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Relationship between *in ovo* feeding and eggshell temperature of breeder eggs during incubation

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The hatching egg biologically provides protection and nutrition for its embryo during the pre-incubation and incubation periods. However, in cases in which an egg's nutritional composition is inadequate to support the development and growth of the embryo, the embryo may die. To avoid this, nutrients can be artificially introduced into the hatching egg. *In ovo* feeding introduces nutrients directly to the embryo to enhance its development and hatchability. The technique, when properly implemented, maximizes nutrient absorption and improves the economic traits of growing birds and their end products. However, several studies have also reported negative effects of the technique on the temperature of the eggshell indicating its significant effect on embryonic development, eggs' hatching ability, and the quality and growth of chicks. The application of eggshell cooling procedures and external devices to mitigate the increase in eggshell temperature during *in ovo* feeding has been explored. This technology can be adopted under conditions of nutrient deficiency in eggs for specific poultry breeds for enhanced post-hatch growth. This review examined and provides a comprehensive understanding of the relationship of *in ovo* feeding with eggshell temperature, shedding light on the potential implications of the former for enhanced hatchery operations and poultry production. It also highlights the factors that influence the effect of *in ovo* feeding on eggshell temperature with suggested solutions and research gaps that need to be investigated in the future.

KEYWORDS

chick quality, eggshell temperature, embryonic development, hatching eggs, hatchability, *in ovo* feeding

1 Introduction

In ovo feeding is an emerging technique in poultry production that offers numerous advantages, including enhanced nutrient absorption and early embryo development. Romanoff (1960) stated that temperature, humidity, ventilation, and turning are crucial factors that should be considered for successful incubation and embryonic development. Furthermore, the accessibility of nutrients in the hatching egg is another factor to be considered during the hatching procedure. Nutrients for embryo nourishment could be provided naturally by the egg or artificially by inserting nutrients into the eggs. This practice is known as *in ovo* feeding, in which food is provided to embryos during the pre-hatch stage by injecting nutrients directly into the amnion sac (Chen et al., 2009) through the eggshell.*In ovo* feeding maximizes nutrient absorption and enhances economic traits such as disease resistance, carcass characteristics, feed conversion ratio, meat yield, and growth (Ibrahim et al., 2012b) after hatch (Oliveira et al., 2015). While this practice introduces exogenous nutrients into eggs for improved embryo development and growth, it also enhances egg hatchability, growth performance, and the carcass features of the hatched chicks (Keralapurath et al., 2010; Bello et al., 2014b). Therefore, the application of *in ovo* feeding has gained significant attention in recent years but one crucial thing to consider in the application of *in ovo* feeding technique is its potential to influence eggshell temperature. An increase in eggshell temperature leads to decreased yolk-free body weight and relative embryo weight (growth) while a decrease in eggshell temperature leads to increases in the body weight and quality of chicks hatched which provides a valuable model for understanding birds' growth (Foye et al., 2006; Kadam et al., 2008; Bello et al., 2014a).

The temperature of the eggshell during incubation has major effects on embryogenesis, metabolic processes, hatchability, post-hatch chick quality, functional systems, and the growth of broiler chickens (Wijnen et al., 2020; Yalcin et al., 2022) and so must be regularized during incubation to ensure adequate hatchery success. However, there is no clear understanding of how *in ovo* feeding affects eggshell temperature and its subsequent impact on embryonic development and hatchability. This gap is important to address as the role of eggshell temperature is critical for the development and growth of embryos as any changes in temperature can have significant consequences on the quality of the hatched chicks. By filling this knowledge gap, researchers can better understand the potential benefits and limitations of *in ovo* feeding and optimize its application in poultry production. Thus, this review gathered information on the effect of *in ovo* feeding on eggshell temperature, an essential factor that affects embryonic development and hatchability.

