

Potential Implication of *in ovo* Feeding of Phytochemicals in Poultry Production

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Abstract

Hatchery's goals include maximizing revenue by achieving high hatchability with day-old birds of excellent quality. The advancement of technology has benefited the poultry sector since breeding and genetics technology have increased the rates of meat maturation in developing birds in a short period of time. Excessive use of in-feed antibiotics has been shown in studies to increase the chance of resistance to human infections. Bacterial resistance and antibiotic residues in animal products raised concerns about using antibiotics as growth promoters, eventually leading to a prohibition on using in-feed antibiotics in most industrialized nations. *In ovo* technology is a novel method for delivering bioactive chemicals to developing avian embryos. *In ovo* feeding technologies may provide additional nutrients to the embryos before hatching. The introduction of bioactive compounds has the potential to assist in decreasing and eventually eliminating the problems associated with traditional antibiotic delivery in chicken production. Phytobiotics were advocated as an alternative by researchers and dietitians. So far, several studies have been conducted on the use of phytochemical feed additives in poultry and swine feeding. They have primarily demonstrated that phytobiotics possess antibacterial, antioxidant, anti-inflammatory, and growth-stimulating properties. The antioxidant effect of phytobiotics can improve the stability of animal feed and increase the quality and storage duration of animal products. In general, the existing documentation indicates that phytobiotics improve poultry performance. To effectively and efficiently use the *in ovo* technique in poultry production and advance research in this area, it is important to have a thorough understanding of its potential as a means of nutrient delivery during the critical stage of incubation, its effects on hatching events and posthatch performance, and the challenges associated with its use. Overall, this review suggests that *in ovo* feeding of phytobiotics has the potential to improve the antioxidant status and performance of chickens.

Lay Summary

Due to their role in improving the hatching and performance of chickens, *in ovo* herbs have become increasingly popular in the poultry sector. Additionally, the quest for new growth promoters in livestock production has recently increased as a result of the restrictions on antibiotics in some countries. It has been proven that the use of *in ovo* with herbs can help increase chicken productivity. This review compiles the information available on *in ovo* feeding of phytobiotics for improving poultry productivity and highlights future perspectives.

Key words: antioxidant, growth promoter, *in ovo*, phytobiotics, stress

Abbreviations: BWG: Body weight gain, FCR: Feed conversion ratio, FI: Feed intake, g: Gram, GALT: Gut-Associated Lymphoid Tissue, GIT: Gastrointestinal tract, GSE: Grape seed extract, IOF: *In ovo* feeding, IOI: *In ovo* injection, Kg: Kilogram, Mg: Milligram, NC: Nanocurcumin, PFA: Phytochemical feed additives, RV: Resveratrol.

Introduction

The perinatal period from late-term embryo to a few days post-hatch is important for the development of the gastrointestinal tract and the immune system in poultry. In contrast to mammals that influence the growth of the fetus during pregnancy, avian species can only depend on the composition of the egg and egg microenvironment (Uni and Ferket, 2004). This critical period is crucial for the development of the gastrointestinal tract and the immune system of poultry (Uni and Ferket, 2004; Uyanga et al., 2022). This restriction on nutrient reserves may prevent freshly hatched chicks from growing and developing to their full potential (Uni and Ferket, 2004). *In ovo* feeding technologies may be used to get around this restriction by giving the embryo extra nutrients before it hatches (Uni et al., 2005).

In ovo technology was used for vaccination in poultry hatcheries many years ago (Ricks et al., 1999). Since chicken has a limited amount of nutrients available for the development of embryos, attention has focused on providing additional nutrients to the embryos (Uni et al., 2012). *In ovo* feeding of substances such as antioxidants during incubation may improve the antioxidant status of the chicken embryo and posthatch growth phases (Yigit et al., 2014; El-Saadany et al., 2019). Moreover, *in ovo* feeding of chickens with extracts from numerous plant products has enhanced their defenses against the contagious bursal virus, avian influenza virus, and fowlpox virus (Sood et al., 2013; Nyandoro et al., 2014).

The antioxidant level of the chicken embryo may be enhanced by *in ovo* injection of antioxidants because they have an effective defense against free radicals (Salary et al.,

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2014). Recently, attention has been shifted to the use of herbal additives as growth promoters and antioxidant components from herbs, spices, and their products due to their benefits (Oke et al., 2016, 2017; Oke, 2018; Voemesse et al., 2019; Tokofai et al., 2020; Kpomasse et al., 2021, 2023; Adjei-Mensah et al., 2022). With the growing interest in the use of in ovo phytobiotics, it is crucial to have a thorough understanding of the potential of in ovo feeding of phytobiotics as a method of nutrient delivery during the crucial stages of incubation, hatching events, and posthatch performance and the challenges associated with it to use the technique in poultry production effectively and efficiently and advance research in this area.

Classification of Phytochemicals

Due to the large number of phytochemicals, accurate categorization has been difficult to obtain. Phytochemicals are classified as secondary or major components according to how they function in plant metabolism (Koche et al., 2016). Chlorophylls, pyrimidines, nucleic acid purines, vitamins, amino acids, sugars, and other fundamental components are all found in plants (Harborne, 1984). Secondary components include plant lignans, phenolics, steroids, flavonoids, alkaloids, terpenes, curcumins, saponins, flavonoids, and glucosides (Grashorn, 2010; Dhama et al., 2015; Tiwari et al., 2018). According to a literature review, phenolics are the most abundant and structurally varied plant phyto-components (Huang, 2009).

