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Embryonic Development and Nutritional Modulation through *In Ovo* Feeding in Broiler Chickens

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ABSTRACT This review evaluates current knowledge on *in ovo* feeding (IOF) as a nutritional strategy to improve embryonic development and post-hatch performance in broiler chickens. Modern broiler chickens exhibit rapid growth and substantial early metabolic demand, making the embryonic period a critical phase in which nutrient availability strongly influences physiological maturation. Concurrently, eggs from older breeder hens often display altered yolk composition, reduced albumen quality, and weakened antioxidant status, collectively imposing nutritional and metabolic constraints on the developing embryos. IOF has emerged as a promising approach to alleviate these limitations by delivering nutrients, amino acids, vitamins, probiotics, or other bioactive compounds directly into specific embryonic compartments during late incubation. Evidence from numerous studies indicates that IOF promotes gastrointestinal maturation, enhances immune function, supports metabolic transitions, and improves early growth performance. Administration of amino acids, particularly arginine, tryptophan, and threonine, further contributes to gut health, muscle development, and antioxidant capacity. Despite these demonstrated benefits, variation in injection timing, dosage, and absorption dynamics underscores the need for standardized protocols and deeper mechanistic understanding. Overall, this review provides an updated synthesis of current findings and outlines practical considerations for the effective application of IOF as a nutritional strategy to enhance embryonic development, improve chick quality, and alleviate breeder-age-related constraints in modern broiler production.

(Key words: arginine, broiler chicken, fertile egg, *in ovo* feeding, threonine, tryptophan)

INTRODUCTION

Modern broiler production is showing exceptionally rapid growth and a markedly shortened production cycle, with commercial birds reaching market weight in only 35 to 42 days post-hatch (Uni et al., 2012). Consequently, the approximately 21-day incubation period represents more than one- third of the bird's entire lifespan, making embryogenesis a critical window that determines not only hatchability but also post-hatch performance, robustness, and immune competence (Givisiez et al., 2020). During this relatively brief developmental interval, the embryo undergoes organogenesis, myofiber formation, and the establishment of metabolic regulatory systems, processes that rely entirely on the nutrient reserves contained within the egg and are shaped by the maternal age, genetic line, and physiological condition of the breeder hen (King'ori, 2011; Raffaelli and Stern, 2020; Ding et al., 2022). The fertile egg functions as a self-contained biological system composed of the embryo, yolk, albumen, and shell, each contributing essential substrates and structural support throughout incubation (Wong and Uni, 2020). Because these resources are fixed at the time of lay and cannot be replenished, the embryo must meet all metabolic and biosynthetic needs using a limited nutrient reserve (Foye, 2005). The major factor of this limitation is maternal aging, which alters yolk lipid composition, albumen viscosity, shell quality, and the supply of micronutrients, thereby imposing additional metabolic challenges on the developing embryo (Alo et al., 2024). In response to the limited and nonrenewable nutrient environment of the egg and to mitigate the adverse effects of breeder aging, *in ovo* feeding (IOF) has emerged as an innovative strategy to enhance embryonic nutrition (Uni and Ferket, 2004). Initially IOF introduced as a route for vaccine delivery, IOF was later adapted for the administration of bioactive compounds, nutrients, and probiotics during late incubation (Uni and Ferket, 2004). Typically, IOF is performed between embryonic day (ED) 17 and 19 when the digestive tract and absorptive pathways are functionally competent, and depending on the

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objectives of supplementation, substances may be deposited into the yolk sac, amniotic cavity, or air cell (Pandey et al., 2021). Numerous studies have demonstrated that IOF of amino acids, vitamins, minerals, and probiotics can enhance gastrointestinal maturation, increase digestive enzyme activity, improve oxidative defense, and support superior early growth performance (Foye et al., 2006; Bakyaraj et al., 2012; Yadgary et al., 2013). In addition to its short-term developmental impacts, IOF is increasingly viewed as a tool for early metabolic imprinting, with the potential to modulate gene expression, epigenetic marks, and long-term physiological outcomes (Jha et al., 2019). However, several limitations exist in the practical application of IOF. Optimal dosing, injection volume, and site-specific absorption dynamics remain major variables requiring standardization. Additionally, concerns regarding hatchability, contamination, and mechanical injury underscore the need for refined methodology and clearer mechanistic understanding Optimal dosing, injection volume, and site-specific absorption dynamics remain major variables requiring standardization (Das et al., 2021). Therefore, this review integrates current evidence from developmental physiology and nutritional science to assess IOF as a strategy that extends beyond nutrient supplementation, influencing early-life physiological development and subsequent growth performance (Jha et al., 2019).

EMBRYONIC DEVELOPMENT

1. Embryonic Growth and Physiological Development

Embryonic development represents more than one-third of the broiler's lifespan, making it a critical period in which minor disturbances can substantially influence post-hatching performance and overall productivity (Givisiez et al., 2020). In avian species, development begins with a fertilized egg containing a blastodisc positioned on the yolk surface, which subsequently differentiates into the blastoderm through the formation of epiblast and hypoblast layers (Eyal-Giladi and Kochav, 1976). Early in development, the embryo lies flattened atop the yolk but progressively reorients into a vertical position as it grows within the yolk mass (Starck, 2020). During the first 1 to 2 days, epiblast cells proliferate and migrate to form the primitive streak, from which the ectoderm, mesoderm, and endoderm emerge and establish the foundation of organogenesis (Cinnamon et al., 2025). Embryonic development proceeds through a sequential pattern involving primitive streak formation, establishment of the vascular system, organogenesis, ossification, and feather formation, ultimately resulting in a fully developed chick at hatch (Davey and Tickle, 2007). The ectoderm differentiates into the nervous system, skin, and feathers, while the mesoderm gives rise to the skeletal, muscular, and circulatory systems, and the endoderm develops into the digestive and respiratory systems (Raffaelli and Stern, 2020). The embryonic heartbeat begins around days 2 to 3, followed by the formation of elbow, knee joints, and the beak by days 5 to 6 (Hamburger and Hamilton, 1992; Naieb et al., 2013). Subsequently, feathers and claws emerge between days 11 to 14, and the internal organs migrate into the abdominal cavity from days 15 to 17, completing major structural formation (Hamburger and Hamilton, 1992). During this period, the yolk sac, amniotic fluid, and chorioallantoic membrane (CAM) develop from the ectoderm and support the embryo by providing nutrients and removing waste products (Baggott, 2009). Around ED 17, the embryo begins ingesting amniotic fluid, a process that coincides with rapid structural maturation of the intestinal mucosa and villi (Givisiez et al., 2020). During this late phase of development, oxygen demand increases, yet oxygen diffusion remains limited by the eggshell. Consequently, the embryo repositions its head toward the air cell around ED 18 to prepare for pulmonary respiration (Tona et al., 2003; Dayan et al., 2023). Skeletal muscle development accelerates markedly between ED 15 and 20, peaking shortly before hatch (Fisher, 1958). Myogenesis is regulated by myogenic regulatory factors such as myoblast determination protein (MyoD) and myogenic factor, which act within myotome-derived precursor cells to drive myogenic commitment and differentiation (Ouyang et al., 2017). Extensive myoblast proliferation occurs between ED 5 and 12, after which these cells fuse to form primary myotubes that subsequently develop into secondary fibers (Velleman, 2007). Maturation of myofibers is characterized by sarcomere organization and alignment of Z-lines (Velleman, 2007). Muscle growth consists of hyperplasia and hypertrophy, where hyperplasia occurs primarily during embryogenesis, and the

