

A Review: Nutrition Stimulation with in Ovo Feeding Technology for Optimization of Growth and Development of Prenatal and Postnatal Periods of Chicken

Daryatmo^{a,b}, Niken Ulupi^c, Rudi Afnan^c, Wahyuni^{a,d}

a Students of the Animal Production Science and Technology Study Program, IPB Postgraduate Program b Department of Livestock Production, Faculty of Animal Science, Hasanuddin University c Department of Animal Production Science and Technology, Faculty of Animal Science, IPB University, Bogor d Faculty of Animal Science, Universitas Islam Lamongan

Corresponding author: daryatmo@unhas.ac.id

ARTICLE INFO

Article history: Received 27 December 2023 Revised 30 April 2023 Accepted 16 October 2023

Keywords: In ovo Feeding Nutrition Performance Incubation

IEEE style in citing this article:

Daryatmo, N. Ulupi, R. Afnan, Wahyuni. " Nutrition Stimulation with in Ovo Feeding Technology for Optimization of Growth and Development of Prenatal and Postnatal Periods of Chicken" Jurnal Ternak : Jurnal Ilmiah Fakultas Peternakan Universitas Islam Lamongan, vol. 14, no. 2, pp. 51 - 58, 2023

1. Introduction

ABSTRACT

The improvement in broiler chicken performance is currently thanks to the genetic selection, nutrition, maintenance management, and health and biosecurity programs that have succeeded in improving the maintenance performance of broilers. Such rapid development in the cycle after hatching is undoubtedly also influenced by the embryo's development or the prenatal phase. Unlike other mammals, the process of breeding poultry has its characteristics where the process of embryonic development occurs outside the body of livestock or inside poultry eggs. Chicken embryos rely heavily on the nutrients inside the egg, which provide the energy and amino acid makeup needed for the metabolic needs of the growing embryo during the 21-day incubation process. Chicken embryos are susceptible to a lack of energy during the hatching process. This deficiency can result in weak embryos and, in more severe conditions, embryo death and failure to hatch. The nutritional needs of embryos during incubation can be added to the egg with the in ovo feeding technique. The in ovo feeding technique is a technique that allows the addition of outside nutrients injected into the egg during incubation for the developmental and metabolic needs of the embryo, as well as to improve the nutritional status of the egg. Nutrition stimulation with in ovo feeding technique produces many benefits, such as increasing hatchability, hatching weight, immunity level, reduced bone system disorders, reduced morbidity and mortality after hatching, feed efficiency and weight gain.

> Jurnal Ternak (Animal Science Journal) Faculty of Animal science - Lamongan Islamic University) with CC BY NC SA license.

The development of the poultry industry, especially broilers, is very rapid compared to other livestock sectors, in line with the increasing public need for affordable chicken meat. Various broiler strains with fast-growing characteristics show performance improvements such as body weight parameters of 3.30% per year, FCR (feed conversion ratio) parameters decreasing by 2.55% annually, and maintenance life parameters that are getting shorter [1]. The improvement of broiler chicken performance is the benefit of genetic selection, nutrition, maintenance management, and health and biosecurity programs that have succeeded in improving the maintenance performance of broilers [2].

The shorter maintenance time of broilers until they reach weight according to consumer harvest preferences with an age of 35-40 days of maintenance is equivalent to 70% of the age of the postnatal

Daryatmo et. al ./Jurnal Ternak 14 (2) 2023 pp. 51 - 58

period or after hatching while the age of the prenatal or incubation period is about 30% or 21 days of the entire commercial broiler production cycle. Such a rapid development in the cycle after hatching or postnatal is certainly also influenced by the embryo's development or the prenatal phase. Unlike other mammals, the process of breeding poultry has its characteristics where the process of embryonic development occurs outside the body of livestock or inside poultry eggs. [3][4][5]

Eggs contain complete nutrients and energy for embryonic development needs. Chicken embryos rely heavily on the nutrients in the egg, which provide the energy and amino acid makeup needed for the metabolic needs of a growing embryo during the 21-day incubation process so; that the egg must have a balanced nutritional composition and be sufficient for the metabolic needs of the embryo [6][7].