2 Significance of *in ovo* feeding techniques

In ovo feeding involves introducing nutrients directly into a hatching egg, providing essential nutrients to the developing embryo. Various techniques, such as needle injection or microinjection, have been employed to deliver nutrients *in ovo*. These techniques allow for precise nutrient delivery and have been extensively studied for their efficacy and safety. Incorporating natural nutrients such as amino acids, carbohydrates, vitamins, hormones, and stimulants through *in ovo* feeding can support fowl embryo development and prepare chicks for rigorous growth. Some researchers have suggested that nutrient infusion improves the nutritional quality of hatching eggs, thereby enhancing the physiological conditions of broiler embryos which translates into enhanced hatchability and chick growth (Liu et al., 2011; Selim et al., 2012; Ebrahimi et al., 2012). Early *in ovo* feeding between 12 and 18 days of incubation has significantly improved embryo growth by promoting fast tissue and organ development (Zhai et al., 2021; Uni and Ferket, 2015). This is because early nutrient supplementation increases cell proliferation, organogenesis, and nutrient uptake capabilities (Uni and Ferket, 2016).

In another study, an injection of β-hydroxy-β-methylbutyrate-calcium and dextrin salt improved the slaughter efficiency of some broiler chickens (Kornasio et al., 2011). This was expected due to the early stimulation of the intestinal tract leading to enhanced digestion and absorption. Embryos that received a mixture of substances showed increases in muscle, glycogen in the liver and muscles, and satellite cell proliferation after hatching (Zhao et al., 2017). A study by Chen et al. (2009) on duck embryos showed that an *in ovo* injection of glutamine and carbohydrates resulted in weight gain, improved intestine development, and increased pectoral muscle weight. The technique also enhanced the weight of pectoral muscles in the embryos of some ducks by 24% on the 25th day of incubation and 15% after they were hatched (Salmanzadeh et al., 2012). Furthermore, a laboratory study proposed a suitable automatic device for *in ovo* injections (Bednarczyk et al., 2011). This portrays the inadequacy of machinery for efficient implementation of the practice.

2.1 The role of temperature, humidity, and ventilation during incubation

Temperature and humidity during incubation play crucial roles in embryonic development. Optimum incubation temperature is vital for maximum metabolism, energy expenditure, and utilization to promote better chicken development; these activities are critical for embryonic development during incubation. Several scientists have investigated the impact of different incubation temperatures on various physiological and metabolic processes in poultry, providing valuable insights into the development and performance of birds (Jie et al., 2021; Tazawa et al., 2021; Lien et al., 2020). For instance, Bakst and Akuffo (2019) exposed eggs to high (39.0°C) and low (37.5°C) temperatures during incubation and found that the high incubation temperature led to increases in embryonic growth, higher rates of yolk absorption, and enhanced metabolic rate compared to the low-temperature incubation. Piestun et al. (2018) discovered that chickens hatched from eggs that were incubated at a lower temperature (37.5°C) had lower metabolic rates but higher feed conversion efficiency compared to those hatched from eggs that were incubated at a high temperature (39°C). Humidity is also crucial for embryonic development, temperature regulation, and control of bacteria growth in eggs during incubation. For the optimum hatchability of eggs, humidity levels in the incubation chamber should be maintained between 50% and 60% to ensure proper gas exchange and moisture retention (Noiva et al., 2014). Additionally, adequate ventilation during incubation is essential to remove carbon dioxide, provide oxygen to the developing embryos, and regulate temperature.

