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Recent Technologies of In-Ovo Feeding in Poultry

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Abstract

In ovo feeding is a novel technique used to deliver various biologics and nutraceuticals directly to poultry embryos prior to hatching. The purpose of in-ovo feeding is to improve the overall health and performance of the hatched chicks. These injections are timed based on the embryonic development stage and are usually performed on day 18 or 19 of the incubation period. Early nutritional programming (in ovo injection) is one of the newest and most successful embryos feeding methods to prepare chickens with healthy intestines, good microbiota, enhanced immunity and improved overall growth performance. Nutraceuticals including amino acids, vitamins and carbohydrates were typically employed for early feeding via the in ovo method, along with feed additives like hormones, medications, exogenous enzymes, herbal plants and their derivatives, immunostimulants, vaccines and other biological substances. The main focus of current reviewis to provide an insight into applications and available findings related to in ovo feeding on performance and health parameters of poultry, along with challenges and future perspectives of this technique.

Keywords: In ovo feeding; Nutraceuticals; Immunostimulants; Health.

INTRODUCTION

In ovo feeding, also known as in-ovo nutrition or in-ovo injection, is a technique used in the poultry industry to provide nutrients and other substances directly to developing embryos inside the egg. It involves injecting a small volume of a nutrient solution or other bioactive compounds into the egg's amniotic fluid before incubation.

The purpose of in-ovo feeding is to improve the overall health and performance of the hatched chicks. By providing essential nutrients and bioactive substances directly to the developing embryo, it can enhance their growth immune function and overall vitality. This technique is primarily used incommercial poultry production, including broiler chickens and turkey production.

The injection process is typically performed using automated machines that are capable of precise and sterile injections. The injection needle is inserted through the egg shell and into the amniotic fluid and the desired amount of nutrient solution is injected. The eggs are then placed in an incubator for normal development.

Common substances injected during in-ovo feeding include carbohydrates, amino acids,

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vitamins minerals probiotics, prebiotics and vaccines. These injections are timed based on the embryonic development stage and are usually performed on day 18 or 19 of the incubation period, which is close to hatching.

The advantages of in-ovo feeding include:

1. *Improved chick quality:* In-ovo feeding can enhance the development of vital organs immune system and skeletal structure, resulting in healthier and stronger chicks.

2. *Increased Hatchability:* Providing nutrients directly to the embryo ensures that they are available during critical development stages, which can improve hatchability rates.

3. Reduced post-hatch mortality: In ovo feeding can enhance the immune system of chick making them more resistant to diseases and reducing post-hatch mortality rates.

4. *Cost savings:* In ovo feeding allows for precise delivery of nutrients, minimizing wastage and reducing the over all cost of feed.

5. *Environmental Benefits:* Since in ovo feeding reduces the need for excessive feed, it can also reduce the environmental impact associated with poultry production.

It's worth noting that in-ovo feeding is a specialized technique that requires careful control, expertise and proper equipment. While it has shown promising results in improving chick quality and performance, further research is still being conducted to optimize its implementation and explore its long term effects on the birds.

Recent advancement in poultry sector have produced new methods enabling the poultry industry to increase productivity and precision. Aiming at enhancing production quality, varying protocols of in ovo injection (Oliveira *et al.*, 2023) facilitate the introduction of exogenous substances into the egg to supplement the nutrients that support embryonic development. According to Aladii *et al.* (2018) in ovo feeding is a novel technique used to deliver various biologics and nutraceuticals directly to poultry embryos prior to hatching. The nutritional needs of the chicks have changed over decades. Early nutritional programming (in ovo injection) is one of the newest and most successful embryos feeding methods to prepare chickens with healthy intestines, good microbiota, enhanced immunity and improved overall growth performance. In ovo feeding is a practical solution that is revolutionizing poultry production due to its immediate beneficial effects on post-hatch. Nevertheless, some adverse effects have also been observed. While Subramaniyan *et al.* (2019) reported that in ovo injection of L-arginine (100 µg/µL/egg) benefits embryo survival rate, hatchability and chick weight, Araújo *et al.* (2020) observed that in ovo injection of a product containing can thaxanthin at different concentrations (0.35 to 0.65 mg/ml/egg) impaired hatch ability and chicks' physical quality. This article will provide an in sight into applications and available findings related to in ovo feeding on performance and health parameters of poultry, along with challenges and future perspectives of this technique.