The nutrients in chicken eggs largely depend on the nutritional conditions of the hen. The need for nutrients in eggs is increasing with the rapid metabolism of broiler-strain egg embryos that have been genetically improved. Chicken embryos are susceptible to energy shortages during the hatching process, where this deficiency can result in weak embryos and, in more severe conditions, can result in embryo death and failure to hatch [8][4][9].

The nutritional needs of embryos during incubation can be added to the egg with the in ovo feeding technique. The in ovo feeding technique is a technique that allows the addition of nutrients from the outside that are injected into the egg during incubation for the developmental and metabolic needs of the embryo, as well as to improve the nutritional status of the egg. By injecting into the amniotic layer, an additional solution along with the amniotic fluid can be taken orally by the embryo before hatching, providing nutrients into the intestine of the embryo so that it can stimulate the development of the intestine. In addition, the provision of exogenous nutrients at the embryonic stage of development is so important that it can generate many advantages for post-hatching chickens, such as improving the efficiency of nutrient utilization, immune response, muscle development, and reducing mortality and post-hatching morbidity [10][10][10].

In ovo feeding was first introduced by the in ovo application used for vaccination against Marek's disease on the 18th day of incubation. The results obtained a better level of immunity. The application of [11] in ovo feeding technology until now has developed with various types of nutrients used in eggs. This review article examines the potential for nutritional stimulation of in ovo feeding technology in broilers.

2. State of the Art

One of the processes of growth and development of broiler chickens is influenced by the nutrients contained in the eggs during the incubation period. The faster growth rate after hatching results in more complete nutritional needs with the amount needed in the embryonic phase. The addition of nutrients using the in ovo feeding technique has given good results in various parameters reported by the researchers. In ovo feeding, the technique is expected to be one of the methods to increase the productivity of broiler chickens.

2.1. Growth and Development of Chicken Embryos

Chicken eggs will hatch after 21 days of incubation by going through a series of embryonic developments in a complex manner. The embryo is a multicellular diploid eukaryote that occurs outside the mother's body in the earliest stages of development. During its development, the embryo obtains food and protection from the egg's contents through yolk, albumin, and egg cage.[12][13]

The embryonic development of chickens starts from fertilization, blastulation, gastrulation, neurulation, and organogenesis. Fertilization is the combination of male sex cells and female sex cells to form a zygote, which occurs through mitotic division. In the blastulation process, blastoderm is formed where division occurs in the form of a blastomere mass that forms the basis of the prospective chicken's body. The next stage is Gastrula which forms the gastrulation and the axis of the embryo so that the embryo begins to grow elongated. Furthermore, at the Tubulation stage, the embryo occurs neurulation, namely the formation of a neural roof or forerunner of the central nervous system. And at

the last stage is organogenesis, the embryo develops from a primitive form into a definitive form that has a specific form and likeness within a single species [14].

Embryonic development is divided into three phases, namely; in the first week, it begins with the growth of embryos in the form of primitive stria and the formation of chorioallantoic membranes. In the second week, the embryonic growth process in the form of various organs is getting more and more perfect, as well as the exchange process of O2- CO2, the result of the chorioallantoic respiration process and the last phase includes the accumulation of glycogen in the liver and muscle tissue, the start of respiration in the lungs, the entry of the remaining yolk sac into the body, pipping and hatching.[3]

Exterior factors and the egg's interior influence the embryo development process during incubation. Exterior factors are influenced, such as temperature, humidity, ventilation, egg size, and egg cage conditions [12]. Internal factors can be influenced by genetics or breed and egg nutrition [9][14]. The very high rate of broiler growth and development also has negative impacts, such as the occurrence of ascites, bone abnormalities, and decreased immune system [1]. This is likely to happen because of differences in growth and development in the embryogenesis and post-hatching processes Growth and development in the embryonic period greatly affect the post-hatching broiler growth and development period where good organ growth, especially the digestive tract, will make the absorption of nutrients more optimal. [16]