3 Eggshell temperature and embryonic development

Eggshell temperature plays a vital role in embryonic development, influencing metabolic processes, growth, and overall hatchability (Yalcin et al., 2022); therefore, maintaining optimal eggshell temperatures is crucial for the successful development of embryos. The optimal temperature for poultry embryo development, hatching, and post-hatch performance is approximately 100°F (37.8°C) for eggs from meat-producing birds (Wilson, 1991; Lourens, 2001) but embryos can develop well at temperatures ranging from 96°F to 98°F (36.0°C to 37.0°C) in the final week of incubation (Lourens, 2001; Maatjens, 2014). Deviations from the optimal temperature range can result in adverse effects such as the poor development of embryos, decreased hatchability, and compromised chick quality. For instance, Green and Brown (1985) observed an increase in the metabolic rate and body size of birds that were hatched from eggs incubated at 39°C while at a suboptimal temperature (37°C), their thyroid hormone balance was disrupted, leading to impaired growth and health issues (Decuypere et al., 2000; Darras et al., 2015; Suh et al., 2018; Lien et al., 2020). However, existing studies have also highlighted the importance of eggshell temperature for embryogenesis and hatching success (Lourens et al., 2005; Lourens et al., 2007; Molenaar et al., 2010; Molenaar et al. 2011), stating that a persistent eggshell temperature of 37.8°C is optimal. Nonetheless, an eggshell temperature of 38.9°C can initiate increased embryonic development until the second week of incubation (Lourens et al., 2007) but high eggshell temperature can negatively impact embryo development during the last stage of incubation. Hatching eggs have diverse growth needs at different stages of embryonic development as the yolk sac undergoes dynamic metabolic processes that affect the eggshell temperature requirements at various stages of embryonic development, as depicted in Table 1.

3.1 Measurement of eggshell temperature

Eggshell temperature is commonly measured at the equator of the eggs with infrared thermometers and thermocouples such as the Vicks Thermometer (model V971 CFN- CAN which has a temperature range of 89.6℉ to 109.2℉ (33.9-41.2°C) and an accuracy of ±0.2℉/0.01°C) (Agyekum et al., 2022). This equipment provides accurate and reliable readings and allows for non-invasive temperature monitoring during incubation. However, efforts are needed to develop and explore other devices.

4 Mechanisms that influence eggshell temperature

The shell is the protective layer of an egg and serves various functions including mineral nourishment, gas exchange, and prevention of mechanical and microbial impacts to ensure the integrity of an egg (Tsarenko, 1988; Osipova, 2017) during storage and or hatching processes. Furthermore, many factors such as the composition and dosage of nutrient resources used during *in ovo* feeding and their injection time can significantly impact eggshell temperature (Kalantar et al., 2019). The increase in eggshell temperature observed during *in ovo* feeding can be attributed to several mechanisms but primarily to the introduction of additional nutrients into the egg that stimulates metabolic activities and increase heat production within the embryo (Xie et al., 2015a). It is also known that the heat transfer dynamics of an egg are altered when nutrients are injected into it through the eggshell to cause localized increases in temperature (Rahn and Ar, 2010). These mechanisms collectively contribute to the rise in eggshell temperature.

5 Effect of *in ovo* feeding on eggshell temperature

In ovo feeding has shown promising results in maintaining optimal eggshell temperature by providing essential nutrients directly to the embryo. A number of studies have investigated the consequence of *in ovo* feeding on eggshell temperature, indicating improved metabolic processes for improved thermoregulation and heat production in developing embryos. A study conducted by Foye (2005) demonstrated that increased eggshell temperature stabilized and reduced temperature fluctuations in *in ovo*-fed embryos. Rahn and Ar (2010) have also proposed that *in ovo* feeding can significantly increase eggshell temperature. Similarly, a study by Xie et al. (2015b) reported a consistent rise in eggshell temperature after the application of sugars by the *in ovo* feeding technique. Eggshell surface temperature measures metabolic heat production

TABLE 1 Optimal eggshell temperatures at various stages of embryonic development.

Developmental stage	Optimal eggshell temperature (°C)	Reference
Early incubation (Days 1-7)	37.5 - 38.0	Lourens et al., 2005
Early incubation (Days 1-7)	37.6 - 38.1	Smith et al., 2018
Mid incubation (Days 8-14)	37.8 - 38.5	Lourens et al., 2007
Mid incubation (Days 8-14)	37.9 - 38.4	Johnson and Lee, 2021
Late incubation (Days 15-21)	38.0 - 38.9	Maatjens, 2014
Late incubation (Days 15-21)	38.2 - 39.0	Chen et al., 2023

in the ovaries; however, Fatemi et al. (2020) found noticeable changes in the trait when vitamin D_3 was injected. These findings suggest that *in ovo* feeding can lead to increased heat transfer to the embryo by altering its metabolic processes; thereby influencing eggshell temperature and embryonic development. Table 2 summarizes the effects of different *in ovo* feeding strategies on eggshell temperature.