Methods of in-ovo technology

In-ovo technology refers to various techniques and interventions performed during the incubation period of poultry eggs. These methods aim to improve the health, growth and performance of the developing embryos. Here are some common methods used in in ovo technology.

1. In Ovo Injection: This method involves injecting a small volume of nutrient solutions, bioactive compounds, vaccines or other substances directly into the amniotic fluid of the developing embryo. The injection is typically performed through a small hole in the eggshell using automated injection machines.

2. In Ovo Feeding: In this method, a nutrient rich solution is injected into the egg to provide nutrition directly to the developing embryo. The solution usually contains carbohydrates, amino acids, vitamins and minerals essential for embryonic development. At early stages of embryonic development, components are introduced into the egg protein at 12 mm deep. This allows us to specify components as close as possible to the egg embryo. Nutrients are usually added to the yolk sac during the later stages of embryonic development. The yolk sac is the perfect place to inject substances due to its surface area and ability to digest nutrients. After 17 days of incubation, the yolk sac is resorbed and another region of the egg, the air sac and amniotic membrane is used in egg technology (Kucharska-Gaca *et al.*, 2017). In ovo techniques most commonly use an insulin syringe or device that can inject a larger group of eggs. The needles used in such devices are 18.4 mm long and 1.27 mm in diameter and are designed to reach the amniotic membrane (Zhai, 2011).

3. In Ovo Vaccination: Vaccination is an important aspect of in-ovo technology Vaccines can be administered directly into the egg to stimulate the immune system of the developing embryo. This

method helps to protect the chicks against various diseases even before they hatch.

4. In Ovo Gene Editing: Recent advancements in biotechnology have enabled the use of in-ovo gene editing techniques. This method involves modifying the genetic material of the embryo to introduce or suppress certain traits, such as disease resistance or improved growth.

5. In Ovo Light Treatment: Light plays a crucial role in the development of embryos. In-ovo light treatment involves exposing the developing embryos to specific wave lengths and intensities of lightto optimize their growth, circadian rhythms and overall development.

6. In Ovo Electrolyte Supplementation: Electrolyte solutions can be injected into the egg to maintain proper fluid balance and provide essential electrolytes to the developing embryo. This method helps prevent dehydration and supports the development of vital organs.

7. In ovo Heat Treatment: Thermo-balance is an equilibrium between ambient heat and heat produced by living organisms and animal be in the thermoneutral range for optimal production. Chickens perform best in the thermoneutral zone, which ranges between 18 and 22 °C. Thermal manipulation during incubation has been shown to mitigate the effects of heat stress in poultry (Al-Zghoul *et al.*, 2019).

These methods of in-ovo technology require specialized equipment, such as automated injection machines, precise temperature and humidity control in the incubator, and careful handling of the eggs. They are primarily used in commercial poultry production to improve the quality, health, and performance of the hatched chicks.

Why in-ovo juvenile nutrition be done?

In commercial poultry production systems, chicks are deprived of food and water for approximately 24 to 48 hours during transfers between hatcheries and production facilities. This delay adversely affects early feed in take, organ development, natural exposure to microbiota and chick immunity. Early feeding of developing embryos with in ovo inoculation of nutrients and feed supplements at 18 days post-hatch significantly reduced the adverse effects of starvation and improved poultry hatching during the pre and post-hatch periods. In addition, feeding early in ovo supplements provides more protection against a variety of infectious agents than post-hatch supplements. (Arian *et al.*, 2022).

Sites of in ovo injection

In ovo injection sites includes amniotic fluid, allantoic, chorioallantoic inoculation, air cell, yolk sac and chick embryo inoculation. Recently, a study showed that injection or delivery of vaccines into the amniotic fluid and body of developing embryo provided significant post-hatch protection (Roto, Kwon, and Ricke 2016). Care must be taken when passing the needle through the amnion fluid because improper insertion of the needle into the egg can damage the embryo or spread the vaccine to other parts of the egg. Depending on the choice of substance to be injected, the correct injection site and position is required.

