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In Ovo Feeding: A Revolutionary Approach In Poultry Nutrition

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Abstract

IN OVO feeding (IOF) is a new nutritional technique which involves putting nutrients straight into the growing embryo while it is being incubated. This method has drawn a lot of interest since it can improve hatchability, improve post-hatch performance, and enhance embryonic development in chicken. The main advantages, workings, and difficulties of IOF are examined in this review. In order to promote immunological response, intestinal health, and physiological growth, nutrients such carbs, amino acids, vitamins, minerals, probiotics, and plant extracts are frequently given. IOF stimulates the chick's innate and adaptive immunological responses, improves intestinal architecture, encourages early microbial colonization, and increases energy availability during late incubation. Furthermore, it has been demonstrated that IOF increases feed conversion and growth rates while lowering mortality and early-life stress. In order to further promote embryonic development, recent developments have included the use of bioactives like curcumin, garlic, and ginger in addition to hormone and growth factor supplements like thyroxine and IGF-1. But the method is not without its drawbacks. Outcome variability brought on by variations in nutrition type, dosage, timing, and injection technique continues to be a major obstacle. Widespread commercial usage is further restricted by the risk of contamination and the absence of established processes. Notwithstanding these drawbacks, IOF has the potential to completely transform the production of poultry, especially in systems devoid of antibiotics. These restrictions are being addressed by developments in biotechnology and automated delivery methods, which establish IOF as a game-changing instrument for effective and sustainable chicken production.

Keyword: In ovo feeding, embryonic nutrition, poultry development, hatchability, immune function.

Introduction

The poultry industry is one of the fastest-growing sectors in global agriculture, driven by the increasing demand for high-quality animal protein [1]. Achieving optimal chick development and growth from the earliest stages improves overall performance and reduces production costs [2]. Nutrient delivery usually starts after hatching. Recent research, however, has demonstrated a crucial window for intervention during the embryonic stage [3]. The long-term preservation of hatching eggs when the incubator's ability to lay eggs is insufficient is one element that can impact broiler output growth [3, 4].

The developing embryo depends only on the yolk, albumen, and amniotic fluid for nourishment during incubation [5, 6]. However, the embryo's metabolic needs surpass the resources available near the end of incubation (about day 17), especially for glucose and amino acids [6]. Development may be hampered by this nutritional deficiency, leading to weak chicks and a delayed time to post-hatch adaptation [7]. Studies have indicated that storing hatching eggs lowers the hatching rate, but longer egg storage times may negatively impact embryonic development and hatching rate [4].

Initially investigated in the early 2000s, in ovo feeding (IOF) entails injecting exogenous materials straight into the egg before hatching [7]. This method makes it possible to improve immune system maturation, nutritional utilization, and embryonic development [8]. Even before hatching, nutrients are absorbed by the gastrointestinal tract, promoting metabolic programming, gut development, and enzyme activity [5, 8, 9, 10].

Nutrients are introduced to the embryonic environment before ovo injection, and the chick uses them after hatching. According to El-Senousey et al.

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[11], these nutrients and yolk sac reserves can support the system and metabolism, allowing hens to continue growing, maturing, and eating healthily. Compounds that may enhance the intestinal health of chicks throughout the rearing phase have been studied in relation to in ovo injection [12].

This review will highlight recent studies on in ovo feeding techniques, the benefits of using in ovo feeding, and the effects of nutrients injected on growth performance and animal health post-hatching. In addition, this review will mention the most significant challenges and limitations of in ovo feeding technology and the recent advances in research trends.

Benefits of In Ovo Feeding

Improved Hatchability and Chick Quality

Several studies have shown that IOF can increase hatchability rates by lowering late embryonic mortality. According to Kadam et al. [7], chicks from IOF-treated eggs frequently have more developed organs, a greater body weight, and improved yolk absorption. Providing extra nutrients before the bird starts external feeding promotes improved organ development, especially in the immunological and gastrointestinal systems. Uni & Ferket [8] discovered that IOF promotes enterocyte proliferation, crypt depth, and villus height—all necessary for effective post-hatch nutrition absorption. Furthermore, the liver's glycogen stores and enzymatic activity are enhanced, promoting early development and metabolism [13].