Phytobiotics or phyto-genic feed additives can be classified based on their processing features and source. The four main categories of phyto-genic feed additives are plants, spices, essential oils, and oleoresins (Windisch et al., 2008; Yang et al., 2009; Huyghebaert et al., 2011; Gheisar et al., 2015). Plants refer to non-permanent blooming and non-woody plants, such as herbs and shrubs, which are used as a source of phyto-genic feed additives. Whole plants, flowers, leaves, roots, and can be used to produce phyto-genic feed additives (Grashorn, 2010). Spices are plants that have a dense odor or flavor and are commonly used as food additives to enhance flavor. Examples of spices that are commonly used as phyto-genic feed additives include garlic, ginger, cinnamon, and oregano. Essential oils are particularly volatile lipophilic components that are extracted from plants. They are often used as natural fragrances, flavorings and for their therapeutic properties. Extraction of essential oil can be made from different plant parts, like roots, flowers, and leaves using different methods, such as steam distillation (Vankar, 2004). Oleoresins are extracted from spices and are a concentrated form of the plant's active compounds. They are obtained by solvent extraction and contain both the plant's essential oil and non-volatile components. Oleoresins are commonly used in the food industry as flavorings and colorings (Procopio et al., 2022). Categories of phyto-genic feed additives have their own unique set of properties and benefits. The utilization of phytobiotics as feed additives has been found to enhance animal performance and health in the poultry industry (Alghirani et al., 2021).

Beneficial Effects of Phytochemicals on Animal Performance and Health

Phyto-genic feed additives are a class of bioactive compounds derived from several sources of herbs like plants, spices, and

other botanicals. These compounds have been found to have various beneficial effects on animal performance and health when included in animal diets. They are also known as phyto-genics, a term used to describe natural feed additives derived from plant sources (Windisch et al., 2008; Grashorn, 2010; Tiwari et al., 2018). Phyto-genic feed additives have been used in animal feed for many years, and their popularity has recently increased as consumers demand natural and organic products (Alagawany et al., 2021). These compounds have been shown to have a wide range of beneficial effects on animal health, such as improving gut health, enhancing immune function, and increasing feed efficiency (Alagawany et al., 2021). They are believed to work by stimulating the natural processes in the animal's body, leading to improved overall health and performance. Thousands of phytochemicals have been identified to be beneficial due to numerous advantages and have biological activities such as growth-promoting effects such as anticancer, antibacterial, antioxidant, anti-diarrheal, analgesic, easy availability, and low cost (Mahima et al., 2012; Rahal et al., 2014; Dhama et al., 2015, 2018; Yadav et al., 2016; Tiwari et al., 2018).

Phyto-genic feed additives have been found to have a wide range of beneficial effects on poultry nutrition. One of the most significant benefits of phyto-genic feed additives is their ability to stimulate feed intake, which is likely due to the improved palatability of the diet. Additionally, phyto-genic feed additives have been shown to improve nutrient digestibility, resulting in enhanced feed conversion ratio (FCR) and weight gain (Grashorn, 2010; Dhama et al., 2015; Gheisar et al., 2015; Yadav et al., 2016).

Phytobiotics also have the potential to improve gut microflora by preventing the development of dangerous bacteria while encouraging the development of beneficial populations. This can lead to improved gut health and reduced risk of disease. Some phyto-genic feed additives also have antimicrobial properties, which can further contribute to the reduction of disease incidence in poultry (Grashorn, 2010; Dhama et al., 2015; Gheisar et al., 2015; Yadav et al., 2016). Another beneficial effect of phyto-genic feed additives is their coccidiostatic effect, which can help to prevent and control coccidiosis, a common poultry disease caused by a protozoan parasite. Phyto-genic feed additives can also have immunostimulating effects, which can improve the overall immune response of poultry and reduce the risk of infectious diseases (Grashorn, 2010; Dhama et al., 2015; Gheisar et al., 2015; Yadav et al., 2016). Furthermore, some phyto-genic feed additives have anti-helminthic effects, which can help to control intestinal parasites in poultry. This can be particularly important in free-range and organic poultry production systems where outdoor access increases the risk of parasitic infections (Grashorn, 2010; Dhama et al., 2015; Gheisar et al., 2015; Yadav et al., 2016). The antioxidative properties of phyto-genic feed additives can improve the stability of animal feed and the quality and storage time of animal products such as meat and eggs (Gheisar et al., 2015). This can lead to a reduction in waste and an increase in the shelf life of these products. Phyto-genic feed additives have a wide range of beneficial effects on poultry nutrition and can be a valuable tool for improving the health and performance of poultry (Gheisar et al., 2015).

Mechanism of Action of Phytochemicals

Phytochemicals have been related to several different mechanisms of action. Inhibition of microorganisms,

disruption of metabolic processes, and modulation of signal transduction pathways and gene expression are all possibilities (Kris-Etherton et al., 2002; Surh, 2003; Omojate et al., 2014). Plant extracts and essential oils may act against bacterial strains in a variety of ways, including disruption of enzymes involved in cellular energy production and the synthesis of structural components, increased permeability and loss of cellular components, destruction or inactivation of genetic material, and interaction with the phospholipid bilayer of the cell membrane. The mechanism of action is thought to break the cytoplasmic membrane, affecting the proton motive force, electron flow, active transport, and cell coagulation (Kotzekidou et al., 2008).

Incubation of Eggs

There are two types of incubation: natural and artificial (Booth, 2004). Incubation is simply the embryo's development within the egg under appropriate environmental conditions (Berntsen and Bech, 2016). For natural incubation, the broody hen, preferably with good mothering instincts, once laying ends, begins to sit on the eggs, ruffles her feather, spreads out her wings, and makes a special clucking sound (Head, 2011). The hen tries to maintain the general conditions around the eggs, including temperature and humidity, and turns the egg at intervals to ensure the survival of the embryo, normal formation and development of the embryo. In closed systems, feed and water are often provided close to the mother hen for easy access and to be still close to the eggs to avoid cold shock resulting from the mother hen staying away from the eggs for an extended period of time (Spotila, 2004). This incubation period lasts for an average of 21 d in chickens.