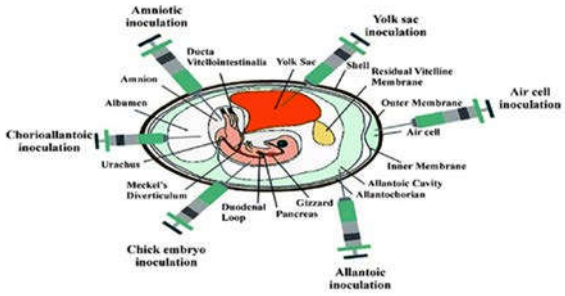

photo courtesy: Arian et al.,2022

Recent researches on in-ovo feeding

Sr no.	Nutrient injected	Amount of injected nutrient	Day of injection	Route of injection	Effects on performances	Reference
1	L-Glutamine and L-leucine	0.6 ml (1% wt/vol) in 0.4% NaCl solution	Day 17	Intra-amniotic administration	↑in progenitor cell count 36%. ↑ in region of the villi 1.9- and 1.3-fold longer in the treatment group.	Reicher et al. 2022
2	Nano- silver	Silver nanoparticle + (Methionine-10 mg + Arginine-25 mg)	Day 18	Air cell administration	AgNPs with amino acids enhanced immune response and resistance against ND virus	Bhanja et al., 2022

table cont.....

3	L-arginine	0.5 mL of Arg solution	Day 18	amnion	Arg increased the antioxidant capacity of the breast muscle in the starter period.	Lu et al., 2022
4	Alcoholic garlic	50, 100, and 150 µL.egg-1 of an alcoholic garlic extract	Day 0	Egg blunt end	garlic extract injection with 150 µL.egg-1 improved hatchability and reduced embryonic mortality.	Mahjar et al., 2022
5	Vitamin D	2.4 µg vitamin D3, 2.4 µg 25- hydroxyvitamin D3 (OHD3), 2.4 µg D3 + 2.4 µg 25OHD3.	Day 18	Egg embryo	↑body weight and breast meat yield of broilers ↓circulating plasma -1-acid glycoprotein (AGP) concentrations	Fatemi et al., 2021
6	D-Glucose monohydrate and ascorbic acid	Dextrose 24 mg, vitamin C 10 mg, dextrose 24 mg + vitamin C 10 mg in a 0.5- and 0.1-ml solution, respectively	Day 25 (geese)	Albumin	↓ in hatchability by 30, 26.11 and 40.58% in dextrose-, vitamin C- and dextrose + vitamin C-treated groups. ↑ in embryo mortality by 367.2% in dextrose and dextrose + vitamin C groups.	Baykalir, Mutlu, and Erisir 2021
7	Canthaxanthin	rate of 0.0, 0.35, 0.45, 0.55, and 0.65 mg/0.5 mL of sterilized and distilled water	17.5th day	Amniotic cavity	↓hatching rates and a longer hatching period CCX showed an improvement in oxidation status of chicks, the hatchability and performance of broilers decreased	Araújo et al., 2020

Nutrients for in ovo feeding

The main purpose of in ovo nutrition is to accelerate embryo development after hatching. Carbohydrates such as maltose, sucrose and glucose generally serve as a source of glucose and are essential for hatching and hatchling development. Filho *et al.* (2018) and Xu *et al.* (2019) stated that dietary amino acid supplementation promotes gastrointestinal tract development and hatchability. Nutrients that can be used in ovo nutrition include carbohydrates, all amino acids, fatty acids, vitamins and other regulatory factors.

Applications of in-Ovo Feeding

In-ovo feeding has various applications and potential benefits in the poultry industry. Here are some of the main applications of in-ovo feeding:

1. Improved Chick Quality: In-ovo feeding can enhance the development of vital organs, skeletal structure, and immune system of the developing embryo. This can result in healthier and more robust chicks at hatch, with improved post-hatch performance.

2. Nutrient Delivery: In ovo feeding allows for precise delivery of essential nutrients directly to the developing embryo. This ensures that nutrients are available during critical stages of embryonic development promoting optimal growth and development.

3. Immune System Enhancement: In ovo feeding can include the administration of immune stimulating substances such as vaccines or immune modulating compounds. This helps to prime the immune system of the developing embryo improving disease resistance and reducing post-hatch mortality.