Enhanced Immune Function

By delivering immunomodulatory drugs or nutrients such as zinc and arginine, IOF can improve disease resistance and decrease reliance on antibiotics by fortifying innate and adaptive immune responses [8]. IOF can prime the immune system by providing immune-stimulants or essential nutrients that influence immunological responses, such as arginine, zinc, or vitamin E. Kadam et al. [7] observed enhanced weight and function of the thymus and bursa of Fabricius in chicks treated with IOF. Antibody levels against common poultry infections have enhanced with injections of particular vitamins and amino acids [14].

Gut Health and Microbiota

Intestinal villi and digestive enzyme activity are encouraged to mature earlier by IOF. When used with probiotics or prebiotics, it promotes the colonization of beneficial microorganisms, improving gut resilience and nutritional absorption [7]. Chicks that get in ovo injections of prebiotics, probiotics, or synbiotics have a better gut flora even before they hatch. Increased populations of *Bifidobacteria* and *Lactobacillus* species inhibit the colonization of pathogenic bacteria such as *Salmonella* [12]. Resistance to leaky gut and systemic infections is increased when the gut epithelium's tight junctions are more intact [15].

Early Growth and Feed Efficiency

Chicks treated with IOF have far superior post-hatch development because of increased metabolic programming and food use. Production is more economical due to decreased feed conversion ratios (FCR) [8]. Numerous advantages of in ovo feeding (IOF) substantially impact the performance, health, and early development of poultry. IOF is a proper hatchery management technique because of its immunological, physiological, and financial benefits. Chicks that get nutrient supplements before hatching have higher metabolic activity and are better equipped to consume feed, which increases their desire and improves their ability to recognize feed, ultimately resulting in improved growth performance [16].

Reduced Post-Hatch Stress

When they hatch, chicks experience physiological stress from outdoor exposure, transportation, and lack of food or water. Injecting carbohydrates in ovo reduces stress-related weight loss by providing instant energy reserves after hatching [17, 18]. Chicks with higher metabolic activity can better regulate their body temperature and adjust to their surroundings [18].

Long-Term Economic and Performance Gains

For poultry producers, the combined physiological benefits of IOF result in financial gains since improved immunological condition and early development lower early chick losses, which lowers mortality. The use of antibiotics will be reduced, and antibiotic-free production systems will be supported by uniform chick quality and size, consistent performance, and natural immunological enhancement [7].

Substances Injected in In Ovo Feeding

A variety of physiologically active substances are introduced into the body during in ovo feeding (IOF) to promote embryonic growth and early post-hatch performance [19]. Certain physiological objectives, such as enhanced immunity, gut maturation stimulation, post-hatch performance support, or embryonic growth, are taken into consideration when choosing these drugs. The main types of chemicals used in IOF are listed below, along with information on their mechanisms and supporting research. Table 1 summarize the main effect (benefits) from injected substance used in IOF.

Carbohydrates

In late development, the embryos produce glucose by gluconeogenesis from fat or protein, first from amnion and albumen and later from muscle, and by glycolysis from their liver's glycogen reserve [20]. During the last phases of incubation, embryos experience glucose depletion. By reducing the catabolism of body reserves, in ovo administration of simple carbohydrates like glucose, maltose, and sucrose helps maintain the energy supply during the late incubation and early post-hatch phase. Uni & Ferket [8] and Ferket [17] showed that in ovo carbohydrate infusion enhances intestinal growth and hatchling body weight.

Glucose, maltose, sucrose, and dextrin are among the most commonly used carbohydrates in IOF due to their role as immediate energy sources during the energy-deficient late incubation phase, supporting thermoregulation and early chick activity. In ovo glucose injection has been demonstrated to improve chick weight, shorten hatching time, and increase glycogen stores in the muscles and liver [8].

In broiler chicken eggs, the IOF of carbohydrates through amnion on embryo day (ED) 17.5 improved the hatching process, early growth, and breast muscle hypertrophy from hatch to post-hatch Day 25. It also raised liver glycogen by two to five times [20]. Similarly, the IOF of carbohydrates improved myoblast proliferation on ED 19, which persisted until Day 5 of chicks, and increased glycogen reserves, BW, and pectoral muscle weight in both the pre-hatch and post-hatch periods [21]. By stimulating goblet cells to make mucin or by upregulating the expression of the mucin gene [22], as well as by increasing the surface area of the villus, the IOF of carbohydrates may contribute to the development of the gastrointestinal tract.