On the other hand, artificial incubation uses an incubator to control the environment around the egg in a way similar to natural incubation to ensure the survival and proper development of the embryo (Ospina et al., 2018). One of the advantages of artificial incubation over natural incubation is that it ensures the hatching of more eggs compared to broody hens, which have limits.

In this artificial incubation system, the eggs are exposed to these plants' phytochemicals. Research by Widowski et al. (2022) and Iyasere et al., (2022) shows that the mother hen's environment may affect the egg composition, leading to physical and behavioral effects in the chicks. Maternal exposure to environmental stressors may affect offspring, so if the mother hen is in a good environment, it will affect the embryo positively. While the mother hen may pass on several nutrients to the eggs, eggs may also be injected *in ovo* with rich phytochemicals, which will affect the physical and behavioral properties of the chicks when they hatch and grow (Shehata et al., 2021).

The quality of day-old chicks is often affected by what they are exposed to during the incubation phase (Bergoug et al., 2013). This adds to why the eggs in incubators must be provided with the right conditions. Research by Hedlund and Jensen (2021) shows that incubation and hatching cause long-term stress in chickens. Hence, it is worth looking at what can be done to reduce this stress effect on chickens. A substance that has been used previously is phytochemicals. Phytochemicals are chemical compounds derived from plants, and they help in providing resistance against microbial infections. One of the outstanding properties of phytochemicals is their role as antioxidants. They have the

potential to boost the immune system, prevent the growth of cancerous cells, and regulate hormones.

In Ovo Technology

Broilers spend 30% to 40% of their lifespan inside the egg, while the market age continues to decline along with advances in genetic and nutritional knowledge (Hulet et al., 2007). The incubation and neonatal stages of broiler chickens account for almost half of the bird's productive lifetime (Proszkowiec-Weglarz et al., 2019). The period between embryonic day eighteen and four days posthatch is important for the chick's survival and growth since it marks the physiological and metabolic shift from egg to feed nutrition (Proszkowiec-Weglarz et al., 2019). As a result, the embryonic life stage now has a lot more significance. *In ovo* feeding appears to boost the late-term broiler's energy status by helping the chick reach its potential for late embryonic and early posthatch growth (Uni et al., 2005). The chick will have its first meal around embryonic day 18, when it consumes the amniotic fluid prior to internal pipping (Ferket and Gernat, 2006). *In ovo* feeding involves injecting liquid feed components into an egg's amnion many days before hatching. *In ovo* feeding is thought to be a viable option of administering nutrients to embryos prior to hatching. At a later stage of development (day 18 of incubation), the nutrients supplemented to the developing embryo through the amniotic sac to provide a constant supply of vital nutrients during the first few days (3 to 4 d) of the posthatch phase, promoting metabolism and intestinal development (Kadam et al., 2013b).

The foremost egg injection device was commercially available in 1992, with a capacity of 20,000 to 30,000 eggs per hour (Johnston et al., 1997; Jochemsen and Jeurissen, 2002). It was created to deliver vaccinations but has been tested for other uses, such as *in ovo* injections.

Figure 1 shows the various *in ovo* injection methods that have been used to examine the effects of various bioactive substances. The efficacy of *in ovo* feeding can be influenced by the site of injection (Avakian et al., 2002; Williams, 2007; Williams and Hopkins, 2011; Peebles, 2018). Generally, the timing and type of the biological substance determine the route of administration. When the injection is done in the initial stages of incubation, the air sac is usually the target (Berrocoso et al., 2017). When the embryo in the egg consumes the amniotic fluids later in the incubation process (Ma et al., 2020), the amnion is intended to provide chemicals in the amniotic fluid. The ability of the amnion to aspirate and digest the vaccine for more thorough systemic assimilation in the embryo has made it the best site for administration (Peebles, 2018). However, the findings of Kop Bozbay et al. (2016) revealed that *in ovo* injection of propolis and injection sites had no effect on hatching traits, including chick survival, chick weight at hatch, or hatchability. A typical injection depth to target the amnion is 2.49 cm from the top of the big end of the broiler hatching egg (Zhai et al., 2011).

Advantage of Early Feeding and *In Ovo* Applications in Poultry

In modern hatchery practices, the timing of hatching can vary between poults and chicks (Careghi et al., 2005). Typically, they hatch out of the egg at different times, with chicks hatching first, followed by poults around 36 to 48 h later. Once the majority of eggs have hatched, the chicks and poults are removed from the hatcher. After hatching, the chicks

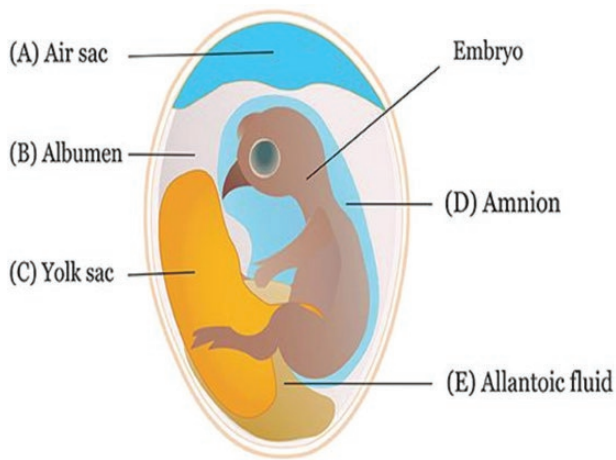


Figure 1. Different Sites of *in ovo* injection (Das et al., 2021).

