



In-ovo Feeding Technique as Pre-hatching Nutritional Strategy on Chicken Productive Performance: Applications, Advances and Future Perspectives

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ABSTRACT

This review underscores the transformative potential of in-ovo injection (IOI) in modern poultry production. By enabling the precise delivery of nutrients, vaccines, antioxidants, and nanomaterials directly into developing embryos, IOI addresses key developmental challenges, such as early nutrient deficiencies and delayed post-hatch feeding. This technique enhances hatchability, chick vitality, and post-hatch performance by improving gastrointestinal development, nutrient absorption, and stress resilience through targeted supplementation with amino acids, carbohydrates, selenium, and vitamin C. A major advantage of IOI lies in its support of sustainable poultry practices. By reducing antibiotic dependency, especially through the use of nano-compounds like nano-copper and nano-zinc, IOI contributes to combating antimicrobial resistance. These materials offer superior bioavailability and antimicrobial properties, promoting growth and gut health. The inclusion of prebiotics and probiotics further enhances the early establishment of a beneficial gut microbiome. The IOI has evolved from a vaccine delivery method into a scalable, automated system that improves consistency, reduces labor costs, and ensures uniform treatment across large batches of eggs. However, widespread adoption faces limitations due to high initial costs, technical complexities in injection timing and dosage, and risks associated with improper substance use. Despite these challenges, continued research and technological advancements are expected to optimize IOI protocols and expand its applications. With innovations in biotechnology and nutrient delivery systems, IOI stands as a promising tool for improving poultry productivity, health, and sustainability. Ultimately, IOI offers a strategic solution to meet the rising global demand for efficient and high-quality poultry production. This review critically examines pre-hatch nutritional interventions in poultry, with emphasis on in-ovo nutrient injection (IOI). It evaluates the implications of delayed post-hatch feeding, compares IOI with conventional methods, and assesses the effects of various in-ovo nutrients on embryonic development, health, and productivity. Limitations and future directions for optimizing IOI in commercial poultry systems are also discussed.

Keywords: Chickens, Hatching, Nutritional, Strategy.

INTRODUCTION

The poultry industry has undergone remarkable transformation over the past few decades, becoming a central pillar in global food security through advancements in genetics, nutrition, housing and management practices (Maharjan and Liang 2020; Olejnik et al. 2022; Acheampong 2024; Brassó et al. 2025). Poultry meat is now one of the most consumed sources of animal protein worldwide, supported by improvements in feed efficiency, growth rates, and disease prevention strategies (Neeteson et al. 2023; Korver 2023; Vlaicu et al. 2024). Modern

broiler chickens, for instance, can achieve market weight within a few weeks, offering an affordable and sustainable protein source for the growing global population. In the pursuit of improved efficiency and sustainability in poultry production, considerable attention has been directed toward optimizing embryonic development and early post-hatch performance. Among the innovative strategies explored, IOI has emerged as a promising technique, enabling the direct delivery of bioactive substances such as nutrients, vaccines, antioxidants, and nanomaterials into the developing embryo. Over the past few years, the effects of feeding schedules, early post-hatch feeding and in-ovo

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nutrient administration on the performance of young chicks have been extensively studied (Hassan et al. 2021; Hassan et al. 2022; Sarhan et al. 2023; Hassan et al. 2023). Originally developed in the early 1980s for vaccine delivery, particularly against Marek's disease, IOI has evolved into a multifunctional strategy aimed at improving embryonic development and post-hatch outcomes. The advent of automated systems, such as the Embrex Inovoject®, has enabled high-throughput and precise delivery, significantly reducing labor while ensuring uniform treatment (Peebles 2018). One of IOI's key advantages is its ability to address nutrient deficiencies during the perinatal period, when chicks may experience delayed access to external feed (Givisiez et al. 2020). Through early nutrient delivery, IOI enhances gastrointestinal development, nutrient absorption and immune function.