number of muscle fibers established during this period does not increase after hatching (Smith, 1963). Post-hatch muscle accretion is mainly due to hypertrophy, supported by the fusion of satellite cells derived nuclei into existing muscle fibers (Allen et al., 1979). Satellite cells, located between the basal lamina and sarcolemma, remain essential for postnatal muscle repair and hypertrophic expansion (Mauro, 1961).

2. Metabolic Transitions during Incubation

In the early stages of embryonic development, oxygen availability is limited due to the immature state of the CAM (Moran, 2007). As a result, the embryo initially relies on anaerobic glycolysis supported by a small endogenous carbohydrate reserve (Givisiez et al., 2020). Glucose derived from the yolk and albumen is metabolized to pyruvate and subsequently converted to lactate. Until sufficient oxygen becomes available, lactate accumulates and is later transported to the liver for gluconeogenesis (Christensen et al., 2003). This Cori cycle remains active during the first week of incubation, when CAM function is still insufficient (De Oliveira, 2007). Because carbohydrates in the yolk are limited, they sustain embryonic metabolism only during the initial days of incubation (Freeman and Vince, 1974). By approximately day 8 of incubation, the CAM becomes functional, allowing efficient gas exchange and ensuring the increasing oxygen requirements of the rapid developing embryo (Phillips and Williams, 1944; Baumann and Meuer, 1992). This transition coincides with a major metabolic shift in which fatty acid oxidation becomes the predominant energy pathway, while reliance on carbohydrates declines (Foye et al., 2006; De Oliveira, 2007). Yolk lipids originate from the maternal liver, where they are synthesized, packaged into very-low-density lipoproteins (VLDL), and transported to the ovary for deposition into developing oocytes (Hall and Mckay, 1993; Walzem, 1996). Thus, the lipid profile of the yolk reflects the composition of maternal VLDL (Speake et al., 1998). During incubation, the yolk sac membrane (YSM) plays a central metabolic role by absorbing yolk lipids and delivering them to the embryo (Noble and Cocchi, 1990). Lipids are stored in cytoplasmic droplets, hydrolyzed through lysosomal fusion (Lambson, 1970), and re-esterified in the endoplasmic reticulum before being secreted as VLDL for embryonic utilization (Speake et al., 1998). By day 14 of incubation, the embryo has completed most structural development and enters a plateau phase of oxygen consumption (Christensen et al., 1996). During this stage, energy metabolism shifts again toward carbohydrates because oxygen availability becomes limiting relative to metabolic demand (Donaldson and Christensen, 1991). Since eggs contain minimal free carbohydrate, glucose during this stage is synthesized de novo from endogenous proteins and amino acids (Matthews and Holde, 1990; Christensen et al., 2001). As hatching approaches, the embryo begins ingesting amniotic fluid and accumulates glycogen within the liver and skeletal muscle to prepare for the high metabolic demands of internal and external pipping (Donaldson and Christensen, 1982; Moran, 2007). These glycogen reserves support muscle activity, thermoregulation, and basal metabolism, and remain the sole energy source until exogenous feed becomes available post-hatch (De Oliveira et al., 2008). Near day 21, the YSM becomes a primary site of glucose metabolism and is characterized by substantial glycogen accumulation and upregulation of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (Christensen et al., 2001; Yadgary and Uni, 2012). As glycogen stores are depleted, the embryo increasingly relies on protein catabolism, primarily utilizing amino acids from the pectoral muscles for gluconeogenesis (Keirs et al., 2002; Foye et al., 2006). Collectively, the YSM, liver, and skeletal muscle operate as coordinated metabolic organs that sustain the energy requirements of the developing embryo. During mid-incubation, the YSM hydrolyzes yolk lipids into fatty acids and glycerol, which are utilized for β-oxidation and gluconeogenesis, respectively (Klasing, 1998; De Oliveira et al., 2008). The liver, initially immature, begins storing glycogen during mid-incubation and becomes the principal site of gluconeogenesis following internal pipping, maintaining glucose homeostasis when yolk carbohydrate reserves are exhausted (De Oliveira et al., 2008). Meanwhile, skeletal muscle, particularly the pectoralis and pipping muscles, accumulates glycogen and undergoes active glycogenolysis and proteolysis immediately before hatch to support the intense muscular activity required for hatching (Pulikanti et al., 2010).

