase in the quails by 14.33%; in the chickens, by 1.97%; in the guineafowls, by 1.29%; and in the turkeys, by 4.43%, versus the control.

DISCUSSION

It should be noted that in our studies we used biostimulants on eggs of different quality (from young and old parent stocks, as well as parent stocks at the peak of productivity). In this study, the hatchability of young poultry from the control batches exceeded standards for such crosses and breeds, and therefore we consider the result to be positive and of great economic importance, especially for expensive poultry (guineafowls). For example, the cost of one hatching egg of the grey-speckled guineafowl is 0.8 US Dollars and more (subject to the Dollar exchange rate in April 2022).

Improved hatching rates of the young poultry occur due to a decrease in basic incubation waste (early dead, blood ring, black rots, dead-in-shell in the first and second half of incubation, late dead, weak, or crippled), which is associated with additional administration of these biostimulants that are intensively consumed by the body, especially under stress. These substances prevent hypoxic, and hypoenergetic conditions in embryos, and reduce the effects of oxidative stress, as we proved in a series of previous studies [17-19].

The shell permeability for biostimulants has already been proven by Russian scientists Karmoliev R.Kh. et al. [20], therefore the effectiveness of this method is beyond doubt.

Thus, administered biostimulants can be not only an effective means of improving hatching rates of young poultry, but the non-invasive method of biostimulant administration by a sprayer or a cold fogger can be used.

CONCLUSION

In this paper, we presented main differences between the non-invasive method and the method of injecting biostimulants into the egg, indicated benefits and drawbacks of such methods, and presented the results of our own studies that confirm high effectiveness of the biostimulant composition in the non-invasive method of administration on the poultry farms.

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