undergo various hatchery treatments, such as sexing, vaccination, and beak trimming, to ensure their health and welfare (Glatz, 2000). Depending on the hatchery's protocols, these treatments can take a few hours to a few days. During this time, the chicks are held in the hatchery before being transported to the production farm. During transportation, the chicks may undergo a further holding time of up to 72 h. This period can be stressful for the chicks, as they are deprived of food and water, leading to a period of starvation. However, the residual yolk that is still present in their bodies after hatching provides them with the energy needed for growth and maintenance during this time. The yolk is a crucial source of nutrition for the chicks, as it contains essential nutrients such as proteins, fats, and minerals (Lesnierowski and Stangierski, 2018). It also provides antibodies that help protect the chick from diseases until its own immune system develops (Lesnierowski and Stangierski, 2018). The yolk is gradually absorbed by the chick's body over several days, providing a steady supply of energy and nutrients during the early stages of life (Sklan and Noy, 2000). Restriction in feed access does not only impairs the chicks' growth but also their immune system's ability to fight off pathological diseases (Willemsen et al. 2010; Bhuiyan and Iji, 2015).

In ovo administration of various compounds such as bacteriophages, electrolyte solutions, glycerol, hormones, organic acids, peptides, silver nanoparticles, trace elements, amino acids, vitamins, carbohydrates, and plant extracts directly into the egg during incubation is well documented in the literature (Roto, Kwon, and Ricke 2016). One of the potential benefits of *in ovo* technology is that it can stimulate favorable immunological responses in birds, leading to improved disease resistance and overall health (Jha et al., 2019). Additionally, *in ovo* technology can address perinatal nutritional deficiencies in birds (Jha et al., 2019). During the perinatal period, which encompasses the time before and after hatching, birds may experience nutritional deficiencies due to the transition from embryonic yolk nutrition to exogenous feeding (Proszkowiec-Weglarz et al., 2019). This can be compounded by the long hatchery window of 24 to 36 h and time-consuming hatchery logistics such as sorting, sexing, vaccinations, beak trimming, and chick transport (Noy and Uni, 2010). By administering nutrients and other compounds directly into the egg during incubation, *in ovo* technology can potentially mitigate these perinatal nutritional deficiencies

(Kadam et al., 2013). For example, *in ovo* administration of amino acids, vitamins, and trace elements can help support the development of the chick's immune system, muscles, and other tissues (Bakyara et al., 2012). Furthermore, *in ovo* administration of other compounds such as bacteriophages, organic acids, and plant extracts can also help to promote gut health and reduce the risk of disease (Yadav et al., 2016). This is particularly important in commercial poultry production, where disease outbreaks can have significant economic impacts.

In ovo technology offers several additional benefits; one potential advantage is that it can stimulate the colonization of the embryonic gut with beneficial microbiota (Shehata et al., 2021). The embryonic gut is a sterile environment before hatching, and establishing a healthy gut microbiome is critical for proper immune system development and the bird's overall health (Shehata et al., 2021). *In ovo* administration of probiotics or phytobiotics can help promote the growth of beneficial gut bacteria and improve gut health, potentially reducing the risk of disease (Rubio, 2019). *In ovo* technology can also promote the development of the embryonic gastrointestinal tract (GIT) and gut-associated lymphoid tissue (Siwek et al., 2018). The GIT is responsible for the digestion and absorption of nutrients, while the gut-associated lymphoid tissue plays a critical role in immune function (Ahluwalia et al., 2017). *In ovo* administration of nutrients and other compounds can help support the development and maturation of these systems, improving the bird's overall health and disease resistance (Shehata et al., 2021). Recently, *in ovo* sexing has been proposed as an application of this technology to address an important animal welfare and ethics issue in commercial layer production (Gautron et al., 2021). Male chicks are of limited value in the egg-laying industry and are typically culled by maceration or suffocation shortly after hatching. *In ovo* sexing can determine the sex of the chick prior to hatching, allowing for the selective culling of male embryos and eliminating the need for posthatch culling (Jennison, 2021). This approach has the potential to significantly reduce the number of male chicks culled, improving animal welfare and reducing the environmental impact of commercial layer production. *In ovo* technology has the potential to address several significant challenges in poultry production, including disease control, perinatal nutrition, and animal welfare (Ferket, 2009). While further research is needed to understand this technology's benefits and limitations fully, it represents a promising approach to improving the efficiency and sustainability of the poultry industry.

Effect of *in ovo* Administration of Phytogetic Substance on Poultry Animals

Nutritional Need of Embryos

Energy is stored in the embryo of fast-growing chicks after incubation for rapid growth and development (Gous, 2010). During the last phase of embryogenesis, the chick depletes the energy stored in the embryo through gluconeogenesis, which occurs mainly in the yolk sac and liver (Fasenko, 1996). The glycogen produced is then stored in the liver, muscle, and yolk sac for hatching purposes and growth and development posthatch (Fasenko, 1996). Over the years, chicken strains have been selectively bred to grow faster and reach market size quickly (Humphrey et al., 2014). This has led to changes

in the bird's genetic makeup, with the liver maturing earlier in heritage lines (Humphrey et al., 2014). The embryonic development phase, therefore, has become a critical period to prepare and equip newly hatched chicks for this fast-growing process (Ravindran and Abdollahi, 2021).

To ensure optimal growth and development of the chick embryo, researchers have focused on supplying various phyto-genic nutrient substrates to the developing embryos (Abd El-Hack et al., 2022). Phyto-genic substances are derived from plants and have been found to have potential energy for animals (Karásková et al., 2015). In the context of *in ovo* injection, phyto-genic substances can be delivered directly to the developing embryo through the eggshell, which may improve nutrient absorption and utilization.