Feed additives are used to enhance feed efficacy and promote birds' health (Al-Marzooqi et al. 2024), which align with global efforts to reduce antibiotic use in animal agriculture. Recently, biotechnology has expanded IOI applications through the inclusion of nano-based compounds. Nano-copper and nano-zinc, for example, improve nutrient bioavailability, exhibit antimicrobial and antioxidant properties and support immune and skeletal development (Khalil et al. 2023; Issa et al. 2024; Hassan et al. 2025). Moreover, in-ovo administration of antioxidants like vitamin C and selenium has been shown to alleviate the negative effects of heat stress, a growing concern in warmer regions (El-Senousey et al. 2018). Probiotic and immune modulator can also be delivered in-ovo to support early gut microbiota development and disease resistance. Amino acids such as arginine and methionine support muscle growth and immunity, while carbohydrates like glucose and maltodextrin serve as immediate energy sources, enhancing hatchability and early growth (Zangeronimo et al. 2023). Technical variables such as injection timing, volume, and site must be carefully managed to prevent embryo injury or inconsistent outcomes. Additionally, the high cost of automation may restrict its use among small-scale

producers (Roto et al. 2016). This review aims to focus on pre-hatch strategies such as in-ovo nutrient injection. It highlights the causes and consequences of delayed feeding, compares IOI with conventional methods, and examines nutrient types and their impacts on chick development, health, and productivity. The review also addresses limitations and future prospects for optimizing IOI in modern poultry systems.

Causes of delayed access to feed in chicks

Delayed access to feed in newly hatched chicks is primarily due to the hatch window (HW), the time gap between the first and last chick hatching in a batch, which can range from 24 to 72 hours (Khaliduzzaman et al. 2022). This variation is influenced by breeder flock age, egg characteristics (e.g., weight, shell thickness, porosity), and embryo sex (Willemsen et al. 2010). Chicks from older hens and lighter eggs often hatch earlier, and female embryos typically hatch before males. Additionally, hatchery practices, such as blending eggs from different flocks and variations in pre-incubation egg storage conditions, can further widen the HW (Erataral 2023). In addition, the typical commercial approach to handling broiler chicks involves hatching at a central hatchery, followed by grading, counting, vaccinating and transporting the chicks to the broiler farm (Fig. 1). Beyond hatching, significant delays occur during hatchery processing. Early-hatched chicks may wait 24–48 hours for access to feed and water due to procedures such as sorting, sexing, vaccination, and transport (Giersberg et al. 2021). Environmental stressors, including darkness, loud noise, dust, and temperature fluctuations during transport and handling, can further impact chick welfare (Riber and de Jong 2023). Although commercial hatcheries prioritize operational efficiency and hygiene, these practices may compromise early nutrition (Yamak et al. 2023). Innovations like early feeding systems in hatcheries are being developed to offer chicks immediate access to feed, water, and light post-hatch (Hanna et al. 2024), addressing concerns over delayed nourishment and supporting optimal development and health.