Results from the IOF on carbohydrates improving hatchability were inconsistent. IOF of carbs delayed the hatching process in broiler hens [23], reduced hatchability in turkeys [24], and boosted hatchability in a study by Uni et al. [20]. In domestic pigeons, hatchability was enhanced by injecting 200 µl of a 0.75% saline solution with a lower level of carbohydrates (1.5% maltose and 1.5% sucrose, or 2.5% maltose and 2.5 % sucrose). In contrast, hatchability was decreased by injecting a high level of carbohydrates (4.5% maltose and 4.5% sucrose) [25]. One possible explanation for the reduced hatchability is hyperglycemia brought on by an excessive injection of carbohydrates into the embryo. According to Ding et al. [26], a dose of glucose that is closer to 1 g/kg can cause hyperglycemia in the embryos, which can result in abnormalities of the heart and/or limbs, disturbed cell proliferation, apoptosis, and, ultimately, embryonic death and decreased hatchability. These are explained by variations in the kind and amount of carbs that are injected. Among other things, the IOF of carbohydrates may raise the body weight of hatchlings, decrease embryonic metabolism, and raise the egg's internal temperature [23].

Amino Acids

Arginine, threonine, methionine, and lysine are essential and functional amino acids frequently utilized in IOF procedures. Protein synthesis, tissue growth, immunological function, and enzyme production are all aided by in ovo amino acid supplementation [18]. For instance, injecting arginine in ovo improves immunological response and nitric oxide generation, while combining cysteine and methionine boosts antioxidant qualities through glutathione formation. Additionally, the synthesis of gut mucin and barrier function are supported by in ovo injection of threonine [20]. Yehia et al. [18] found that injecting methionine and lysine enhanced post-hatch performance and survivability.

Eggs incubated under heat stress (39.6°C for 6 h/d) from ED 10 to ED 18 received an injection at ED 17.5 of a mixture of L-methionine and L-cysteine (5.9 and 3.4 mg, respectively) [27, 28]. Because methionine and cysteine are injected under heat stress, this combination decreased the level of heat shock protein-90, indicating lower levels of oxidative stress [28].

According to a study by Tako et al. [29], IOF of 1,000 µg of the leucine metabolite hydroxy-beta-methylbutyrate (HMB) through amnion on ED 17.5 increased enterocyte proliferation and differentiation, and on ED 20, decreased protein degradation, increased the surface area of the villus at Day 3, and raised BW at Day 10. In Turkey, the IOF of arginine, egg white protein, and HMB at ED 23 enhanced growth performance and the jejunal absorption of alanine and glucose post-hatch period [30].

A recent study by Ma et al. [31] found that the IOF of HMB may enhance hatchability and raise the growth of the broiler's breast muscle and body weight during the post-hatch phase. According to the scientists, the activation of muscle protein, which can restore muscle cells in the late embryonic stage when increased energy requirement is satisfied by generating glucose from muscle through gluconeogenesis, was the cause of the muscular hypertrophy [20].

Vitamins and minerals

In ovo supplementation of vitamins, especially vitamin C, D3, and E, is crucial for supporting antioxidant capacity, cellular metabolism [6], and bone development [32]. When birds experience stress from exposure to warming near the end of incubation, vitamin C helps reduce the stress-related reactions [33]. While the IOF of vitamin C did not impact feed intake and growth metrics during the starting phase, it did boost hatchability [34, 35].

Zhang et al. [36] found that IOF of 3 and 6 mg of ascorbic acid increased body weight (BW), average daily gain (ADG), and average daily feed intake (ADFI) during the grower phase (Day 14–28).

However, a high dose (36 mg) had an adverse effect on growth performance, and 12 mg beneficially decreased plasma Malondialdehyde during the grower and finisher phase of a bird's life. Another study found that ascorbic acid IOF increased post-hatch growth, improved leg muscle development, and elevated plasma antioxidant levels in broilers. IOF of vitamin C improved hatchability, reduced embryonic mortality, and raised the plasma level of vitamin C in freshly born chicks [35, 37].