Studies have shown that the late embryonic phase, specifically between days 14 and 18 of incubation, is crucial for supplying phyto-genic nutrients (Oladokun and Adewole, 2020; Oladokun et al., 2022). This period is characterized by a high caloric demand to fuel the hatching operation, and the embryo begins to consume the nutrients in the egg towards the end of incubation.

In Ovo Phytobiotics on Hatchability

Different authors have documented the effects of *in ovo* phyto-genic nutrients on embryo growth and hatchability. This review protrudes different phyto-genic substance use as *in ovo* supplements with a different result. Coskun et al. (2014) found that the injection of sunflower pollen extract into the amnion did not affect hatchability. Therefore, providing phyto-genic nutrients during this phase can help increase nutrient reserve to support organs and structural development posthatch. *In ovo* injection of phyto-genic nutrients during embryonic development has emerged as a promising strategy to improve the hatchability and posthatch performance of chickens in the context of fast-growing strains (Oladokun and Adewole, 2020).

Using *in ovo* injection is a potential strategy for improving chickens' hatchability and posthatch performance. This technique involves the injection of various substances, such as nutrients, drugs, or vaccines, directly into the egg during the incubation period (Bello et al., 2013; Li et al., 2016). The perinatal period, which encompasses both the pre and posthatch period, is a critical stage in the development of the chick embryo. During this period, the embryo has a high caloric demand to fuel the hatching process (Ferket and Uni, 2006). The availability of nutrients for *in ovo* injection is essential for embryonic development and hatchability. As the incubation period progresses, the embryo consumes the nutrients in the egg and begins to use its body reserves to prepare for emergence (Ferket, 2006). Therefore, it is crucial to provide the developing embryo with adequate nutrients to prevent weight loss and ensure successful hatching.

Several studies have demonstrated the potential benefits of *in ovo* phyto-genic feed additives injection for improving hatchability and posthatch performance in chickens. For instance, *in ovo* administration of nutrients, such as amino acids, vitamins, and minerals, has been shown to improve hatchability, chick weight, and FCR (Bello et al., 2013; Li et al., 2016). Additionally, *in ovo* vaccination against various pathogens, such as Marek's disease virus, has been shown to enhance immune responses and reduce mortality in chickens (Peebles et al., 2017). The use of *in ovo* phyto-genic feed additives injection is a promising approach for improving

the hatchability of chickens. Providing the developing embryo with adequate nutrients and vaccines through *in ovo* injection can support healthy embryonic development, reduce weight loss during the perinatal period, and improve hatchability.

In contrast, N'nanle et al. (2017) reported that *in ovo* administration of Moringa oleifera extract improved hatchability and resulted in heavier chickens at hatch. Similarly, Ngueda et al. (2021) found that injection of Manihot esculenta extract solution on the 18th day of incubation improved hatchability and reduced total incubation duration. In other studies, Hajati et al. (2014) reported that *in ovo* injection of grape seed extract (GSE) increased hatchability, while Heidary et al. (2020) found that *in ovo* administration of nano curcumin decreased hatchability. Morovate et al. (2016) observed no differences in the hatchability of birds that were administered *in ovo* Silybum marianum extract. Additionally, Fazli et al. (2015) reported that garlic and tomato extract increased the percentage of male chicks, while Mahjar and Al-Salhi (2022) found that *in ovo* injection of garlic extract influenced the hatchability of fertilized broiler eggs. Oke et al. (2021) discovered that *in ovo* inclusion of black cumin at a 2-mg dose increased hatchability when broiler birds were thermally challenged during incubation. Peşmen (2022) also reported no significant difference in hatchability when *in ovo* injection of black cumin was administered to broiler birds. Akosile et al. (2023b) administered clove and cinnamon *in ovo* to broiler birds and concluded that hatchability was significantly influenced compared to the control birds. Overall, these results suggest that the effects of *in ovo* administration of these extracts on hatchability can vary depending on the specific extract and dosage used. However, most studies found that the solutions of phyto-genic feed additives injected were safe for the developing embryos, and some extracts showed a dose-dependent effect on hatchability.

In Ovo Feeding and Chick Quality

Through various assessment methods and supplementation strategies of supplementing eggs in the hatchery with an adequate amount of phyto-genic feed additives to enhance the growth of the embryo to produce effective chick weight and good chick quality parameters, it is possible to optimize chick quality and promote the healthy growth of broiler chickens. However, there is limited research on the effects of *in ovo* phyto-biotic supplementation on chick weight and quality.

Ngueda et al. (2021) studied the *in ovo* feeding of Manihot esculenta extract solution at day 18 of embryonic development of broilers and reported improved chick weight and chick quality. As comparison to the saline solution and the negative control groups, the authors reported that the weights of 1-d-old chicks in the Manihot esculenta extract group were higher. Similarly, *in ovo* pollen extract injection (PEI) into the amniotic fluid of fertile broiler eggs on the 16th day of incubation increased subsequent chick weight from 70.1% to 73.5% among the control and PEI groups, respectively, to improve the genetic potential for late embryonic and early posthatch growth (Cocksun et al., 2014). In contrast, Morovate et al. (2016) and Fazli et al. (2015) also concluded that no differences were observed in increased weight at hatch of birds that were administered *in ovo* Silybum marianum extract and garlic extract.