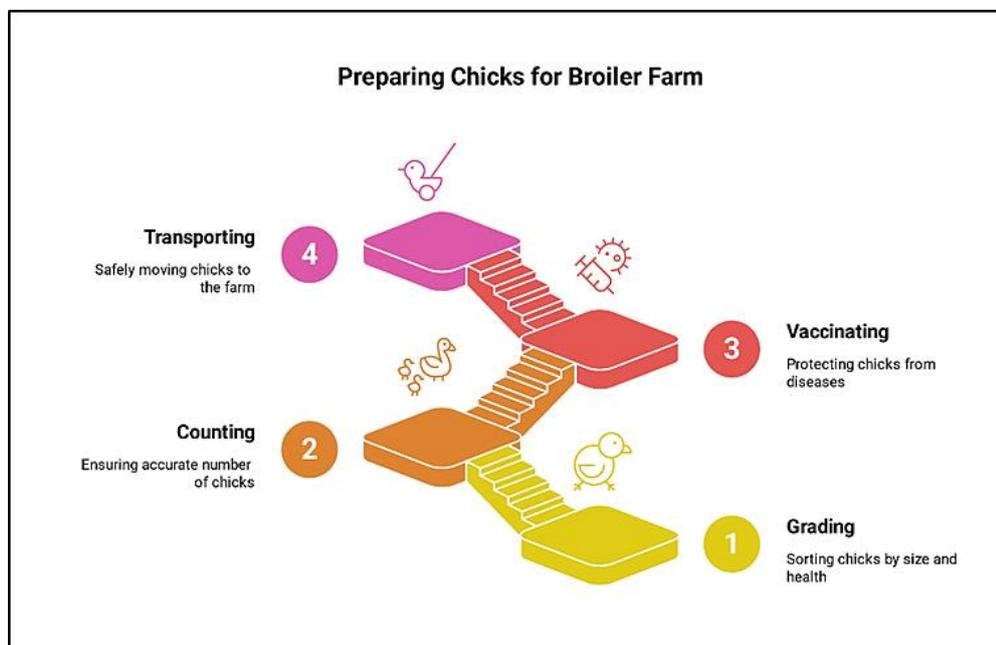


Fig. 1: Overview of hatchery processing steps: grading, counting, vaccinating, and transporting newly hatched chicks to the broiler farm.

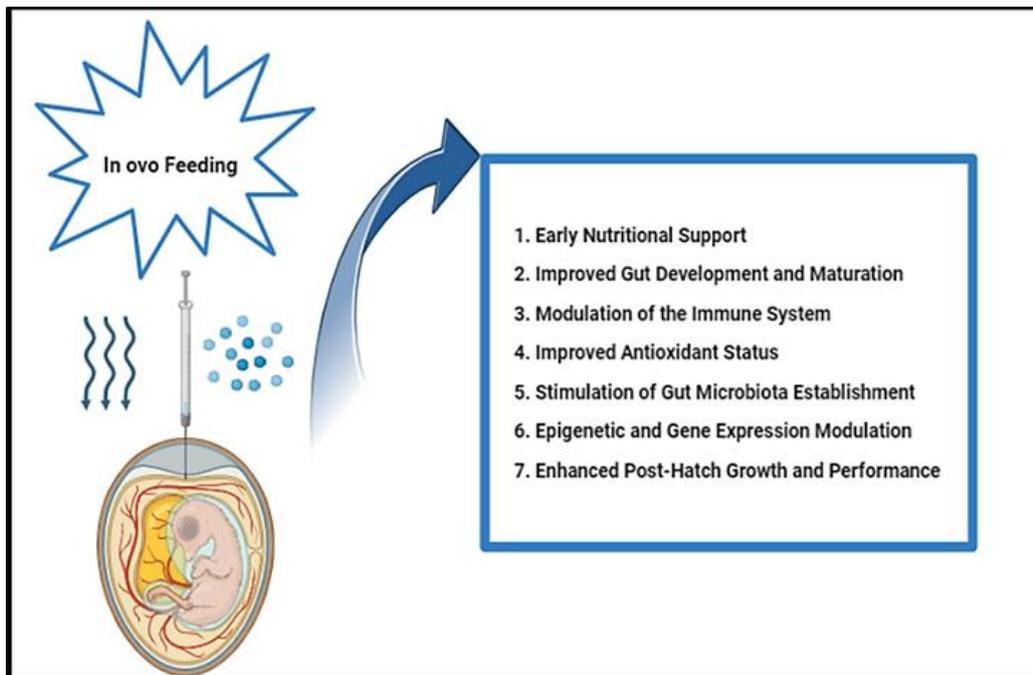


Fig. 2: Summary of the Mode of Action of In Ovo Feeding in Poultry.

In-Ovo injection technique: precision and technological advancement

Fig. 2 summarizes the mode of action of in ovo feeding in poultry involves the early delivery of nutrients or bioactive substances directly into the developing embryo, typically during the late stages of incubation. This early intervention provides immediate nutritional support, enhancing energy metabolism. It improves gut maturation, boosts immune and antioxidant systems, and promotes early microbial colonization. This approach also influences gene expression, resulting in better hatchability, early growth, and overall chick health and performance in the post-hatch period. It typically performed between days 17 and 19 of incubation, IOI targets specific embryonic compartments such as the amniotic fluid, yolk sac (YS), or allantois, depending on the desired biological outcome (Roto et al. 2016). The amniotic route is commonly used for rapid nutrient absorption, while the YS allows for sustained release (Hassan et al. 2024). Modern IOI systems utilize automated machinery equipped with sterilization protocols, optical sensors, or ultrasound-guided tools to ensure accurate injection depth and minimize the risk of contamination or embryonic injury (Guérin et al. 2024; Niloofar et al. 2024). These systems enhance efficiency and safety, making them suitable for large-scale poultry operations. Automation reduces labor demands and improves consistency, enabling the simultaneous administration of vaccines, nutrients and growth promoters in a single step (Pandey et al. 2021; Abd El-Azeem et al. 2024). Ultimately, IOI supports early organ development, strengthens immunity, and enhances growth performance, aligning with sustainable poultry production goals (Khalil et al. 2023; Issa et al. 2024).