3. Nutrient Absorption and Transfer in Late-Term Embryos

During late incubation, particularly around ED 19, the yolk sac becomes internalized into the abdominal cavity and accounts for approximately 15 to 20% of the embryo's body weight (BW), serving as the primary nutrient reservoir until hatch (De Oliveira et al., 2008). Although lipid uptake from the yolk is minimal during early embryogenesis, lipid mobilization accelerates markedly during the final phase of development, providing the major metabolic fuel for the embryo (Yadgary et al., 2010). Docosahexaenoic acid, which is critical for neural and retinal development, is preferentially mobilized during this stage and coincides with the near doubling of embryonic brain weight (Wong and Uni, 2020). Within yolk sac epithelial cells, lipid-soluble nutrients are esterified by cholesterol acyltransferase, packaged into low- density lipoproteins, and transported via the yolk vein to the embryonic liver (Noble, 1986; Shand et al., 1993). Hydrolysis of lipids by the yolk sac lipases release free fatty acids and glycerol, with the former undergoing β-oxidation to supply ATP and the latter serving as a substrate for gluconeogenesis (Romanoff, 1960; Foye et al., 2007). Yolk absorption proceeds rapidly during the final hours before hatch and is largely completed approximately 14 hours pre-hatch (Freeman and Vince, 1974). As this process concludes, the yolk sac regresses and is incorporated into the abdominal cavity, continuing to supply nutrients during the immediate post-hatch period (Wong and Uni, 2020). During late embryogenesis, direct nutrient absorption through the yolk stalk also becomes increasingly important. The yolk stalk, which connects the yolk sac to the jejunum, expands substantially as hatch approaches, forming an open conduit for nutrient transfer (Noy et al., 1996). From approximately ED 18 onward, intestinal growth accelerates dramatically. Relative intestinal weight increases disproportionately to BW as villus height (VH), enterocyte proliferation, and crypt activity intensify (Uni et al., 2003; Tako et al., 2004). Concurrently, the expression and activity of digestive enzymes and nutrient transporters located on the brush-border membrane rise sharply (Uni et al., 1999). For instance, sucrase-isomaltase and sodium-glucose cotransporter 1 become highly expressed immediately before hatch, enabling efficient digestion and absorption during the initial feeding period (Uni et al., 1999; Foye et al., 2007). Throughout most of incubation, hepatic metabolism relies primarily on fatty acid oxidation (Surugihalli et al., 2022). Glucose is synthesized endogenously and stored as glycogen in the liver, yolk sac, and skeletal muscle when available in excess (Noble and Cocchi, 1990; Speake et al., 1998). As the embryo transitions into the pre-hatch period, physiological hypoxia develops due to high metabolic demand relative to oxygen availability (Dayan et al., 2023). Under such conditions, carbohydrate-based metabolism predominates, supported by rapid hepatic and muscular glycogenolysis that elevates circulating glucose (De Oliveira et al., 2013). Hepatic gluconeogenesis is similarly activated to produce glucose from amino acids and other non-carbohydrate precursors (Givisiez et al., 2020). These processes are tightly regulated by endocrine mediators, including insulin, glucagon, and thyroid hormones, to maintain glucose homeostasis (Lu et al., 2007; Moran, 2007). As pulmonary respiration initiates, the yolk sac becomes a major site of glucose production, characterized by substantial glycogen accumulation and elevated expression of gluconeogenic enzymes such as PEPCK and G6Pase (Uni et al., 2005; De Oliveira et al., 2008). The embryonic endocrine system tightly regulates nutrient absorption and metabolic transitions during late development. Thyroid hormones and corticosterone play central roles in elevating metabolic rate and promoting tissue maturation before hatch (Groef et al., 2013). The somatotropic axis, regulated by growth hormone- releasing hormone, thyrotropin-releasing hormone, and somatostatin, modulates pituitary growth hormone (GH) secretion, which subsequently stimulates insulin-like growth factor 1 (IGF-1) production to promote muscle and skeletal development (Kim, 2010). By late embryogenesis, the hypothalamo-hypophyseal portal system is fully formed, enabling adrenal corticosterone secretion to be regulated by adrenocorticotropic hormone and corticotropin- releasing factor (Jenkins and Porter, 2004). Elevated corticosterone enhances GH secretion (Porter, 2005), and together they increase circulating triiodothyronine (T_3) levels, promoting metabolic activation, muscle differentiation, and lung maturation required for hatch (Groef et al., 2013). Pancreatic hormones also contribute to metabolic regulation. Embryonic β-cells begin secreting insulin around ED 11 to 12, promoting glycogen synthesis in response to rising blood glucose (Kikuchi et al., 1991; Givisiez et al., 2020). IGF-1 and

insulin-like growth factor 2 (IGF-2) are expressed throughout embryogenesis, with IGF-2 playing a particularly critical role in fetal tissue growth (Kocamiş and Killefer, 2003). Thus, nutrient absorption and metabolic regulation during late embryogenesis depend on the coordinated activity of the yolk sac, liver, and intestine. Disruptions in any of these systems can impair post-hatch viability and early growth performance (Christensen et al., 1999; Applegate et al., 2005).

BREEDER HEN AGE

1. Breeder Age on Egg Quality

The age of the breeder hen exerts a substantial influence on external and internal egg quality (Tona et al., 2003; Alo et al., 2024). As hens progress in age, egg weight and yolk proportion increase, providing a larger potential energy reservoir for the developing embryo (Vieira and Moran, 1998; Peebles et al., 2001). However, this increase in yolk volume is accompanied by a decline in albumen quality; older hens produce albumen with reduced viscosity and lower protein concentration, weakening its protective capacity and diminishing its contribution to embryonic nutrition (Tona et al., 2004; Fasenko, 2007). Consequently, despite the larger yolk mass, the overall nutritional composition of the egg becomes imbalanced, with relatively lower concentrations of amino acids, vitamins, and minerals in the albumen fraction (Yildirim and Yetisir, 2004). Eggshell quality also deteriorates progressively with breeder age. Reductions in shell thickness and structural strength, combined with increased shell porosity, elevate the risks of water loss and microbial penetration during incubation (Tůmová and Gous, 2012). These age-related structural changes contribute to reduced fertility and hatchability, a pattern associated with metabolic strain in older hens, imbalanced yolk nutrients, compromised embryonic vascular development, and oxygen limitations during late embryogenesis (Fasenko, 2007). Although chicks from older breeders tend to be heavier at hatch, they often exhibit reduced vitality, increased susceptibility to dehydration, and decreased resilience to stressors, which can impair early post-hatch growth (Vieira and Moran, 1998; Hudson et al., 2004). Increased shell porosity, in particular, exacerbates excessive moisture loss and hypoxic conditions during incubation, thereby altering embryonic energy metabolism and intensifying oxidative stress (Tona et al., 2004). Breeder age is also closely related to alterations in the oxidative status of the developing embryo. Older hens accumulate greater amounts of reactive oxygen species due to prolonged metabolic activity and chronic exposure to environmental challenges (Liu et al., 2018; Gu et al., 2021). Numerous studies have reported a decline in total antioxidant capacity (TAC) and antioxidative enzymes such as superoxide dismutase (SOD) and glutathione (GSH), along with increased concentrations of malondialdehyde (MDA) in aged breeders (Liu et al., 2018; Chang et al., 2024). This compromised antioxidant defense heightens the embryonic vulnerability to oxidative stress, contributing to the elevated mortality observed in embryos from older hens (Zhang et al., 2025). In addition, proper skeletal development in avian embryos depends heavily on calcium derived from the eggshell, which supplies approximately 80% of total calcium required for embryogenesis (Tuan, 1988; Dieckert et al., 1989). Although the yolk provides roughly 30 mg of calcium, the shell contributes nearly 800 mg by the time of hatch (Alfonso-Torres et al., 2006; Yair and Uni, 2011). As breeder hens age, however, the amount of calcium deposited per shell remains relatively constant despite increasing egg size, resulting in a lower calcium density within the shell (Alfonso-Torres et al., 2006). This reduction weakens the shell structure and increases fragility, ultimately contributing to decreased hatchability and higher late embryonic mortality (Elibol and Brake, 2002).