Antioxidant enzyme levels, immune organ development, and hatchability were markedly enhanced by in ovo injection of vitamin E [38]. Vitamin E and C injections give the embryo immunological modulation, metabolic control, and antioxidant protection. Oxidative stability and hatchability are enhanced by vitamin E. As potent antioxidants, vitamins E and C reduce oxidative stress in developing embryos [37, 38]. In Chinese yellow broiler chicken eggs, El-Senousey et al. [11] also showed that the IOF of 3 mg vitamin C through the air sac at ED 18 enhanced immunity and raised antioxidant synthesis at Day 1.

Vitamin D3 injections in ovo improved calcium metabolism and bone mineralization. Vitamin D is necessary to transfer calcium through CAM and its use from the eggshell [39]. A lack of vitamin D in eggs can lead to hypoxia, a calcium deficiency, and a failure to reach the pipping pre-hatching posture, which is necessary for the switch to pulmonary respiration. It can also raise the risk of late embryonic death [39]. Eggshell thickness may increase as a result of less calcium dissolution. According to Peebles & Brake [40], an embryo from an egg with a thinner shell has a stronger shell, can pip successfully, and has a greater hatchability.

When 1.2 μg of vitamin D3 or its more active form, 25(OH)D3, is injected in ovo, the blood level of 25(OH)D3 in the broiler embryos on Day 19.25 rises. Hatchability and hatchling BW are unaffected by this injection, nevertheless [41]. However, another study conducted by the same researchers revealed that by decreasing the inflammatory response indicator at post-hatch age, the in ovo injection of a greater dose (2.4 μg) of 25(OH)D3 at Day 18 had boosted the breast meat yield and BW [42].

Mineral supplementation in ovo is crucial for immunological response, bone formation, and enzyme activation [6]. Antioxidant enzymes such as glutathione peroxidase and superoxide dismutase require zinc and selenium. Selenium lowers oxidative stress, but zinc boosts enzyme activity and immunological responses [29]. Skeletal development is supported by calcium or vitamin D3 administration in ovo [42]. Zinc is necessary for DNA replication, cell division, immunological regulation, and improving chick immune function and antioxidant status when fed in ovo [29].

Probiotics, and Prebiotics

Probiotics (such as Lactobacillus and Bacillus subtilis) can be delivered in ovo to improve gut health, lower the pathogen load, and promote early gut colonization with good bacteria [43, 44]. Probiotic bacteria such as Lactobacillus species, Bacillus subtilis, and Enterococcus are administered intravenously to alter the gut microbiota [45]. While prebiotics act as a substrate for good bacteria and boost broiler performance, probiotics primarily work to strengthen the intestinal barrier and compete with infections [3, 46].

Based on flux balance studies, probiotic strains could be chosen for IOF to help produce the metabolites at the ideal level. In order to alter the metabolic environment of the embryo's gut, more effective strains of microorganisms should be injected because only a small number can be done [44]. Certain bacteria use dietary prebiotics as substrates, which promotes the growth and abundance of such bacteria [47]. Prebiotic-induced in ovo stimulation results in early-life microbial regulation, which can have long-term effects on immunity, nutrition absorption, and gut histomorphology [48]. The common prebiotics that are supplemented and proven to be advantageous for poultry growth and development are inulin, galactooligosaccharides (GOS), and mannanoligosaccharides (MOS) [49].

In slow-growing hens, the GOS injected in ovo can reduce immunological responses from heat stress. When given at 12 ED, GOS (3.5 mg/egg) decreased the expression of cytokines (IL-10 and IL-1p40) in hens with chronic HS and decreased the expression in chickens with acute HS [50]. Another study on heat-stressed chickens found that IOF of GOS (3.5 mg/egg at ED12) enhanced FCR, decreased foot-pad dermatitis, and raised BW and daily feed intake. Slawinska et al. [12] state that these directly connect to HS.

Plant Extracts, Natural Bioactives, and Phytochemicals

Natural antioxidants and phytochemicals in ovo supplements lower oxidative stress, control inflammation, and boost immunity. Natural substances with antibacterial, anti-inflammatory, and antioxidant qualities include curcumin, resveratrol, ginger, and garlic extract. According to El-Shall et al. [51], in ovo curcumin enhanced the oxidative state, growth rate, and liver health of chicks. Additionally, they discovered that taking garlic oil supplements in ovo affects gastrointestinal and immunological function [51].