Methods of the IOI Technique

Manual IOI: Traditional Methods

Before the development of automated in-ovo injection (IOI) systems, manual methods were commonly used to deliver bioactive substances into developing embryos. These techniques, though effective in research

and small-scale operations, demand high precision to avoid embryonic injury and contamination (Ahmed et al. 2023). Manual IOI typically involves selecting viable, fertilized eggs via candling, disinfecting the shell, and injecting substances into targeted embryonic compartments like the yolk sac, amnion, or allantois using a fine needle. The injection site is then sealed with sterile wax or adhesive to prevent moisture loss and contamination (Pandey et al. 2021). This method offers flexibility in controlling the injection's volume, location, and type of substance, making it ideal for experimental use (Kadam et al. 2013). However, it is labor-intensive, time-consuming, and prone to human error, limiting its use in commercial hatcheries. Despite these drawbacks, manual IOI remains valuable in research for evaluating bioactive compounds (El-Sabrou et al. 2019).

Automated IOI systems

Automated in-ovo injection (IOI) systems mark a major advancement in poultry production, offering efficient, accurate, and biosecure delivery of bioactive substances to embryos. These systems are now standard in commercial hatcheries, ensuring uniform application of vaccines, nutrients, and growth enhancers during incubation to improve hatchability and chick performance (Peebles 2018). Automated IOI enables high-throughput processing of thousands of eggs per hour, with advanced needle designs that minimize eggshell damage and reduce contamination risks (Ahmed et al. 2023; Guérin et al. 2024). Imaging and sensor technologies aid in accurately targeting compartments like the yolk sac or amnion (Kadam et al. 2013), while integrated disinfection and sealing mechanisms maintain hygiene and egg viability (Pandey et al. 2021). These systems enhance precision, consistency, and scalability, making them indispensable in modern hatcheries (El-Sabrou et al. 2019). Despite high initial costs limiting use in smaller operations, their advantages far outweigh manual methods (Niloofar et al. 2024). Compared to manual methods, automated systems offer unparalleled advantages (Table 1).

Table 1: A Comparison Table: Manual vs. Automated IOI Systems

Aspect	Manual IOI	Automated IOI
Efficiency	Time-consuming and labor-intensive, suitable for small-scale operations.	High-throughput systems process thousands of eggs per hour, ideal for large-scale hatcheries.
Accuracy	Relies on operator skill; prone to inconsistencies in dosage and target site precision.	Advanced imaging and sensing technologies ensure precise targeting and consistent dosages.
Cost	Low initial investment; accessible for small-scale producers and researchers.	High initial costs for equipment and maintenance; cost-effective in large-scale operations.
Biosecurity	Disinfection of eggshells and sealing with wax or adhesive reduce contamination risks but depend on manual handling.	Integrated sterilization mechanisms and minimal manual handling enhance biosecurity.
Scalability	Not practical for large-scale operations due to its labor-intensive nature.	Designed for scalability, handling tens of thousands of eggs daily.
Applications	Ideal for research and experimental settings, allowing flexibility in treatment types and volumes.	Primarily used in commercial settings for vaccinations, nutrient delivery, and IOF.
Advantages	Flexible and precise for specialized treatments; cost-effective for small sample sizes.	Highly efficient, uniform, and biosecure; reduces labor costs and human error.
Challenges	Prone to operator error, including inaccurate targeting and inconsistent dosing.	High installation and maintenance costs; less accessible for smaller operations.
Technological Features	Basic tools: syringes, fine needles, and manual sealing materials.	Includes advanced needle designs, imaging technologies, integrated sterilization, and automated sealing mechanisms.
Future Directions	Remains relevant in niche research settings for its adaptability.	Advances in AI and robotics are expected to further improve precision, efficiency, and cost-effectiveness.