2. Nutritional Imbalance in Older Breeders

Eggs produced by older broiler breeders are typically larger and contain a higher yolk to albumen ratio, a characteristic that contributes to an inherent nutritional imbalance within the egg (Şahan et al., 2014). Previous studies have shown that the yolk of aged hens contains a greater lipid content but a lower protein content than that of younger hens (Yadgary et al., 2010). Although this shift increases the amount of available metabolic energy for the developing embryo, it simultaneously limits the supply of indispensable amino acids required for cellular differentiation, tissue formation, and overall embryonic growth (Yadgary et

al., 2010). Interestingly, a comparative evaluation of yolk from 32- and 52-week-old breeders reported higher concentrations of essential amino acids, including Met, Cys, Lys, Thr, Trp, Arg, and Ile, in yolks from the older flock (Santos et al., 2022). Nevertheless, the overall decline in yolk protein density and the disproportionate expansion of lipids indicate a reduction in protein quality relative to embryonic requirements. Moreover, eggs from older hens contain lower levels of antioxidant compounds such as carotenoids and vitamin E, rendering embryos more susceptible to oxidative damage (Cherian, 2008). Resultant oxidative stress can induce lipid peroxidation, DNA damage, and mitochondrial dysfunction, ultimately compromising embryonic viability and post-hatch physiological resilience (Li et al., 2020). Breeder age also influences yolk lipid composition, particularly the relative distribution of major fatty acids (Nielsen, 1998; Burnham et al., 2001). Because the embryo relies heavily on yolk lipids to meet its energetic demands, age-related shifts in fatty-acid profiles can directly affect hatchability and early growth (Washburn, 1990; Speake et al., 1998). With advancing maternal age, yolk typically exhibits increased proportions of polyunsaturated fatty acids (PUFA), especially arachidonic acid (C20:4 n-6), whereas linoleic acid (C18:2 n-6) and myristic acids (C14:0) tend to decrease (Cherian, 2008; Yilmaz-Dikmen and Sahan, 2009). A reduction in these fatty acids has been associated with lower hatchability and elevated late-stage embryonic mortality, likely reflecting age-related declines in fatty-acid synthesis deposition efficiency and yolk formation (Yilmaz-Dikmen and Sahan, 2009). In addition, limited expression of metabolic enzymes and restricted oxygen availability during embryogenesis constrain the effective utilization of yolk fatty acids (Uni et al., 2003; Addo et al., 2018). Because PUFA are highly prone to peroxidation, their increased proportion heightens the embryo's vulnerability to oxidative injury (Panda and Cherian, 2014). Beyond yolk changes, deterioration in albumen quality constitutes a second major physiological constraint in eggs from older hens. The thinning of the eggshell accelerates CO_2 diffusion, thereby reducing the CO_2 binding capacity of the albumen and elevating albumen pH (Nasri et al., 2020). Elevated pH interferes with embryonic cellular metabolism and diminishes the buffering capacity of the albumen, creating a less favorable developmental environment (Chang et al., 2024). Albumen viscosity is primarily maintained by the structural integrity of the ovomucin-lysozyme complex, but an alkaline shift destabilizes this complex, promoting albumen liquefaction and reducing viscosity (Kato et al., 1970; 1985). These changes impair the albumen's protective functions and diminish its role as a reservoir for amino acids and antimicrobial proteins, thereby compounding the nutritional and oxidative imbalance already present in the yolk (Jalili-Firoozinezhad et al., 2020). Collectively, alterations in yolk and albumen composition exert detrimental effects on embryonic development, reducing post-hatch vitality, stress tolerance, and immune competence (Cherian, 2008; Yadgary et al., 2010). Thus, although eggs from older hens may appear advantageous due to their larger size, they frequently contain physiological and nutritional deficiencies that constrain hatchability and chick quality (Fasenko et al., 1992). Therefore, targeted nutritional strategies are essential to correct these imbalances and mitigate the developmental limitations associated with eggs from aged broiler breeders.

IN OVO FEEDING

1. Physiological Benefit

IOF refers to the delivery of nutrients, amino acids, vitamins, or bioactive compounds directly into the egg during embryogenesis to support embryonic development and improve post-hatch growth (Das et al., 2021). A broad range of substances, including carbohydrates, amino acids, vitamins, probiotics, and prebiotics, has been administered through this technique, which is generally performed during the late stages of incubation (Uni and Ferket, 2004; Kucharska-Gaca et al., 2017). Among the factors determining IOF efficacy, the injection site is particularly critical because it dictates the route of absorption, affects the safety of the procedure, and influences nutrient utilization efficiency (Das et al., 2021). Commonly targeted anatomical sites include the amniotic cavity, yolk sac, allantoic cavity, and air cell. The amniotic cavity is the most frequently used site, as embryos begin swallowing amniotic fluid during late development (Uni et al., 2003). Nutrients injected into the amnion enter the gastro-