4. Reduction of Post-Hatch Stress: By providing essential nutrients before hatching in-ovo feeding helps reduce the stress experienced by chicks during the transition from the egg to the external environment. This can lead to better adaptation, improved feed in take and reduced mortality.

5. Precision Feeding: In ovo feeding allows for accurate and targeted delivery of nutrients,

enabling precise nutrition management. This can optimize nutrient utilization, reduce feed wastage, and improve feed efficiency in the early stages of chick development.

6. Environmental Sustainability: In-ovo feeding can contribute to environmental sustainability in poultry production. By improving nutrient utilization and reducing feed wastage, it helps minimize the environmental impact associated with excessive feed consumption and waste disposal.

It's important to note that while in-ovo feeding has shown promising results, its commercial implementation and optimization require careful research, development, and technical expertise. The specific applications and benefits may vary depending on the nutritional needs and goals of the poultry production system.

In ovo feeding improves growth and production performance of poultry birds

A variety of chicken species may perform better through out the embryonic and post-hatch stages with food supple mentation utilising in ovo technology. The weight and quality of day old chicks were improved, as were the muscular growth and energy metabolism at hatch by the in ovo supplementation of branched chain amino acids on the 22nd day of incubation containing l-leucine and l-valine injected into the amniotic fluid of turkey poults (Kop-Bozbay and Ocak, 2019). Similar to the previous study, Gao *et al.* (2018) found that adding 1% arginine solution to the in ovo diet at 17.5 days of incubation significantly increased the embryo weight at the 19th day, improved post-hatch performance and increased digestive enzyme activity and relative organ weight in broiler chicks. The main purpose of in ovo feeding is to expose the developing poultry embryo with a variety of nutrients which are required to stimulate the development of gastro intestinal tract (GIT), improve immunity, antioxidant defence, enhance beneficial microbial population, increase the digestion and absorption and lessen the negative effect of starvation.

In ovo feeding enhances immune performance of poultry birds

Greater immuno competence was not developed into commercial strains of chicken because it would have a detrimental effect on growth and productivity. Through the in ovo supplementation of nutraceuticals, immunostimulants, vaccinations, bioactive chemicals and other feed additives, researchers and scientists have recently concentrated on improving immunocompetence. According to Li *et al.* (2016), in ovo injection of folic acid (vitamin B) improved the plasma concentration of lysozyme and immunoglobulin A and M, upregulated immune related gene expression and increased folic acid concentration in broilers. In ovo injection of threonine (0.5 mg/ml) on day 11 of incubation dramatically increased the gene expression of immunoglobulin A in freshly hatched Japanese quail chicks, according to research by Kermanshahi *et al.* (2017). It may be concluded that potential applications of nutraceuticals via in ovo injection stimulates the immune response in many poultry species.

In ovo feeding of nutraceuticals improves antioxidant status of poultry birds

In the current scenario with the advent of modernisation of poultry technology, the scientific community is focused to improve the antioxidant defences of poultry birds through early stage feeding of nutraceuticals and other biological substances by using the in ovo technique (Arain *et al.* 2022). In ovo injection of Galacto Oligosaccharides (GOS) as prebiotic on the 12th day of incubation of egg mitigated the adverse effect of heat stress and enhanced the health and production performance of broilers (Slawinska *et al.* 2020). It can be suggested that in ovo inoculation of nutraceuticals mitigated the negative effect of oxidative stress and improved antioxidant defence of developing embryo and post-hatch poultry birds.

Effect of in ovo nutrition on gut development

The GIT of new born hatched avian chicks has limited capability to metabolise the protein and carbohydrate rich diet. Recently, research by Tasharofi *et al.* (2018) reported that intra yolk sac injection of dextrose and albumin on 8th day of incubation of egg significantly improved the post hatch performance and improved GIT development and energy reserve of hatchlings. Additionally, in ovo supplementation of raffinose (1.5, 3.0 and 4.5 mg/egg) on day 12th of incubation is improved the intestinal morphology and immunity, which indicated the improved gut health of chicks (Berrocoso *et al.* 2017). Conclusively, it turns out that early in ovo feeding is crucial for enhancing digestive organ development as well as productive performance of poultry birds.