The digestive tract has a mucous lining of the small intestine, a highly functional epithelium composed of absorptive. Sensory cells, which are constantly updated by a multipotent intestinal stem cell (ISC) located inside the crypt that turns into functionary cells lining the intestinal villi in chicken embryos (Gallus gallus), villi formation occurs on the 15th day of embryonic development or incubation. At the same time, the crypt develops on day 21 (the day of hatching). The stimulation of nutrients can enhance the development of this small intestine through [17][18] in ovo feeding [10]. At the time of hatching, early feeding stimulates the completion of the maturation of the small intestine through the expansion of the epithelium of crypt and villi, the mediation of cell proliferation and differentiation, and the activation of nutrient transporters, digestive enzymes, and mucus secretions [16].

2.2. In Ovo Feeding Technology

Early access to nutrients is fundamental in the early stages of growth and utilization of nutrients by chicks. The rapid growth of body weight, the efficiency of feed utilization, and gaining marketable weight early, this improvement in [3][19] prenatal development can be achieved by in ovo feeding. Supplementation of macro- and micronutrients and probiotics also causes good results on hatchability and disease resistance in chicks [20].

In ovo feeding is a technique of administering additional essential substances into the egg. The in ovo feeding application was first used for vaccination against Marek's disease (MD) on the 18th day of incubation and produced a better vaccine and effect. Previously, the Marek vaccination process was carried out by manual injection through subcutaneous. Morevoer, after the success of the in ovo technique for vaccination, Embrex Inc. produced the Inovoject R engine, which became the first automatic machine introduced in the United States (Sharma & Burmester, 1982) [21].

Automatic in ovo injection machines until now are produced by various companies that hatcheries use around the world. The results of using some automatic in ovo injection machines show different results on hatching performance parameters, with consideration given to the age of the floc breeder and the age of egg transfer. The advantages of automatic [22] in ovo injection include a faster vaccination process and a more significant number of eggs, reduced labor costs, reduced human error risk, and the immune system can be given from the beginning. The injection process in ovo is carried out quickly due to the limited time during the transfer of eggs from the setter to the hatcher unit,

Daryatmo et. al ./Jurnal Ternak 14 (2) 2023 pp. 51 - 58

which is carried out at an age between 17.50 and 19.20 incubation days or sometimes differs depending on the type of hatching machine used [21] [23].

In ovo feeding injection can be done through the embryo directly and through other parts of the egg such as yolk sac, albumin, amnion, allantois and chorion as well as air pockets so that the nutrients given can be absorbed The position of the injection in ovo depends on the age of hatching or embryo The egg to be injected[3], [23] in ovo feeding[24] at the beginning of hatching or the early stage of embryo growth is given in the position of the yolk closest to the initial embryo while in the late stage or age of 17-19 days the embryo can be given in the amniotic position [25] Next various studies have been carried out with various types of natural nutrients such as amino acids, carbohydrates, vitamins, probiotics, minerals that can support the development of embryos and chickens post-hatching for an intensive [24]maintenance system The addition of nutrients in the amnion through in ovo feeding in the embryonic period can accelerate intestinal development and increase the ability of chickens to digest nutrients after hatching[20]

3. Method

The method used in compiling this review article is the literature study technique. Criteria in the form of primary data include national and international journals in English That discuss the process of growth and development and nutrition, which has an important role, and the application of in ovo feeding techniques in the incubation process. The articles that have been collected are then selected by looking at the collection of journal contents with review articles to be made.

Nutrition	Types of	Time	The Effect of In Ovo Feeding	Reference
Group	Nutrition	incubation (day to)		
Amino acids	Arginine	18	 Increased hatching weight Increased body weight at the age of 42 days 	[27]
	Glutamine	17	 Stimulation of the small intestine of the embryo Increase the size of the small intestine and the number and size of intestinal villi 	[17]
	Leucine	14 and 19	- Increased Tolerance to heat in broilers	[28]
	Methionine	18	- Longer intestinal morphometry	[29]
	Threonine	18	 Morphological and functional improvement of the intestinal mucosa Increased performance of chicks when hatching and at the age of 21 days post- hatching. 	[30]
Mineral	Selenium	18	 Improvement of the humoral and cellular immune system Prevents oxidative stress Preventing the incidence of necrotic enteritis disease 	[31]
	Combination of minerals (Fe,Zn, Mn,Cu,Ca,P)	17	- Increased concentration of Ca and P and Percentage of chicken bone weight	[32]

Table 1. In Ovo Feeding with Different Types of Nutrients

Nutrition Stimulation with in Ovo Feeding...... Jurnal Ternak (Animal Science Journal) with CC BY NC SA license.