intestinal tract and are absorbed through the intestinal mucosa, mimicking natural ingestion and offering an efficient uptake pathway (Uni and Ferket, 2004). The yolk sac serves as the primary nutrient reservoir for the embryo (Kucharska-Gaca et al., 2017). Compounds injected into the yolk are absorbed via the YSM and subsequently transported into the embryonic circulation (Li et al., 2016). Although yolk injection does not typically compromise hatchability, its absorption rate may be slower than that observed after amniotic administration (Das et al., 2021; Zhu et al., 2021). In contrast, the air cell provides a technically simple entry point due to its spacious structure; however, nutrients delivered into this region must be absorbed through the chorioallantoic vasculature (Siwek et al., 2018). Consequently, injections applied too close to hatch may interfere with pulmonary transition and compromise respiration function (Peebles, 2018). Albumen injection leverages the physiological transfer of albumen into the amniotic cavity near ED 19, where it becomes available for oral ingestion by the embryo (Pandey et al., 2021). The proteins are subsequently digested and absorbed through the intestinal epithelium (Peebles, 2018). The optimal timing of IOF is dependent on the biochemical characteristics of the substance delivered (Das et al., 2021). As the embryo approaches the final stages of development, intestinal absorptive pathways and metabolic systems become increasingly functional, enabling more effective utilization of the administered nutrients (Uni et al., 2005). Supplementation during this period can partially compensate for the nutrient gap that newly hatched chicks experience before first access to feed, thereby supporting digestive maturation and immune system development (De Oliveira et al., 2014; Tako et al., 2014; Stawinska et al., 2014). Consequently, IOF has gained prominence as an early nutritional intervention within broiler production systems seeking enhanced growth and improved feed efficiency (Uni and Ferket, 2004; Bakyaraj et al., 2012; Lugata et al., 2024). Late-term embryos experience progressive hypoxia and rely heavily on hepatic glycogen reserves and muscle protein catabolism to support hatching processes (Vieira and Moran, 1999; Christensen et al., 2001). Supplementation with carbohydrates, amino acids, or antioxidants via IOF has been shown to enhance liver glycogen deposition, reduce proteolysis, and improve metabolic resilience (Givisiez et al., 2020). IOF also enhances intestinal morphology, increasing VH and crypt depth (CD), thereby improving digestive capacity (Uni and Ferket, 2004). Specific amino acids, such as Arg, Thr, and Gln, stimulate enterocyte proliferation and mucin synthesis, strengthening mucosal integrity and pathogen resistance (Bhanja et al., 2014). In addition, IOF promotes the development of lymphoid organs and increases lymphocyte activity, leading to enhanced innate and adaptive immune responses (Peebles, 2018). Certain amino acids additionally contribute to cytokine regulation and exhibit anti-inflammatory effects (Ruth and Field, 2013). Hormonal modulation has also been reported, with corticosterone-related pathways implicated in promoting cellular growth and protein synthesis (Kucharska-Gaca et al., 2017). Despite these advantages, IOF carries technical risks. Penetration of the eggshell and embryonic membranes may physically damage the embryo or introduce bacterial contamination, potentially reducing hatchability (Abd El-Ghany, 2025). Therefore, meticulous hygienic procedures, including the use of sterile needles and thorough disinfection of the eggshell surface, are essential to minimize these risks (Elliott et al., 2020).

2. Applications of *In Ovo* Feeding

in ovo technology has been widely adopted in research settings and commercial poultry production (Kucharska-Gaca et al., 2017). One of the earliest and most successful applications is the administration of Marek's disease vaccines into the amniotic cavity on day 18 of incubation, a practice that has been automated in more than 90% of U.S. hatcheries (Ricks et al., 1999; Peebles, 2018). Modern hatchery equipment is capable of sterilizing each needle, detecting viable embryos, and injecting the amniotic fluid or air cell at extremely high throughput, with processing capacities ranging from roughly 12,000 to over 70,000 eggs per hour (Das et al., 2021). Recent research has expanded IOF applications to include a variety of nutrients and bioactive substances such as amino acids, carbohydrates, electrolytes, antioxidants, plant extracts, and other functional compounds, to enhance embryonic development and enhance post-hatch performance (Uni and Ferket, 2004; Peebles, 2018). In particular, IOF-based nutrient supplementation has been recognized as an effective strategy to minimize the nutrient gap experienced by chicks prior to their

first feed access and to reduce early post-hatch mortality (Pinchasov and Noy, 1993; Vieira and Moran, 1999). For instance, the injection of vitamin C has been reported to enhance hatchability, improve bone strength, and support intestinal health (Soltani et al., 2019). A recent meta-analysis further indicated that IOF of vitamin C is particularly effective in lowering feed conversion ratio (FCR), while vitamin E tends to increase hatchling BW (Ncho et al., 2024). The technology has also been applied to microbial supplements; for example, IOF of probiotics, especially *Bifidobacterium* strains, into the yolk has been shown to increase post-hatch BW and improve FCR (Leão et al., 2021). Among phytogenic additives, cinnamon extract has attracted attention due to its capacity to reduce MDA levels and improve feed efficiency (Akosile et al., 2023). Resveratrol administration has likewise been associated with increased hatchability and greater hatchling BW (Elsaadany et al., 2019). Commercially, automated injection systems have demonstrated high throughput and precision, capable of treating tens of thousands of eggs per hour with minimal hatchability loss (McGruder et al., 2011; Zhai et al., 2011). Consequently, IOF has developed from an experimental concept into a nutritional strategy that enhances immune function, growth performance, and nutrient utilization efficiency, and it is anticipated to play an increasingly central role in future commercial poultry feeding practices.