In Ovo Phytobiotics and Performance Effect

Modern broiler lines are developed to mature quickly and reach a high body weight at the point of sale (Asche et al., 2018). These strains are chosen for characteristics that improve the FCR and shorten the duration to market weight (Lippens et al., 2000; Tůmová et al., 2002). Consequently, during the incubation phase, the day-old chick acquires a substantial quantity of body weight, and this weight increase persists after hatching (Decuyper et al., 2007). In reality, a broiler chicken's body weight can grow by up to 5,000%, or 2 kg, in 5 to 6 wk. This rapid development strains the bird's physiological systems, including its nutrient needs to support this fast growth and development, the bird's food must provide adequate nutrients (Ravindran and Abdollahi, 2021). Meeting these nutritional needs during embryonic development is critical to ensuring the chick has the necessary reserves to support growth and development after hatching.

The effects of different types and doses of *in ovo* phyto-genic feed additives on hatching and performance of chickens are shown in Table 1. It has been mostly reported that *in ovo* supplementation of phyto-genic substances has been shown to influence chicken performance parameters and has a growth-promoting effect on poultry. Ngueda et al. (2021) proposed that the early feeding of Manihot esculenta extract via an *in ovo* route on the 18th day of incubation is utilized by chick embryos along with yolk sac reserves and promotes the growth performance than those of the other group. Feed intake was higher in the Manihot esculenta group and FCR showed no significant differences across the treatments. Injection of GSE into broiler eggs at the dose rates of 3 (GSE) and 4 (GSE) into the airspace on the 5th day of incubation. Feed intake, body weight, body weight gain (BWG) and FCR appeared with no significant differences (Zeinab et al., 2019). Similarly, Hajati et al. (2014) investigated the effect of (3 mg/egg), (4.5 mg/egg), and (6 mg/egg) GSE on ileal microflora, performance, hatchability, antioxidant activity, and yolk sac weight of broiler chickens. The findings demonstrated that, in comparison to control groups, *in ovo* treatment of 4.5 mg GSE or vitamin C enhanced the average daily weight gain and daily feed consumption of chickens. *In ovo* feeding (IOF) of black cumin (2, 4, 6, and 8 mg) into the airspace of the egg at 17.5 d of incubation improved the final weight of the birds injected with black cumin as compared with the control. The feed intake was similar, while the FCR of the in the *in ovo*-treated birds was lower than that of the control birds (Oke et al., 2021).

In the literature, reports of production parameter values are not always consistent. According to recent findings, several phyto-genic compounds have increased feed intake while decreasing the FCR, resulting in unforeseen consequences. Heidary et al. (2020) studied the influence of IOF of nanocurcumin (NC) on growth immune responses, performance, intestinal morphology, and antioxidant status of broiler chickens challenged with thermal stress. The findings demonstrated that the IOI of broiler embryo with 0.01, 0.03, and 0.05 mL/egg of NC during the 17.5th day of incubation had no influence on daily weight gain, feed intake, or FCR from days 1 to 10, nor did it affect body weight on those days. Treatments had a significant impact on feed intake from days 1 through 24, so all groups receiving NC had lower average feed intake than control groups. Additionally, the study Nnanle et al. (2017) revealed influence of *in ovo*

supplementation of 0.5, 5, and 50 µg/mL of Moringa oleifera leaves at 18 d of incubation in the air chamber of broilers' hatching eggs. The authors revealed that the IOI nutrient of Moringa oleifera leaf extract did not affect the feed intake and BWG up to 7 wk of age. However, the authors observed increased body weights with increased extract concentration. The doses of the various bioactive compounds, environmental factors, and managerial variations may be attributed to these contradictory results.

Recently, El-Kholy et al. (2021) conducted a study on *in-ovo* injection of herbal extracts clove extract (0.1 mL), (thyme extract (0.1 mL), and cinnamon extract (0.1 mL) on immunological and physiological responses and posthatch performance of broilers on day 10 of incubation. The authors indicated that *in-ovo* administration of herbal extracts influence the FCR, daily weight gain, hatching weights, final weights, and weight gain during various experimental times. The BWG and FCR of chickens hatched from eggs injected with herbal extracts were superior to those of the control group. While research has shown interesting finding on the use of bioactive substances in *in ovo* supplementation to improve chicken hatchability and posthatch performance, there are still challenges that must be overcome before these technologies can be adopted on a larger scale by the poultry industry.

One of the difficulties is creating *in ovo* supplementation standards that can be replicated and implemented reliably across various production methods. This would necessitate taking into account variables such as bioactive substance timing, dose, and transport mechanism, as well as genetic and environmental factors that can impact the reaction of the chicken embryo. To resolve these issues, additional study is required to investigate the impacts of various bioactive substances and supplementation methods on performance parameters, as well as to create standardized procedures and recommendations for *in ovo* supplementation. This would allow the poultry business to more easily and effectively implement these technologies to enhance the performance and profitability of their operations.

In Ovo Feeding of Phytobiotics and Antioxidant Status

Optimal antioxidant status is crucial during the perinatal development of chicks. According to Oke et al. (2020) and Zhang et al. (2018), heat stress is known to destabilize the redox status and cause oxidative damage in poultry and it is characterized by reduced levels of cellular antioxidant activity. Recent studies also showed that administering a mixture of essential oils via the *in ovo* route enhanced the blood antioxidant status of broiler chickens without impairing growth performance parameters (Gouda et al., 2020; Oladokun et al., 2021). Using *in ovo* black cumin extract, Oke et al. (2021) indicated that the use of 6 mg of the extract meliorated the antioxidant parameters of broiler chicken at day 21 of incubation. Similar to this, the study of Akosile et al. (2023a) showed that the antioxidant status of broiler chicks was enhanced at hatch using 2 mg clove. In addition, Farag et al. (2023) demonstrated that the *in ovo* feeding of polyphenolic compounds of chicoric and rosmarinic improved oxidative stress in chicks, while Abdelghani et al. (2023) also demonstrated that 3 mg/egg of soy isoflavone had a positive effect on the antioxidant status of newly hatched chicks.