	Zn	9	- Prevents oxidative processes in	[33]
			the heart of the embryo	
	Cu	18	Increases the Percentage of pectoral muscles	[34]
Vitamin	Vitamin A	18	- Boosts the immune system	[35]
	Vitamin C	17	- Hatchability increases	[36]
	(Ascorbic Acid)		- The development of leg muscles and the systemic antioxidant capacity of broilers at the age of 42 days.	[37]
	Vitamin D3	18	- Weight gain	[38]
			- Increased Percentage of breast meat	
			- Decrease in plasma concentrations of glycoprotein acid (AGP)	
	Vitamin E	18	 Improved hatchability and doc quality Improved physiological response. 	[39]
	Vitamin K	18	 Performance improvements Immune system enhancement Bone growth 	[40]
Probiotics	Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium, and	18	 Week 1 performance improvement As an immunomodulator of immunity, gene expression 	[41]
	Bifidobacterium bifidum			
	Enterococcus faecium and Bacillus subtilis,	17	A decrease in the number of Salmonella enteritidis in chicks (immunity is getting better)	[42]
Carbohydrates	Carbohydrates	14,5	 Increased hatchability Increased hatching weight Increased pectoralis muscle size 	[43]

4. Results and Discussion

The type of nutrition used for injection in ovo feeding has different roles and functions in the growth and development of embryos [26]. Some types of nutrients have a specific influence on the development of embryos in the hatching period so that they can produce performance and support the desired genetic expansion. These functions such as for the process of catabolism and anabolism of proteins, and energy sources, activation of the immune system and the development of the intestinal mucosal area [5][10]

Based on various studies, it is shown that stimulation of nutrition in ovo feeding can improve the performance of chickens in the prenatal and postnatal periods. However, various studies have also shown different results, such as decreased hatchability due to the application of in ovo feeding technology. Proper in ovo feeding application can result in better hatching performance and growth of chickens after hatching. The success of the in ovo technique is influenced by sharing factors such as the location and route of injection dose incubation age or embryo cleanliness of the premises,

equipment, and preparation for the implementation of in ovo feeding [25][44][23][45][46][21]. In addition to this, the type and volume of nutrient solvent liquid can also affect hatching performance [6]. Too much solvent liquid will affect the level of moisture in the egg, which can cause embryo death and hatching failure [24].

5. Conclusion

Nutrition stimulation with in ovo feeding technique produces many benefits such as increasing hatchability, hatching weight, immunity level, reducing bone system disorders, reduced morbidity and mortality after hatching, feed efficiency, and weight gain. This can provide an overview of the importance of feeding earlier to optimize broiler performance and be more efficient. Although there are differences in the results about the effectiveness of in ovo feeding applications in various other studies, of course, this is an opportunity to improve the ability of in ovo feeding techniques to be better with various nutritional potentials and other techniques.