IN OVO FEEDING OF AMINO ACID

1. *In Ovo* Feeding of Arginine

Arg serves as a precursor for nitric oxide (NO), polyamines, creatine, and proline, and plays vital roles in angiogenesis, protein synthesis, and immune activation (Wu et al., 2009; Fathima et al., 2024). Polyamines and NO acts as key mediators of angiogenesis (Wu et al., 2009). NO generated from Arg enhances CAM vascularization, thereby improving oxygen and nutrient delivery to embryonic tissues (Fathima et al., 2024). Arg also activates the mammalian target of rapamycin signaling pathway, stimulating myofibrillar protein synthesis and cell proliferation, while simultaneously upregulating antioxidant enzymes to mitigate oxidative stress (Khajali et al., 2020; Linh et al., 2021). Across numerous studies (Table 1), IOF of Arg has consistently demonstrated beneficial effects on hatchability, early growth, gastrointestinal maturation, immune function, and metabolic regulation in broiler chickens. Arg is most commonly injected into the amniotic cavity or air cell between ED 14 and ED 18, with effective concentrations ranged from 0.5 to 2%. Improvements in hatchability, embryonic survival, and chick weight at hatch have been widely reported (Nayak et al., 2016; Subramaniyan et al., 2019; Nabi et al., 2025). Enhanced feed efficiency and accelerated BW gain (BWG) has been observed following IOF of Arg. Early post-hatch growth is particularly responsive, IOF of Arg increased BWG during 1−7 days (Gao et al., 2016) and improved FCR during 1−21 days (Saki et al., 2013), enhanced FCR at 30 days (Omidi et al., 2020), and increased feed intake (FI) and BWG at 42−48 days (Saki et al., 2013). Additional increases in FI from 11−42 days and greater BW at 24 and 42 days have been observed (Tahmasebi and Toghyani, 2016). One of the most prominent consequences of the IOF of Arg is accelerated intestinal morphogenesis. Supplementation increases VH, VH to crypt depth ratio (VH:CD), and reduces CD, thereby improving absorptive capacity (Gao et al., 2016). Activities of digestive enzymes, including alkaline phosphatase (AKP), maltase, and sucrase rise accordingly, accompanied by upregulation of inducible NO synthase (iNOS) (Gao et al., 2016). Arg also elevates the expression of gut hormones, such as ghrelin, vasoactive intestinal peptide (VIP), and glucagon-like peptide-2 (GLP-2), which collectively support early intestinal functional maturation (Gao et al., 2016). Arg exerts strong immunomodulatory effects. Increased expression of NO synthase (NOS), iNOS, Toll-like receptor-2 (TLR-2), and TLR-4 has been observed in the duodenum, jejunum, and ileum at 21 days (Gao et al., 2017). Humoral immune indicators, including secretory immunoglobulin A (sIgA), interleukin-2 (IL-2), interleukin-4 (IL-4), and serum immunoglobulin A (IgA), are elevated following IOF of Arg (Gao et al., 2017), and higher sheep red blood cell (SRBC) antibody titers have been reported (Toghyani et al., 2019). Arg also strengthens the intestinal barrier function by upregulating *Mucin-2*, *claudin-1*, *zonula occludens-1* (*ZO-1*), and *ZO-2* (Gao et al., 2017). Furthermore, Arg enhances the expression of key nutrient-sensing receptors, including taste

Table 1. Effects of *in ovo* feeding of arginine in broiler chickens[1]

Broiler strain	Breeder age	Injection site	Injection day	Inclusion level	Positive effects[2]	References
-	52 week	Amniotic fluid	d 5	2%	Growth performance (1−21 d FCR↓, 42−48 d FI↑, ↑BWG), serum parameter (Haematocrit↓, BV↓, SV↓, H:L ratio↓, NO↑)	Saki et al. (2013)
-	50 week	Albumen	d 10	0.5, 1, 1.5%	Growth performance (42 d BWG↑, FCR↓)	Azhara et al. (2016)
Cobb 400	34 week	Amniotic fluid	d 18	0.5%	Hatching performance (hatch weight↑), growth performance (1−21 d BW↑)	Nayak et al. (2016)
-	34 week	Amnion	d 17.5	1%	Growth performance (1−7 d BWG↑), organ weight (liver↑, proventriculus↑, gizzard↑), gut health (duodenum VH↑, CD↓, VH:CD↑), hormone (duodenum ghrelin↑, VIP↑ and GLP-2↑), enzyme activities (duodenum AKP↑, maltase↑, sucrase↑, iNOS↑)	Gao et al. (2016)
-	-	Air sac	d 14	7%	Growth performance (24 and 42 d BW↑, 11−42 d FI↑), organ length (42 d jejunum↑, ileum↑), gut health (11 d jejunum CD↓)	Tahmasebi and Toghyani (2016)
Arbor Acres	34 week	Amniotic fluid	d 17.5	1%	Growth performance (1−42 d BWG↑, FI↑), gut health (42 d jejunum VH↑, CD↓, VH:CD↑, 21 d jejunum Mucin-2↑, claudin-1↑, ZO-1↑, ZO-2↑), cellular signaling (21 d jejunum mTOR↑, S6K1↑, 4E-BP1↑)	Gao et al. (2017a)
Arbor Acres	34 week	Amniotic fluid	d 17.5	0.5, 1, 2%	Growth performance (7−21 d BW↑, 1−21 d ADG↑, ADFI↑), organ weight (liver↑, proventriculus↑, gizzard↑, duodenum↑, jejunum↑, and ileum↑, hormone (jejunum ghrelin↑ and GLP-2↑), enzyme activity (jejunum amylase↑, trypsin↑, and lipase↑, jejunum AKP↑, maltase↑, sucrase↑), sensing receptors (jejunum T1R1↑, T1R3↑, CaR↑, PRC6A↑), nutrient transporters (jejunum SLC7A4↑, SLC7A6↑, SLC7A7↑, SLC3A1↑, SLC6A19↑, SLC1A1↑, SGLT1↑, GLUT2↑, GLUT5↑, FABP1↑), serum parameter (Arg↑, Ile↑, Leu↑, Met↑, Val↑, Pro↑)	Gao et al. (2017b)
Arbor Acres	34 week	Amnion	d 17.5	1%	Immune signaling (21 d duodenum NOS↑, iNOS↑, jejunum NOS↑, iNOS↑, ileum iNOS↑, duodenum and ileum TLR-4↑, jejunum TLR-2↑ and TLR-4↑), serum parameter (iNOS↑ and NO↑), organ weight (thymus↑), immune indicator (duodenum, jejunum, and ileum sIgA↑, IL-2↑, IL-4↑, serum IgA↑, IL-2↑, IL-4↑)	Gao et al. (2017c)
Arbor Acres	34 week	Amnion	d 17.5	1%	Organ weight (0, 3, 7, 21 d ↑breast), plasma parameter (21 d TP↑, ALB↑, T3↑, and T4↑), breast muscle amino acid concentrations (21 d Thr↑, Val↑, Leu↑, Phe↑, Lys↑, Arg↑, Gln↑, and Ala↑), cellular signaling (21 d breast muscle mTOR↑, S6K1↑)	Yu et al. (2018a)
Arbor Acres	34 week	Amnion	d 17.5	1%	Liver parameter (0 d glycogen↑, 0, 21 d glucose↑, 0 d G6P↑, 0 and 7 d PEPCK↑, 0 d glycogen synthase↑, 0 d glycogen phosphorylase↓), plasma parameter (0 d glycogen↑ and insulin↑), muscle parameter (0, 3, 21 d glycogen synthase↑, 0, 21 d glycogen phosphorylase↓)	Yu et al. (2018b)
-	-	-	d 8, 14, 18	0.1, 1, 2.5%	Hatching performance (1%-d 14, 18 survival rate↑, d 14 hatching rate↑, chick weight↑), stress indicator (1%-d 14 breast HSP47↓, HSP60↓, HSP70↓), muscle differentiation (breast myogenin↑, MyoD↑)	Subramaniyan et al. (2019)
Ross 308	-	Air sac	d 14	7%	Growth performance (1−42 d BWG↑, FI↑), serum parameter (31 d SRBC↑, 14 d AST↑)	Toghyani et al. (2019)
-	24 week	Amniotic fluid	d 14	0.5, 1%	Growth performance (30 d FCR↓), gut health (caecum *Coliform*↓, *E. coli*↓, *Lactobacillus*↑)	Omidi et al. (2020)
-	-	-	d 18	1%	Antioxidant capacity (0 d breast MDA↓, GSH↑, 21 d breast TAC↑)	Lu et al. (2022)