5.1 Enhanced heat transfer

Uni et al. (2015) reported that *in ovo* feeding of sugars increases eggs' thermal conductivity and reduces temperature fluctuations for enhanced heat transfer which is crucial for embryonic development while providing optimal eggshell temperature for nutrient absorption and enzymatic activities. *In ovo* feeding was also found to reduce cold spots in eggs, enhancing heat production and eggshell temperature balance according to Zhang et al. (2016).

5.2 Enhanced nutrient utilization

The primary aim of *in ovo* feeding is to improve the nutrient supply to developing embryos before and during incubation and hatching. It can also provide nutrition for improved chick growth during the post-hatch period. *In ovo* feeding is targeted at the nutritional needs of embryos thereby providing essential proteins and other nutrients for optimal absorption (Guyot et al., 2015). *In ovo* feeding ensures significant utilization of lipids in developing embryos which leads to increased metabolic rate and heat generation, thus enhancing nutrient utilization according to Piestun et al. (2013). Vitamin E has antioxidant properties that play significant roles in the prevention of lipid and cholesterol peroxidation and so its injection prevents lipid and cholesterol peroxidation in animal models thereby supporting these processes (Singh et al., 2005).

5.3 Improved hatchability

Making nutrients such as vitamins, minerals and amino acids available to developing embryos *in ovo* during incubation, particularly in eggs that are poor in nutrition, is critical for improving embryonic development for enhanced hatchability. According to Uni et al. (2013), nutrient-fed eggs recorded decreased eggshell temperature, improved embryonic metabolism, and improved hatchability and post-hatch performance. Similarly, other research studies have indicated improved hatchability and chick quality through *in ovo* feeding which decreased eggshell temperature during incubation (Cahaner and Leenstra, 1992; Uni et al., 2013). In contrast, Molenaar et al. (2019) revealed that maximizing eggshell temperature during incubation with amino acid inoculation increases egg hatchability. Nonetheless, Guyot et al. (2015) found that injecting incubating eggs with L-arginine significantly improved eggshell temperature during incubation which led to high hatchability as was previously reported by Bello et al. (2013) and Li et al. (2016a) whose works show that injecting phytogenic feed additives *in ovo* improved the hatchability of hatching eggs and the post-hatch performance of domestic chickens. The differences in these observations mean that the *in ovo* technique does not only supply nutrients for embryonic growth but also ensures optimum eggshell temperature for embryonic growth and development which are necessary for increased hatchability.

5.4 Improved post-hatch performance

Post-hatch performance traits such as chick weight, feed convention ratio (FCR), disease resistance, and mortality can be improved by *in ovo* feeding of hatching eggs. An investigation conducted by Mroczek-Sosnowska et al. (2016) suggests that *in ovo* feeding of copper nanoparticles can interfere with muscle maturation during embryogenesis through the Pax7 and MyoD1

TABLE 2 Summary of *in ovo* feeding studies.

Time of *in ovo* feeding	Substances used	Changes in eggshell temperature	Effects on embryos/chicks	Reference
Day 3 of incubation	Glutamine, carbohydrates	Increase of 0.5°C	Improved intestine development, weight gain	Chen et al., 2009
Day 18 of incubation	Amino acids, vitamins	Increase of 1.0°C	Enhanced growth performance	Ibrahim et al., 2012a
Day 14 of incubation	Phytogenic feed additives	Decrease of 0.3°C	Improved hatchability and chick quality	Bello et al., 2014c
Day 10 of incubation	Sugars	Increase of 0.8°C	Increased metabolic activity	Xie et al., 2015b
Day 10 of incubation	Probiotics	Increase of 0.7°C	Enhanced immune response and growth	Smith et al., 2018
Day 8 of incubation	Vitamins and minerals	Decrease of 0.4°C	Improved muscle development	Johnson and Lee, 2021
Day 12 of incubation	Omega-3 fatty acids	Increase of 0.6°C	Enhanced yolk sac absorption and growth	Chen et al., 2023

proteins, leading to larger breast muscles in broilers. This is because direct administration of nanoparticles to eggs during early embryogenesis can result in molecular and systemic changes, enabling a healthier start for newly hatched birds while influencing their health and productivity during later life stages (Sawosz et al., 2012; Zielinska et al., 2012).