Table 1: Effects of different types and doses of *in ovo* phytobiotics on hatching and performance of chickens

Extracts	Dose	Hatch (%)	FI	BWG	FCR	Reference
Nano curcumin	0.01	100 ^a	15.89	13.56	1.007	Heidary et al., 2020
	0.03	86.67 ^b	15.89	11.89	1.003	
	0.05	86.67 ^b	17.43	12.09	1.125	
	Control	100 ^a	18.62	12.29	1.291	
Grape seed extract	3 mg	84.28 ^{bc}	29.03 ^{bc}	21.46 ^{abc}	1.35	Hajati et al., 2014
	4.5 mg	92.85 ^a	28.15 ^b	21.71 ^{ab}	1.35	
	6 mg	75.71 ^c	30.94 ^a	20.45 ^{bcd}	1.37	
	Control	80.00 ^c	27.42 ^d	19.74 ^d	1.39	
Black cumin	2 mg	86.48 ^{cd}	4963.8 ^b	2087.5 ^{bc}	2.38 ^b	Oke et al., 2020
	4 mg	87.75 ^{bc}	5215.3 ^{ab}	2175.0 ^b	2.40 ^b	
	6 mg	91.93 ^{ab}	5321.8 ^{ab}	2455.0 ^a	2.17 ^c	
	8 mg	87.75 ^d	4887.0 ^b	2030.0 ^b	2.41 ^{ab}	
	Control	86.07 ^{cd}	4835.3 ^b	1900.00 ^c	2.55 ^{ab}	
Silybum marianum extract	0 mg/kg	86.14	96.99	2179.96 ^a	1.63	Morovate et al., 2016
	100 mg/kg	85.88	96.18	2125.20 ^b	1.65	
	200 mg/kg	84.79	97.86	2142.54 ^{ab}	1.67	
Tomato and garlic	Tom	68	107	2263	2.02	Fazli et al., 2015
	Gar	72	111	2388	1.99	
	Control	77	110	2346	2.01	
Plant extracts	Cinnamon		3390.00	2639.10 ^b	1.29 ^b	El-Kholy et al., 2021
	Thyme		3525.50	2752.60 ^a	1.29 ^b	
	Clove		3365.14	2799.80 ^a	1.20 ^b	
	Control		3604.08	2172.10 ^c	1.66 ^a	
Clove and cinnamon	Clove 2 mg	92.0a				Akosile et al., 2023a
	Clove 4 mg	74.0e				
	Cinnamon 2 mg	89.0b				
	Cinnamon 4 mg	81.0d				
	Control	87.0c				
Moringa	0 g	68.8 ^b	23.4 ± 3.77			N'nanle et al., 2017
	0.5 g	93.8 ^a	26.2 ± 4.39			
	5 g	71.1 ^b	28.5 ± 4.81			
	50 g	73.9 ^b	33.3 ± 5.41			
Pollen Extract	Pollen extract	73.1 ^b				Coşkun et al., 2014
	Control	82.3 ^a				
Manihot esculenta extract	<i>M. esculenta</i> extract	91.30 ± 2.00 ^a	6921.84 ± 36.62	1933.42 ± 20.05 ^a	2.32 ± 0.08	Ngeda et al., 2021
	Control	82.90 ± 2.00 ^c	6499.95 ± 34.19	1861.69 ± 20.02 ^b	2.48 ± 0.08	
Resveratrol	52 µg RV/egg	86.81 ^c				EL-Saadany et al., 2019
	50 µg RV/egg	93.89 ^a				
	Control	87.31 ^c				

Gut Development and *In ovo* Phytogetic Nutrition

In ovo injection of phytogetic substances involves the injection of phytogetic nutrients directly into the developing embryos, which provides several benefits. One of these benefits is that the nutrients provided are exposed to the tissues of the GIT after the embryo orally consumes the amniotic contents prior to pipping (when the chick begins to break through the eggshell) (Shehata et al., 2021). This early exposure to nutrients helps to accelerate the development of the GIT, resulting in a greater digestive and nutrient-absorptive capacity in the hatchling (Shehata et al., 2021). This means that the chick is better able to digest and utilize the nutrients in its diet after hatching. However, it should be noted that the digestive tract of hatchlings has a limited ability to digest and utilize diets rich in proteins and carbohydrates (Kadam et al., 2013).

Therefore, *in ovo* feeding provides an opportunity to enhance the nutrient profile of the chick before hatching, which can improve the overall health and growth performance of the chick posthatch (Kadam et al., 2013). Additionally, *in ovo* feeding can reduce the stress on the chick during the immediate posthatch period when feed intake is typically low due to stress from the hatching process (Uni and Ferket, 2004).

The small intestine is a vital organ in the digestion and absorption of nutrients in newly hatched chicks (Ravindran and Abdollahi, 2021). It is responsible for breaking nutrients into their basic components, such as proteins into amino acids, carbohydrates into simple sugars, and fats into fatty acids and glycerol (Ravindran and Abdollahi, 2021). These nutrients are then absorbed into the bloodstream through the intestinal wall and transported to the body tissues for growth and

development. Although the small intestine is fully formed and functional at hatch, it undergoes further development and maturation as the chick transitions from dependent on the yolk sac to exogenous nutrient sources, such as feed. This development and maturation include an increase in villi height, crypt depth, and surface area, which provide a larger surface area for nutrient absorption. The intestinal morphology and function can be influenced by various factors such as diet, management, and early nutrition interventions (Lucas, 1998). Therefore, proper nutrition during the embryonic phase is crucial for the development of the small intestine in newly hatched chicks, which will affect their ability to digest and absorb nutrients and impact their overall growth and development (Lucas, 1998).