Table 1. Continued

Broiler strain	Breeder age	Injection site	Injection day	Inclusion level	Positive effects[2]	References
LiFeng	50 week	Amniotic cavity	d 17.5	1.2%	Antioxidant capacity (1 d serum GPx ↑, 21 d serum CAT ↑), cellular signaling (1 d liver NF-kB ↓)	Ge et al. (2025)
-	-	Amniotic fluid	d 17	5%	Hatching performance (hatchability ↑), organ weight (thymus ↑), carcass trait (42 d carcass ↑), plasma parameter (42 d ALT ↓, AST ↓)	Nabi et al. (2025)

[1] FCR, feed conversion ratio; FI, feed intake; BWG, body weight gain; BV, blood viscosity; SV, serum viscosity; H:L ratio, heterophil to lymphocyte ratio; NO, nitric oxide; VH, villus height; CD, crypt depth; VH:CD, villus height to crypt depth ratio; VIP, vasoactive intestinal peptide; GLP-2, glucagon-like peptide-2; AKP, alkaline phosphatase; iNOS, inducible nitric oxide synthase; NOS, nitric oxide synthase; TLR-4, toll-like receptor 4; TLR-2, toll-like receptor 2; sIgA, secretory immunoglobulin A; IL-2, interleukin-2; IL-4, interleukin-4; IgA, immunoglobulin A; ADG, average daily gain; ADFI, average daily feed intake; T1R1, taste receptor type 1 member 1; T1R3, taste receptor type 1 member 3; CaR, calcium-sensing receptor; GPRC6A, G protein-coupled receptor class C group 6 member A; SLC7A4, solute carrier family 7 member 4; SLC7A6, solute carrier family 7 member 6; SLC7A7, solute carrier family 7 member 7; SLC3A1, solute carrier family 3 member 1; SLC6A19, solute carrier family 6 member 19; SLC1A1, solute carrier family 1 member 1; SGLT1, sodium-glucose cotransporter 1; GLUT2, glucose transporter 2; GLUT5, glucose transporter 5; FABP1, fatty acid-binding protein 1; Arg, arginine; Ile, isoleucine; Leu, leucine; Met, methionine; Val, valine; Pro, proline; ZO-1, zonula occludens-1; ZO-2, zonula occludens-2; mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase 1; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; G6P, glucose-6-phosphate; PEPCK, phosphoenolpyruvate carboxykinase; TP, total protein; ALB, albumin; T3, triiodothyronine; T4, thyroxine; HSP47, heat shock protein 47; HSP60, heat shock protein 60; HSP70, heat shock protein 70; MyoD, myoblast determination protein; SRBC, sheep red blood cell antibody titer; AST, aspartate aminotransferase; Coliform, coliform bacteria; E.coli, Escherichia coli; MDA, malondialdehyde; GSH, reduced glutathione; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; ALT, alanine aminotransferase.
[2] The symbol '↑' represented an increase, while '↓' denoted a decrease.

receptor type 1 members 1 and 3 (T1R1, T1R3), calcium-sensing receptor (CaR), and G protein-coupled receptor family C group 6 member A (GPRC6A). These changes coincide with increased expression of amino acid, glucose, and fatty acid transporters, indicating broad improvement in nutrient uptake and metabolic readiness (Gao et al., 2017). Consistent with these changes, circulating concentrations of Arg, Ile, Leu, Met, Val, and Pro increase following IOF of Arg. Arg activates the mTOR-ribosomal protein S6 kinase 1 (S6K1), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) pathway in intestinal and muscle tissues, enhancing protein synthesis and muscle accretion (Gao et al., 2017; Yu et al., 2018). Correspondingly, breast muscle concentrations of several amino acids, including Thr, Val, Leu, Phe, Lys, Arg, and Gln, are elevated (Yu et al., 2018). Energy metabolic indicators also improve, with increased hepatic and muscle glycogen, upregulated glucose-6-phosphate (G6P) and PEPCK, and reduced glycogen phosphorylase activity (Yu et al., 2018). These shifts suggest improved metabolic resilience during the immediate post-hatch transition. Arg enhances antioxidant status by reducing MDA and increasing GSH and TAC in muscle tissues (Lu et al., 2022). Serum glutathione peroxidase (GPx) and catalase (CAT) activities also rise, accompanied by reduced hepatic NF-κB expression (Ge et al., 2025). Downregulation of heat-shock proteins (HSP47, HSP60, and HSP70) following Arg treatment suggests improved stress tolerance and cellular protection (Subramaniyan et al., 2019). Additionally, Arg positively influences microbial ecology by reducing Coliform and *Escherichia coli* counts while increasing *Lactobacillus* populations in the cecum, indicating a shift toward a healthier microbial environment (Toghyani et al., 2019).

2. *In Ovo* Feeding of Tryptophan

Trp is an essential amino acid that contributes not only to protein synthesis but also to the biosynthesis of serotonin and melatonin, two key molecules involved in stress regulation and behavioral stability (Moreira Filho et al., 2019). Serotonin derived from Trp supports neural development, whereas melatonin exerts antioxidative and immunomodulatory effects, protecting embryonic tissues from oxidative injury (Ebrahimi et al., 2025). Trp also participates in niacin metabolism,

indirectly reducing embryonic mortality during late incubation through improved redox balance (Qaisrani et al., 2018). Approximately 95% of Trp catabolism occurs through the hepatic kynurenine pathway, supporting the synthesis of niacin and nucleotide intermediates (Badawy, 2017). In addition, Trp-derived indole compounds have been shown to enhance intestinal epithelial function by upregulating tight junction proteins, improving mucosal integrity, and facilitating more efficient nutrient transport (Li et al., 2021). The functional relevance of Trp during embryogenesis has been demonstrated in broiler chickens (Table 2). Nayak et al. (2022) reported that IOF of 0.5% Trp into the amniotic cavity at ED 18 improved both carcass characteristics and early intestinal development. Notably, broiler chickens injecting Trp exhibited a higher proportion of breast muscle at 28 days, indicating an early advantage in lean tissue accretion. Improvements in intestinal architecture were also observed, with duodenal samples collected at 4 days post-hatch showing increased VH, reduced CD, and an elevated VH:CD ratio, collectively indicating enhanced absorptive capacity and mucosal health. These findings suggest that IOF of Trp during late embryogenesis may contribute to the establishment of a more functionally mature intestine, providing a physiological foundation that supports superior growth performance during subsequent production phases.