The IOI position and timing

The timing and site of IOI are critical for optimizing the effectiveness of delivered substances such as vaccines, nutrients, and bioactive compounds, as well as ensuring embryo safety and hatchability. Typically, IOI is performed during the late stages of incubation, between day 17 and 18, when organogenesis is nearly complete and the embryo has developed immunocompetence (Uni and Ferket 2004; Ferket et al. 2006). The most commonly used injection sites include the air cell, yolk sac (YS), amniotic fluid, allantoic membrane, and directly into the embryo (Saeed et al. 2019). Among these, the amniotic route is preferred due to its higher efficacy embryos naturally ingest the amniotic fluid before hatching, allowing substances to be absorbed via enteric tissues (Peebles 2018). According to Saeed et al. (2019), the choice of injection site and timing directly influences hatchability and post-hatch chick quality. They found that injecting into the amniotic fluid or embryo body rather than the air cell or allantoic cavity provided over 90% protection efficacy, compared to less than 50% in the latter routes. Accurate injection depth is essential: insufficient depth may deposit substances in non-target areas like the air cell, whereas excessive depth may damage the embryo. For specific compounds like amino acids, early-stage injection into the YS at day 7 has also shown potential benefits for hatchability. However, for most applications, late-term delivery to the amniotic fluid, ideally around 18.8 days of incubation, is considered optimal for achieving consistent results in immune response and nutrient uptake (Abd El-Azeem et al. 2024). Overall, understanding embryonic development stages and post-hatch physiology is vital for selecting the appropriate IOI timing and position, which in turn enhances chick performance and survival rates.

Advantages of *in-ovo* nutrient injection technique over traditional feeding strategies

The IOI offers distinct advantages over traditional post-hatch feeding, particularly during the critical perinatal period when embryonic nutrient reserves are limited (Das et al. 2021). In commercial hatcheries, chicks may remain without access to external feed and water for up to 72 hours

post-hatch due to processing and transportation. During this period, they rely solely on residual yolk for energy, which can delay organ development, suppress immunity, and compromise performance (Bhuiyan et al. 2011). Research has demonstrated that IOI can bridge this nutritional gap. For instance, Reicher et al. (2022) reported that injecting a solution of L-glutamine and leucine on day 17 of incubation significantly increased intestinal cell proliferation and differentiation, promoting early maturation of the gastrointestinal tract. This facilitates faster yolk absorption and enhances nutrient uptake post-hatch. Additionally, IOI promotes early colonization of beneficial gut microbiota, supporting immune development and reducing vulnerability to pathogens (Roto et al. 2016). The IOI enables the precise delivery of nutrients, bioactives, or vaccines directly into the egg, ensuring the developing embryo receives essential compounds before hatching (Das et al. 2021). This pre-hatch support improves hatchability, organ development, feed efficiency, and post-hatch growth. Unlike conventional strategies, IOI reduces the lag between hatching and first feed intake, thereby lowering early mortality risks and dependency on antibiotics. Furthermore, IOI's precision delivery minimizes nutrient wastage and enhances bioavailability, making it cost-effective and scalable in commercial systems (Bednarczyk et al. 2021). By integrating nutrition, immunity, and health in a single step, IOI aligns with modern poultry sustainability goals, supporting the production of high-performance, antibiotic-free poultry (Madej and Bednarczyk 2016). Thus, IOI stands out as a transformative tool in contemporary poultry management.

Types of nutrients used in the IOI

The IOI of proteins and amino acids (AAs)

Proteins and AAs are fundamental to embryonic development, as they serve as building blocks for protein synthesis, tissue growth, and immune function. Studies have shown that the injection of AAs *in-ovo* significantly influences embryonic development and post-hatch performance. Moreover, AAs such as L-threonine and L-methionine injected into broiler eggs have been shown to support immune function, improve digestive organ

development, and enhance growth performance (Nabi et al. 2022). The incorporation of these nutrients into the IOF process has become a key tool in the poultry industry, promoting healthier, more robust chicks from hatch. Gonzales et al. (2023) evaluated the effect of IOI of folic acid and sulfur AAs on the mitotic activity of myocytes, performance, relative organ weight, hematological values, and characteristics of broiler chicken carcasses. They found that the inoculation of folic acid on the 1st day of incubation increases gene expression and improves the performance of broilers.