Due to the genetic selection for fast growth in modern poultry, the intestinal development of chicks needs to occur quickly to reach full functional digestive and absorptive capacities to meet the high nutrient demands of rapid growth (Ravindran and Abdollahi, 2021). The small intestine is crucial for the digestion and absorption of nutrients, and any deficiency in its function could lead to poor growth performance and health problems.

Phytogenic feed additives, such as herbal plants and their extracts, have been reported to have positive effects on

the gastrointestinal tract, including spasmolytic and laxative effects (Windisch and Kroismayr, 2006). It has been demonstrated that *in ovo* feeding with bioactive compounds that boost the activity of digestive enzymes enhances digestion in hatchling chicks (Siwek et al., 2018). These bioactive substances can be delivered directly to the developing embryo through *in ovo* injection and subsequently be exposed to the tissues of the gastrointestinal tract after the embryo orally consumes the amniotic contents before hatching. This can accelerate enteric development and increase digestive and nutrient absorptive capacity in the hatchling chicks. Oke et al. (2020) showed the effect of *in ovo* black cumin on the intestinal weight of thermally exposed broiler birds. The result showed that intestinal weight was significantly affected by birds administered *in ovo* black cumin. Similarly, Akosile et al. (2023a, b) showed positive influence of *in ovo* administration of clove and cinnamon on the intestinal weight of broiler birds in hot, humid environments. Villus height in the jejunum was significantly affected on day 10 by *in ovo* injection of nano curcumin, thereby enhancing the intestinal growth and performance ability of the birds (Heidary et al., 2020). The summary of different *in ovo* phytogenic feed additives (PFA) and their effects are shown in Figure 2.

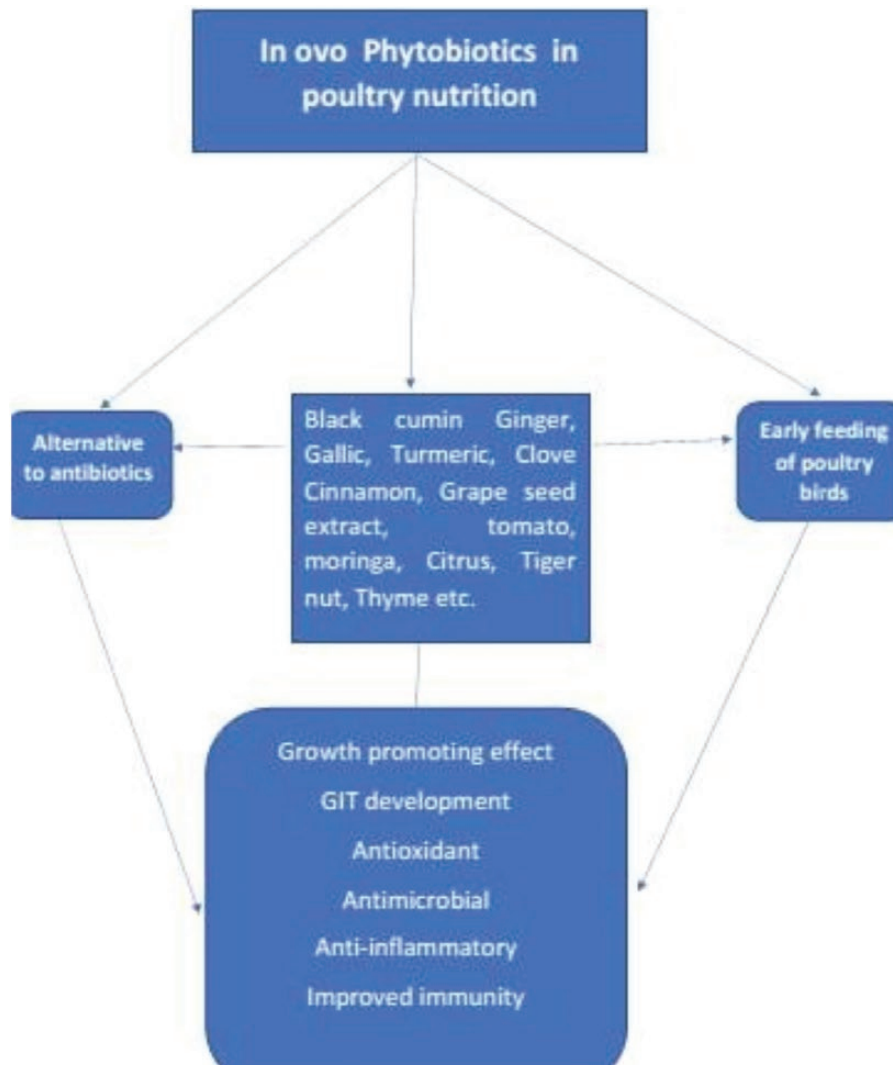


Figure 2: Summary of different *in ovo* phytobiotics and their effects.

Conclusion

This review collates information on the potential of *in ovo* feeding of phyto-genic feed additives as emerging advances in avian sciences to provide insight into the use of the technology. Existing data indicate that *in ovo* supplementation with phyto-genic feed additives is a promising technique and it might positively affect the antioxidant status of embryos, hatching, and posthatch growth performance of chickens. To demonstrate the long-term effects of *in ovo* supplementation and the financial benefits for poultry industry, more research is required. Although some herbs and their derivatives have been explored, more studies are needed on novel phyto-genic feed additives to further increase their scale in poultry production.

Conflict of interest statement

The authors declare no conflict of interest.

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