3. *In Ovo* Feeding of Threonine

Thr is a key component of intestinal mucin and plays a central role in maintaining the structural integrity of the mucosal barrier and supporting immune defense (Kadam et al., 2008). Thr metabolism also provides glycine and serine precursors required for glutathione synthesis, thereby supporting the antioxidant defense system (Huang et al., 2021). Thr is metabolically versatile, undergoing degradation through several enzymatic pathways. It may be cleaved by Thr aldolase to produce Gly and acetaldehyde, or oxidized by Thr dehydrogenase to form 2-amino-3-ketobutyrate, which is subsequently converted to glycine and acetyl-CoA (Strifler et al., 2024). In addition, Thr dehydratase catalyzes the conversion of Thr to α-ketobutyrate, a precursor of propionyl-CoA and succinyl-CoA, allowing Thr-derived carbon to enter the tricarboxylic acid cycle (Ding et al., 2019). IOF of Thr has consistently demonstrated beneficial effects on hatchability, early gut development, growth performance, and antioxidant capacity (Table 3). Improvements in hatching performance have been reported following amniotic injection, with 1% Thr increased chick yield and 5% Thr enhanced hatchability (Mousavi et al., 2009; Nabi et al., 2025). Productive responses during post-hatch growth have also been documented. Kadam et al. (2008) observed that yolk sac injection of Thr at 2.5 to 10% elevated BWG from 21−28 days and increased FI during 14−21 days. Similarly, Tahmasebi and Toghyani (2016) reported that 5% Thr delivered into either the air cell or amnion improved BW at 11, 24, and 42 days and enhanced FI from 1−42 days, ultimately improving carcass weight. Additional evidence showed improved BWG and FCR through 42 days (Mousavi et al., 2019). Furthermore, Nabi et al. (2025) reported enhanced hatchability, thymus weight, and carcass yield with 5% Thr. Thr also play a crucial role in the structural maturation of the small intestine. IOF of 0.5% Thr has been shown to stimulate early duodenal development, characterized by increased VH, reduced CD, and a higher VH:CD ratio at 4 days of age (Nayak et al., 2022). Supporting these findings, Tahmasebi and Toghyani (2016) reported increased jejunal and ileal segments at 42 days. The physiological benefits of Thr extend beyond morphological growth. Enhancements in metabolic and immune parameters have been observed,

Table 2. Effects of *in ovo* feeding of tryptophan in broiler chickens[1]

Broiler strain	Breeder age	Injection site	Injection day	Inclusion level	Positive effects[2]	References
Cobb 400	34 week	Amnion	d 18	0.5%	Carcass trait (28 d breast↑), gut health (4 d duodenum VH↑, CD↓, VH:CD↑)	Nayak et al. (2022)

[1] VH, villus height; CD, crypt depth; VH:CD.
[2] The symbol '↑' represented an increase, while '↓' denoted a decrease.

Table 3. Effects of *in ovo* feeding of threonine in broiler chickens[1]

Broiler strain	Breeder age	Injection site	Injection day	Inclusion level	Positive effects[2]	References
		Yolk	d 14	2.5, 5.0, 7.5, 10%	Growth performance (21−28 d BWG↑, 14−21 d FI↑)	Kadam et al. (2008)
		Amniotic fluid	d 17	1%	hatching performance (chick yield↑), growth performance (1−42 d BWG↑, FCR↓)	Mousavi et al. (2009)
		Air sac	d 14	5%	Growth performance (11, 24, and 42 d BW↑, 1−42 d FI↑), carcass trait (11 d carcass↑), organ length (42 d jejunum↑, ileum↑)	Tahmasebi and Toghyani (2016)
Ross 308		Air sac	d 14	5%	Growth performance (1−42 d BWG↑, FI↑), serum parameter (31 d SRBC↑, 14 d glucose↑, ALB↑)	Toghyani et al. (2019)
		Amniotic cavity	d 18	3, 6, 9%	Organ weight (0 d intestine↑)	Alabi et al. (2020)
Ross 308		Amniotic fluid	d 18	6%	Antioxidant capacity (serum SOD↑), gut health (jejunum VH:CD↑)	Eisa et al. (2022)
		Amniotic fluid	d 17	5%	Hatching performance (hatchability↑), organ weight (thymus↑), carcass trait (42 d carcass↑), serum parameter (42 d ALT↓, AST↓)	Nabi et al. (2025)

[1] BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SRBC, sheep red blood cell antibody titer; ALB, albumin; SOD, superoxide dismutase; VH:CD, villus height to crypt depth ratio.
[2] The symbol '↑' represented an increase, while '↓' denoted a decrease.

including increased SRBC antibody titers at day 31, elevations in serum glucose and albumin (ALB) at day 14 (Toghyani et al., 2019), and increased intestinal mass (Alabi et al., 2020). Furthermore, Thr appears to reinforce antioxidant defenses and intestinal health, as demonstrated by increased SOD activity and improved VH:CD ratio in the jejunum following 6% Thr injection (Eisa et al., 2022).

CONCLUSION

IOF has emerging as an effective strategy to enhance embryonic nutrition in modern broiler production, where rapid post-hatch growth places substantial metabolic demands on chicks. Accumulated evidence indicates that IOF supports gastrointestinal maturation, improves immune competence, and strengthens metabolic resilience, while targeted administration with amino acids such as Arg, Trp, and Thr further promotes muscle development, gut health, and antioxidant capacity. IOF has also been applied to alleviate the nutritional and physiological limitations associated with eggs from older breeders, thereby improving chick quality and early growth. However, variation in injection timing, dosage, and site-specific absorption dynamics highlights the need for further standardization of IOF protocols and a clearer mechanistic understanding of IOF-induced responses. Continued refinement of IOF techniques and additional research will be essential to achieve consistent and predictable improvements in broiler health and growth performance.

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