Hormones and Growth Factors

Growth-promoting substances like thyroxine and IGF-1 (insulin-like growth factor-1) have been shown to affect post-hatch performance and embryonic development rates. Supplementing with this growth factor has been discovered to increase the broiler's post-hatch performance by promoting muscle growth and metabolic rate [52].

Thyrotropin-releasing hormone (TRH) levels in chicken embryos' brains demonstrated that TRH levels rose as incubation came to a close, with the rise in hypothalamic TRH being nearly 15 times greater from ED 14 to the chicks' first day [6, 53]. TRH was administered in ovo through the air cell membrane or the tiny end of the eggs, improving hatchability and increasing embryonic blood plasma T3 and T4 in turkeys [53].

A study by Afsarian et al. [54], broiler chicken eggs were exposed to 15°C for one hour on ED 11, 13, 15, and 17 in order to alter the eggshell temperature, and 65 ng of thyroxine was injected at ED 18. Thyroxine injection and temperature control resulted in more first-grade chicks, a lower yolk sac weight, and a higher body weight at hatch. Furthermore, these hens exhibited a lower mortality rate and developed ascites 22–42 days after hatching when exposed to conditions that cause ascites [54].

Challenges and Limitations

Poultry researchers now face new opportunities and challenges in maximizing poultry output due to ongoing advancements in *in ovo* technology, which have opened up new possibilities for perinatal nutrition [6]. One of the primary issues that impacts the IOF program's response is the absence of defined procedures for the in ovo delivery of chemicals into the eggs [55]. In order to prevent embryonic damage, precise injection location and volume are essential [55]. However, not all hatcheries consistently possess the tools or know-how to apply IOF [7]. Another difficulty is the possibility of contamination from incorrect procedure, which could introduce infections into the egg and result in poor hatchling health or embryonic mortality [8].

Administered digestible nutrients in ovo can improve bird quality, increase glycogen reserves, accelerate the development of the total digestive tract, improve skeletal health, enhance muscle growth rate, increase body weight gain, improve feed conversion, and enhance immune function [5].

Despite the challenges yet to be overcome, the in ovo technique has shown great potential for commercial adaptation in the poultry industry [56]. The automation of in ovo technology has reduced the labor cost, the risks of pathogenic contamination, and the wastage of vaccines and bioactive inoculants, as the in ovo delivery machines can self-sterilize, detect, and inoculate only the viable embryos [56].

Over the past two decades, progress in ovo feeding (IOF) has evolved significantly due to developments in molecular biology, biotechnology, and the worldwide movement to produce poultry without antibiotics. The focus of current research has changed from increasing growth rates to investigating processes that support resilience, long-term health, and sustainable production [6, 52]. IOF might become common in modern hatcheries as sustainability, animal welfare, and product quality gain more attention [56].

Conclusions

One of the newest and most effective ways to nourish embryos for better health and function before and after hatching is by feeding them in ovo. It is essential because it gives the chicks enough nutrition and supplements to eat in the production farm after hatching. Vitamins, amino acids, glucose, and supplements like probiotics, prebiotics, exogenous enzymes, and hormones are utilized in ovo feeding. Numerous studies have demonstrated that injecting various compounds in ovo can reduce mortality, boost immunity, increase body weight at hatch and post-hatch, decrease yolk sac weight, increase the number of quality hatched chicks, and protect against harmful gut microbes through competitive exclusion.

In ovo feeding is a revolutionary concept in poultry science. By bridging the gap between embryonic and post-hatch nutrition, IOF improves chick viability, growth performance, immunological function, and overall production efficiency by supplying certain nutrients and bioactives during crucial developmental phases. Even if there are still standards, cost, and implementation issues, these will probably be resolved with more study and technical developments. IOF will continue to be crucial in determining the direction of poultry nutrition as the industry shifts toward more efficient and sustainable methods.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Animal Production, the University of Jordan, Amman, Jordan.