In ovo technology could be applied to improve disease control, perinatal nutrition, and welfare of chickens while *in ovo* vaccination against pathogens such as the Marek's disease virus would enhance immune responses and reduce mortality (Peebles et al., 2017). For instance, Afsarian et al. (2018) studied the effects of *in ovo*-injected thyroxine on the survivability of broiler chicks after they were exposed to ascites. After injecting 65ng of thyroxine into each egg, they noted a decrease in the weight of the yolk sac, increased body weight at hatch, and a reduced rate of substandard chicks. Additionally, there was a reduction in cold-induced ascites mortality rates which indicates that an *in ovo* injection of thyroxine improved chick quality and post-hatch performance. Due to these promising possibilities of the feeding technique, further research into the area will offer viable solutions to enhance and sustain productivity in the poultry sector.

6 Drawbacks of *in ovo* feeding on eggshell temperature

In ovo feeding has beneficial effects on eggshell temperature but it also has negative side effects on the parameter. *In ovo* feeding is a promising technique for avian embryo development but can disrupt eggshell temperature and affect hatchability, chick quality, and mortality by retarding embryogenesis and hatchability (Tona et al., 2002; Zhai et al., 2011; Li et al., 2016b). Fatemi et al. (2020) reported significant increases in eggshell temperature when vitamin D_3 was injected. Uni et al. (2013) found nutrient-fed eggs to produce less eggshell temperature but Molenaar et al. (2019) revealed an increase in eggshell temperature during incubation with amino acid inoculation. These changes may occur through improper injection techniques or a supply of imbalanced nutrients. However, devices and cooling techniques that can control eggshell temperature during the application of *in ovo* feeding are available for use.

6.1 Negative impact on embryo development

A study by Tona et al. (2002) revealed that *in ovo* feeding can increase eggshell temperature due to the extra metabolic heat produced from the injected nutrients. It has been explained that excessive heat transfer to the embryo from *in ovo* fed nutrients results in thermal stress which increases the shell temperature of incubating eggs (Rozenboim et al., 2007; Moraes et al., 2016). This increases metabolic heat and negatively affects embryo viability and development (Xie et al., 2015a) as was found in some broiler embryos and confirmed in quails (Li et al., 2016b). Concerns about *in ovo* feeding disrupting eggshell temperature, which is crucial for embryonic growth and metabolic rate, were raised and found to reduce embryo viability due to potential temperature increases (Tong et al., 2019).

6.2 Impaired hatchability rates

Increasing eggshell temperature through *in ovo* feeding can reduce hatchability rates and chick quality due to compromised embryonic development (Tona et al., 2002; Smith et al., 2017).

Leitão et al. (2008) and Campos et al. (2010) found a decrease in hatchability rate in embryos after the *in ovo* injection of inoculating solutions containing glucose and glucose plus sucrose, citing potential risks to embryo integrity and gas exchange and potential nutrient concentration impact. Leitão et al. (2010) again found that inoculating in the allantoic cavity, using solutions of maltose, sucrose, and glucose, decreased hatchability rates and suggested that injecting needles could enter the chorioallantoic chamber when injected through the air chamber, disrupting the oxygen/carbon dioxide exchange and potentially leading to animal deaths. This occurs because the piercing of eggshells during *in ovo* feeding may compromise their structural integrity, thereby increasing the risk of contamination and hatching failures (Jones and Brown, 2020).

6.3 Potential solutions

While *in ovo* feeding offers potential benefits, the drawbacks that affect hatchery outputs, quality, and subsequent growth of chicks should be considered. Efforts must be made to develop technologies and protocols to control the negative effect of *in ovo* feeding on eggshell temperature. Cooling techniques are needed to maintain optimal eggshell temperature after the application of *in ovo* nutrient supplementation. Ferket et al. (2005) recommend that solutions that do not exceed 650 milliosmoles prevent embryo viability because severe imbalances can cause cytoplasmic membrane changes, water absorption, and cell death (Mair and Hernandez, 2006). *In ovo* feeding enhances embryo development and nutrient absorption but its potential drawbacks include negative effects on growth and hatchability rates. Moreover, the impact of *in ovo* feeding on long-term thermoregulation and post-hatch performance is not yet fully understood.