The IOI of carbohydrates and lipids

Carbohydrates, particularly glucose and simple sugars, serve as primary energy sources for developing embryos, especially during the final stages of incubation when metabolic demands peak. The IOI of carbohydrates has been shown to enhance embryonic glycogen reserves, supporting energy metabolism, organ maturation, and improved post-hatch growth and survivability. Glucose administration has been linked to better embryonic energy status, increased hatchability, and accelerated development of the digestive tract, promoting efficient nutrient absorption after hatching (Zangeronimo et al. 2023). Additionally, complex carbohydrates such as starch and maltodextrin have demonstrated benefits by stimulating gut development, enhancing villi growth, and increasing digestive enzyme activity, leading to improved feed efficiency and body weight gain. Zangeronimo et al. (2023) conducted a meta-analysis confirming that in-ovo carbohydrate supplementation consistently improves hatchling energy reserves and early performance. Recent research has also explored synergistic effects with other bioactives. Kpodo and Proszkowiec-Weglarz (2023) reported that combining carbohydrates with amino acids improved energy metabolism, protein synthesis, and immunity which fosters early gut microbiota colonization, enhancing gastrointestinal health and reducing pathogen susceptibility. Timing and dosage are critical. Injection during days 17-19 of incubation coincides with peak embryonic energy requirements, maximizing the benefits of supplementation (Zangeronimo et al. 2023). However, excessive doses may induce osmotic stress and reduce hatchability. Advances in automated IOI technology have facilitated precise, large-scale nutrient delivery, making this approach feasible in commercial hatcheries (Dong et al. 2013). Lipids, particularly omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), are essential for membrane integrity, energy storage, and hormone synthesis. IOI of PUFAs has been shown to enhance embryonic viability, reduce oxidative stress, and support immune function and neural development (Bakayaraj et al. (2012).

The IOI of vitamins

Vitamins are essential organic compounds that regulate numerous physiological processes, including metabolism, immune response, and growth. In-ovo injection (IOI) of vitamins has emerged as a promising tool to enhance embryonic development and post-hatch performance in poultry. Among these, vitamin C (ascorbic acid) has received significant attention. Ali et al. (2024) stated that injecting 3mg into the amnion improved

hatchability, embryo and chick weight, reduced mortality, and improved intestinal morphology and liver function (El-Senousey et al. 2018). However, Mousstaaid et al. (2022) noted no detrimental effects at doses up to 25mg. Zhu et al. (2020) showed that yolk injection at day 11 improved immune and antioxidant status. ELDeeb et al. (2020) also confirmed improved hatchability and post-hatch gut development following vitamin C or glucose injection. Vitamin D3 is crucial for bone mineralization and immune regulation. Its IOI enhances skeletal strength and gut immune defense through antimicrobial peptide production. Vitamin B12 supports DNA synthesis, red blood cell production, and energy metabolism. Its IOI improves growth rate, feed efficiency, and reduces mortality. Additionally, vitamin E acts as a potent antioxidant. Araújo et al. (2019) demonstrated that its IOI improves oxidative status, immunity, and overall chick health. Collectively, in-ovo vitamin supplementation represents an effective strategy to optimize chick viability and performance.

The IOI of trace elements

Trace elements such as zinc, copper, selenium, and manganese are essential for avian embryonic development, playing critical roles in enzymatic functions, antioxidant defense, immune responses, and tissue growth. These micronutrients help reduce oxidative stress, enhance bone mineralization, and support immune system maturation factors directly influencing hatchability and early post-hatch performance.