TABLE 1. Main effect from substance injected in ovo feeding

Substance	Main effect
Carbohydrate	*- Helps maintain the energy supply during late incubation and early post-hatch phase* *- Enhances intestinal growth and hatchling body weight* *- Improve chick weight, shorten hatching time* *- increase glycogen stores in the muscles and liver* *- Improved myoblast proliferation*
Amino Acids	*- Enhanced post-hatch performance and survivability* *- Increased enterocyte proliferation and differentiation* *- Decreased the level of heat shock protein-90, indicating lower levels of oxidative stress* *- Decreased protein degradation, increased the surface area of the villus*
Vitamin C	*- Reduce the stress-related reactions and boost hatchability* *- Increased body weight, average daily gain, and feed Intake during the grower phase*
Vitamin D3	*- Improved calcium metabolism and bone mineralization* *- Necessary to transfer calcium through CAM and its use from the eggshell*
Vitamin E	*- Enhance oxidative stability and hatchability* *- Reduce oxidative stress in developing embryos*
Zinc	*- Boosts enzyme activity and immunological responses* *- Improving chick immune function and antioxidant status*
Selenium	*- Lowers oxidative stress*
Calcium	*- Bone formation*
Probiotics	*- Improve gut health, lower the pathogen load, and promote early gut colonization* *- Strengthen the intestinal barrier and compete with infections*
Prebiotics	*- Promotes the growth and abundance of such bacteria* *- Reduce immunological responses from heat stress*
Phytochemicals	*- Lower oxidative stress, control inflammation, and boost immunity*
Hormones	*- Promoting muscle growth and metabolic rate* *- Increase the broiler's post-hatch performance*
TRH	*- Improving hatchability* *- Increasing embryonic blood plasma T3 and T4 in turkeys*
Thyroxine	*- Higher first-grade chicks, and lower yolk sac weight,* *- Higher body weight at hatch and lower mortality rate*

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التغذية داخل المخصب: نهج ثوري في تغذية الدواجن

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الملخص

تعد التغذية داخل البيضة تقنية غذائية حديثة تقوم على إدخال العناصر الغذائية مباشرة إلى الجنين النامي أثناء فترة التحضين. وقد حظيت هذه الطريقة باهتمام واسع نظرًا لقدرتها على تحسين نسب الفقس، وتعزيز الأداء بعد الفقس، ودعم النمو الجنيني في الدواجن. قد أظهرت الأبحاثأهم مزايا وآليات وتحديات هذه التقنية. تُزوَّد الأجنة بالكربوهيدرات، والأحماض الأمينية، والفيتامينات، والمعادن، والبروبيوتيك، والمستخلصات النباتية من أجل تعزيز الاستجابة المناعية، وصحة الأمعاء، والنمو الفسيولوجي، كما تعمل التغذية داخل البيضة على تحفيز الاستجابات المناعية الفطرية والمكتسبة للصيصان، وتحسين بنية الأمعاء، والتشجيع المبكر للمستعمرات الميكروبية، وزيادة توافر الطاقة خلال المراحل المتأخرة من التحضين. علاوة على ذلك، أُثبت أن هذه التقنية تحسن معدلات النمو وكفاءة التحويل الغذائي، مع تقليل نسب النفوق. وقد شملت التطورات الحديثة في هذا المجال استخدام مركبات حيوية نشطة مثل الكركم والثوم والزنجبيل، بالإضافة إلى الهرمونات وعوامل النمو مثل هرمون الثيروكسين، وذلك لتعزيز التطور الجنيني بشكل أكبر. ومع ذلك، لا تخلو التقنية من التحديات؛ إذ يظل تباين النتائج الناجم عن اختلاف نوعية العناصر الغذائية والجرعة وموعد وطريقة الحقن عقبة رئيسية. كما أن خطر التلوث وغياب بروتوكولات قياسية يقيّد من الاستخدام التجاري الواسع. وعلى الرغم من هذه القيود، تُعد التغذية داخل البيضة تقنية واعدة قد تُحدث تحولًا جذريًا في إنتاج الدواجن، خاصة في الأنظمة الخالية من المضادات الحيوية. وتُسهم التطورات في مجال التكنولوجيا الحيوية وأساليب التوصيل الآلي في تجاوز هذه التحديات، مما يرسخ مكانة التغذية داخل البيضة كأداة رائدة لإنتاج الدواجن بكفاءة واستدامة.

الكلمات الدالة: التغذية داخل البيضة، التغذية الجنينية، تطوير الدواجن، نسب الفقس، الوظائف المناعية.