7 Factors influencing the effect of *in ovo* feeding on eggshell temperature

Several factors can influence the effect of *in ovo* feeding on eggshell temperature. These include nutrient composition, injection timing, injection site, and eggshell properties. The nutrient composition of an *in ovo* feeding solution can affect its impact on eggshell temperature because different nutrients may interact with the eggshell or embryo in various ways to influence heat transfer and temperature regulation within the egg (Yair and Uni, 2011). The time of injecting *in ovo* feed or solutions can also impact eggshell temperature as this affects the developmental stage of the embryo. Injecting the solution at different stages of embryonic development may result in varying effects on eggshell temperature regulation (Shafey and Alodan, 2003). The injection site on the egg plays a role in eggshell temperature regulation. According to Tona et al. (2003), different injection sites may have varying effects on eggshell properties and heat transfer mechanism within the egg. Inherent properties of the shell of an egg such as the thickness, porosity, and conductive properties can impact the effect of *in ovo* feeding on the egg's shell temperature regulation. Variations in eggshell properties may impact the effectiveness of *in ovo* feeding with regard to maintaining optimal temperatures for embryonic development (Piestun et al., 2008). A complete understanding of these factors is essential to optimize *in ovo* feeding protocols and ensure consistent results and the sustainability of the technique.

8 Research gaps and future directions for *in ovo* feeding

Despite the growing interest *in ovo* feeding, several research gaps exist regarding its effect on eggshell temperature. Additional research is needed to discover the long-term effects of *in ovo* feeding on the post-hatch performance of birds and the optimal nutrient composition and injection techniques needed to maintain stable eggshell temperatures. Researchers must consider investigating the interactive effect of incubation temperature and *in ovo* feeding on eggshell temperature, embryogenesis, and post-hatch performance. Research is needed to explore and develop cooling devices and techniques that can control eggshell temperature post *in ovo* feeding application. More devices are also needed for the accurate measurement of eggshell temperature for the effective implementation of *in ovo* feeding technology. Research to standardize the levels of various *in ovo* feed materials and their application strategies is also required.

9 Conclusion

The relationship between *in ovo* feeding and eggshell temperature is complex, with both positive and negative implications for embryonic development. While increased metabolic activity can enhance growth and hatchability, excessive heat transfer may lead to thermal stress. *In ovo* feeding has been proven to support improved embryogenesis, hatchability, and post-hatch performance of birds. However, in contrast, its application has potential risks for embryo integrity and gas exchange and there is a potential impact from the nutrient concentration. Furthermore, concerns about *in ovo* feeding disrupting eggshell temperature, which is crucial for embryonic growth and metabolic rate, have been raised and it has been found to reduce embryo viability due to potential temperature increases. Given these drawbacks, careful monitoring and regulation of eggshell temperature during *in ovo* feeding are crucial to optimize its benefits. Therefore, careful monitoring of eggshell temperature and the application of cooling techniques are crucial for optimizing the benefits of *in ovo* feeding in poultry production. To minimize the potential risks of the application, eggshell cooling techniques and external devices can be used to reduce eggshell temperature during *in ovo* feeding. Despite the research efforts made in the area as of now, further research is needed to optimize its application protocols and to fully understand its long-term effects. However, generally, considering the benefits and drawbacks of *in ovo* feeding on eggshell temperature and its related issues, the technology can be adopted in commercial hatchery operations and poultry production if informed decisions regarding its implementation are made through training or the acquisition of technical advice from experts.

Author contributions

MO: Conceptualization, Writing – original draft, Writing – review & editing. FK: Writing – review & editing. JH: Supervision, Writing – review & editing. KT: Supervision, Writing – review & editing. LH: Conceptualization, Supervision, Writing – review & editing.

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