Selenium is a key component of glutathione peroxidase, an enzyme vital for neutralizing oxidative damage (Alagawany et al. 2021). In-ovo selenium supplementation, especially in nano-form, has been shown to improve embryonic development, immunity, and survivability due to its superior bioavailability and antioxidant capacity (Heidary et al. 2020). It also supports thyroid hormone production and immune cell proliferation, enhancing early metabolic and growth processes. Zinc, involved in over 300 enzymatic activities, is crucial for protein synthesis, nucleic acid metabolism, and gut development. In-ovo zinc improves intestinal integrity, bone formation and immune responses. Oladokun and Adewole (2020) reported improved post-hatch performance and enhanced gene expression related to immunity and growth following zinc IOI. Copper supports iron metabolism, hemoglobin synthesis, and energy production. IOI of copper enhances erythropoiesis, angiogenesis, and cardiovascular development, improving oxygen and nutrient delivery (Hassan et al. 2022). Nano-copper also exhibits antimicrobial effects, contributing to better chick health and reduced pathogen load (Issa et al. 2024). Manganese is vital for skeletal development and acts as a cofactor for manganese superoxide dismutase, an antioxidant enzyme. In-ovo manganese improves bone mineralization and hatchability. Overall, combining these trace elements especially in nano-size through IOI offers a comprehensive strategy to enhance chick viability, immune competence and performance post-hatch (Heidary et al. 2020; Matuszewski et al. 2021).

The IOI of bioactive compounds and specialized nutrients

Bioactive compounds such as probiotics, prebiotics,

and symbiotics are increasingly used in IOI to enhance gut health, immune function, and early growth performance. Probiotics like *Lactobacillus* and *Bifidobacterium* species improve intestinal microbiota balance and nutrient uptake in hatchlings (Pender et al. 2017). Melittin, a peptide from bee venom, offers antimicrobial and immunomodulatory benefits, while royal jelly provides essential nutrients such as vitamins, proteins, and fatty acids that support immune activation and antioxidative status, leading to improved growth (Hassan et al. 2021; Khalil et al. 2023). Recent innovations include injecting specialized nutrients such as L-carnitine, which enhances lipid metabolism, mitochondrial energy production, hatchability, and post-hatch growth. Creatine pyruvate, an energy-dense compound, improves muscle development, feed conversion, and growth rates by facilitating rapid ATP regeneration. Enzymes like phytase and protease have also shown promise in IOI, improving early nutrient digestion and absorption. Phytase enhances phosphorus bioavailability, while protease aids protein breakdown, supporting muscle growth and feed efficiency. Incorporating these bioactive agents into IOI protocols can significantly improve chick quality and productivity, while reducing dependence on antibiotics and synthetic growth promoters (Ayalew et al. 2023).

Moreover, the IOI of various nutrients such as proteins, amino acids, carbohydrates, vitamins, minerals, antioxidants, probiotics, and immune enhancers has been shown to enhance embryonic development, hatchability, and post-hatch performance (Table 2). Most injections

were administered between days 14 - 19, commonly into the amniotic sac, with broiler chickens being the primary focus. Nutrients were selected based on specific developmental roles: proteins and amino acids for growth, carbohydrates for energy, vitamins and antioxidants for immune support, and probiotics for gut health. The findings highlight the importance of timing, injection site, and nutrient type in optimizing IOI outcomes. Future studies should aim for standardized protocols and explore synergistic combinations for enhanced efficacy.

Challenges and limitations of using the IOI technique

The effective application of IOI requires precision to deliver nutrients without harming the developing embryo. Critical challenges include selecting the optimal injection site, timing the procedure to align with embryonic development, and maintaining sterility. Variability in egg size and shell strength complicates protocol standardization (Das et al. 2021). Large-scale use also demands sophisticated automated equipment, which may be cost-prohibitive for smaller operations. High initial costs for equipment and training hinder broader adoption. Moreover, the use of advanced additives, such as nanoparticles or nutraceuticals, raises economic concerns due to their higher production costs (Aguilar-Pérez et al. 2023). Improper dosages may lead to toxicity, nutrient imbalances, or embryonic defects, negatively affecting hatchability (Das et al. 2021). The long-term safety of novel additives, particularly nano-particles requires further investigation to ensure animal welfare and food safety.

Table 2: Selective studies for various nutrients used for the IOI.