Effect of in ovo stimulation and epigenetic modifications

It has been demonstrated that administering 0.2 mL of dissolved bioactive stimulus on day 12

of egg incubation may start a whole cascade of events on several phenotypic concentrations, from gene expression regulation to growth efficiency. This process was created and patent by scientists Gulewicz and Bednarczyk (Siwek *et al.*, 2018). Another study showed that maternal betaine supplementation reduces hepatic cholesterol accumulation in chicken off spring by epigenetic modification of SREBP2 and CYP7A1 genes (Hu *et al.*, 2020).

Challenges

While in ovo feeding offers several potential benefits, there are also challenges associated with its implementation. Here are some of the main challenges of in-ovo feeding:

1. Precision and Consistency: In-ovo feeding requires precise and consistent delivery of nutrients or substances to the developing embryos. Ensuring accurate injection volumes, injection sites, and timing of injections can be technically challenging, especially when dealing with a large number of eggs in commercial production settings.

2. Sterility and Biosecurity: Maintaining sterility during the injection process is crucial to prevent eggs in commercial production settings contamination and the introduction of pathogens into the eggs. Proper sanitation protocols equipment sterilization and strict biosecurity measures are essential to minimize the risk of infection.

3. Embryo Sensitivity: Developing embryos are delicate and sensitive to external factors. The injection process itself may cause stress and potential damage to the embryos if not performed carefully. Balancing the injection volume and pressure to avoid excessive manipulation is important to ensure embryo viability.

4. Limited Injection Volume: The amount of solution that can be injected into the egg in limited due to the size and capacity of the egg. This constraint can restrict the range and amount of nutrients or substances that can be delivered, potentially limiting the effectiveness of in-ovo feeding.

5. Cost and Equipment: In-ovo feeding requires specialized equipment, such as automated injection machines, which can be expensive to acquire and maintain. The initial investment, along with the costs associated with training personnel and ensuring proper equipment maintenance, can be a challenge for some producers.

6. Regulatory Approval: In some countries, the use of certain substances or technologies in in-ovo feeding may require regulatory approval. Obtaining regulatory clearance and ensuring compliance with legal and safety standards can be a complex process that adds to the challenges of implementing in-ovo feeding.

7. Long-Term Effects and Research: While in-ovo feeding has shown promising results in improving chick quality and performance, there is still on going research to better understand its long-term effects on the birds. Continued research and monitoring are essential to ensure the safety and efficacy of in-ovo feeding practices.

8. Other limitations: Difficulties relate to the appropriate embryonic development and utilization of injected nutrients. The manufacture of nutrients and supplements that match the internal environment of the egg and satisfy the specific embryo's biological demands is another difficult issue. Limited authentic protocols are available for early in-ovo feeding of additives and bioactive compounds, which affect the desired results and induce serious financial losses in terms of lower hatchability rate up to 10% in comparison to the non-injected control groups (De Oliveira *et al.*, 2014).

Despite these challenges, on going advancements in technology and research are addressing many these issues and helping to improve the effectiveness and practicality of in-ovo feeding techniques poultry production.

Future Research

Future study be concentrated on feeding multiple biological substances and complex nutrients in ovo so that in post-hatch desirable results be obtained in terms of productivity and health. Additionally, there has to be clearance about decrease in hatchability brought on by in ovo feeding of specific nutrients. To achieve various or all desired post-hatch effects, it is necessary to standardise the composition of nutrients, dosages of nutrients, concentration and viscosity of nutrient solutions, volumes of solutions, timing during the pre-hatch period, routes, locations of injection, types of needles, sealing of holes, etc (Pandey *et al.*, 2021).

CONCLUSION

Nutraceuticals including amino acids, vitamins and carbohydrates were typically employed for early feeding via the in ovo method, along with feed additives like hormones, medications, exogenous enzymes, herbal plants and their derivatives,

immunostimulants, vaccines and other biological substances. It has opened up a brand new window for juvenile feeding that offers opportunities for nutritionists to boost poultry production to its optimum level. It is quite likely that in ovo feeding technology will be widely used and accepted as a standard feeding method for poultry if its current limitations are carefully investigated and addressed.

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