Type of Nutrient	Description	Species	Day of Injection	Injection Position	References
Proteins (e.g., Royal Jelly, Albumin)	Supplies critical AA for embryonic development; supports growth and enhances immune response post-hatch.	Chicken (Layer)	16-18	Air Cell or Amniotic Sac	Oliveira et al. 2015; Joshua et al. 2016; Hassan et al. 2025
Amino Acids (e.g., L-Carnitine, Methionine)	Amino acids enhance energy metabolism, reduce oxidative stress, and improve hatchability traits.	Quail	14-18	Amniotic Sac	Nibedita et al. 2016; Gao et al. 2017; Shebl and Hassan 2018
Carbohydrates (e.g., Glucose, Maltose)	Provides an immediate energy source for the developing embryo; improves hatchability and chick vigor.	Chicken (Broiler)	18	Air Cell	Yair et al. 2014; Bhanja et al. 2015
Vitamins (e.g., Vit. E, & D3)	Essential for antioxidant protection and immune enhancement; improves hatchability and post-hatch performance.	Chicken (Broiler)	17-18	Amniotic Sac	Khaligh et al. 2018; Xu et al. 2021
Minerals (e.g., Nano-Zinc, Selenium)	Nano-minerals enhance bioavailability, improve growth, and provide antioxidant effects. Zinc boosts immunity, and selenium supports metabolic functions and oxidative stress management.	Chicken (Broiler)	Day 18	Amniotic Sac	Oliveira et al. 2015; Ghobadi et al. 2015; Nassif et al. 2023
Trace Elements (e.g., Iron, Manganese)	Essential cofactors for enzymatic reactions; enhances blood development and overall metabolic activity.	Chicken (Broiler)	18	Amniotic Sac or Yolk Sac	Mogahid et al. 2018; Yaripour et al. 2024
Antioxidants (e.g., Nano-Copper, Vitamin C)	Protects against oxidative damage, enhances immune function, and promotes healthy embryonic development.	Chicken (Broiler)	Day 17-18	Yolk Sac or Amniotic Sac	Issa et al. 2024; Ali et al. 2024
Hormones (e.g., Thyroxine, Growth Hormone)	Accelerates embryonic development and metabolic activity; improves hatchability and post-hatch growth.	Turkey	Day 19	Amniotic Sac	Hu et al. 2013; Moosavinasab et al. 2015
Immune Enhancers (e.g., Melittin, Beta-Glucans)	Boosts early immune response; enhances disease resistance in the critical post-hatch period.	Chicken (Layer)	Day 17-18	Amniotic Sac	Khalil et al. 2023; Sogunle et al. 2018
Probiotics (e.g., <i>Bacillus subtilis</i> , <i>Lactobacillus</i>)	Enhances gut microbiota development; promotes better nutrient absorption and post-hatch performance	Chicken (Broiler)	Day 17-19	Amniotic Sac or Yolk Sac	Huang et al. 2024; Shehata et al. 2024
Enzymes (e.g., threonine)	Improves nutrient digestibility post-hatch by enhancing enzymatic activity in the digestive system.	Quail	Day 14	Yolk Sac	Kadam et al. 2008

Future perspectives and research directions

Future research should prioritize the development of novel nutrient formulations that maximize bioavailability and efficacy while minimizing potential risks. Encapsulation techniques, such as nano-encapsulation, could enhance the stability and controlled release of bioactive compounds (Bist et al. 2024). Additionally, integrating synergistic combinations of nutrients, such as antioxidants with trace elements, may further optimize embryonic development and post-hatch performance (Das et al. 2021). The rise of precision poultry farming offers opportunities to enhance the application of IOI. Real-time monitoring systems, utilizing imaging or biosensors, could refine the timing and dosage of nutrient delivery to match the specific needs of individual embryos (Bist et al. 2024). Such integration could also help identify eggs with higher developmental potential, thereby improving overall productivity. The IOI holds the potential to improve the sustainability of poultry production by enhancing resource utilization efficiency and reducing dependency on post-hatch feed additives (Abd El-Azeem et al. 2024). Future studies should investigate the long-term effects of IOI on factors such as feed conversion ratios, disease resistance, and environmental footprint. Addressing these aspects could position the technology as a cornerstone of sustainable poultry farming.

Conclusion

The IOI technique represents a significant innovation in poultry science, offering a proactive approach to enhancing embryonic and early post-hatch development. Its ability to deliver targeted nutrients and bioactive compounds has profound implications for improving chick health, productivity, and overall sustainability in poultry farming. While the method is not without challenges, such as technical complexity and economic feasibility, ongoing advancements in automation, nutrient formulations, and nano-technology promise to overcome these barriers. By bridging the gap between embryonic development and post-hatch performance, IOI has the potential to redefine best practices in poultry production, ensuring a more sustainable and efficient future for the industry.

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