6. References

- [1] M. J. Zuidhof, B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson, "Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 20051," Poult Sci, vol. 93, no. 12, pp. 2970–2982, 2014, doi: 10.3382/ps.2014-04291.
- [2] G. S. Borges Pessôa, F. C. de Tavernari, R. A. Vieira, and L. T. Fernando Albino, "New concepts in poultry nutrition," Rev. Bras. Saúde prod. Anim. , vol. 13, no. 3, pp. 755–774, 2012.
- [3] K. K. Pandey, S. Koley, B. K. Ojha, N. Kurechiya, S. Singh, and A. Singh, "In ovo feeding: Viewpoints on the current status, application and prospect in poultry," Indian Journal Of Animal Health, Vol. 60, NO. 2, Dec. 2021, doi: 10.36062/ijah.2021.04021.
- [4] M. Grodzik et al., "Nano-nutrition of chicken embryos. The effect of in ovo administration of diamond nanoparticles and L-glutamine on molecular responses in chicken embryo pectoral muscles," Int J Mol Sci, vol. 14, no. 11, pp. 23033–23044, Nov. 2013, doi: 10.3390/ijms141123033.
- [5] F. Gonçalves et al. , "Literature Review In Ovo Nutrition: Strategy For Precision Nutrition In Poultry Industry," 2013.
- [6] A. M. de A. Campos, H. Santiago Rostagno, P. Cezar Gomes, E. Aparecida da Silva, L. Fernando Teixeira Albino, and E. Terra Nogueira, "Efeito da inoculação de soluções nutritivas in ovo sobre a eclodibilidade e o desempenho de frangos de corte," R. Bras. Zootec, vol. 40, no. 8, pp. 1712–1717, 2011, [Online]. Available: www.sbz.org.br
- [7] O. T. Foye, Z. Uni, and P. R. Ferket, "Effect of In ovo feeding Egg White Protein, β-Hydroxyβ-Methylbutyrate, and Carbohydrates on Glycogen Status and Neonatal Growth of Turkeys," Poult Sci, vol. 85, pp. 1185–1192, 2006.
- [8] T. M. Shafey, M. A. Alodan, I. M. Al-Ruqaie, and M. A. Abouheif, "In ovo feeding of carbohydrates and incubated at a high incubation temperature on hatchability and glycogen status of chicks," South African Journal of Animal Sciences, vol. 42, no. 3, pp. 210–220, 2012, doi: 10.4314/sajas.v42i3.2.
- [9] Z. Uni, "The effects of in ovo feeding," 2017. Accessed: Oct. 02, 2022. [Online]. Available: https://zootecnicainternational.com/featured/effects-ovo-feeding
- [10] Z. Uni and R. P. Ferket, "Methods for early nutrition and their potential," Worlds Poult Sci J, vol. 60, no. 1, pp. 101–111, Jun. 2004, doi: 10.1079/wps20040009.
- [11] J. M. Sharma and B. R. Burmester, "Resistance of Marek's Disease at Hatching in Chickens Vaccinated as Embryos with the Turkey," 1982. [Online]. Available: http://www.jstor.org/uRL:http://www.jstor.org/stable/1590032Accessed:11-12-201508:48UTC
- [12] T. W. Smith, "Avian Embryo," Mississippi State University, pp. 4–10, 2004.
- [13] F. Fitriani, H. Husmimi, D. Masyitha, and M. Akmal, "Histological Embryonic Development of Chickens in the One to Seven Days Incubation Period," Journal of Agripet, vol. 21, no. 1, Apr. 2021, doi: 10.17969/agripet.v21i1.18449.

- [14] V. Hamburger and H. L. Hamilton, "A series of normal stages in the development of the chick embryo," Developmental Dynamics, vol. 195, no. 4, pp. 231–272, 1992, doi: 10.1002/aja.1001950404.
- [15] M. Buzała, B. Janicki, and R. Czarnecki, "Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review," Poult Sci, vol. 94, no. 4, pp. 728–733, Jul. 2014, doi: 10.3382/ps/pev015.
- [16] N. Reicher, T. Melkman-Zehavi, J. Dayan, and Z. Uni, "It's All About Timing: Early Feeding Promotes Intestinal Maturation by Shifting the Ratios of Specialized Epithelial Cells in Chicks," Front Physiol, vol. 11, Dec. 2020, doi: 10.3389/fphys.2020.596457.
- [17] N. Reicher, T. Melkman-Zehavi, J. Dayan, E. A. Wong, and Z. Uni, "Nutritional stimulation by in ovo feeding modulates cellular proliferation and differentiation in the small intestinal epithelium of chicks," Animal Nutrition, vol. 8, no. 1, pp. 91–101, Mar. 2022, doi: 10.1016/j.aninu.2021.06.010.
- [18] Z. Uni, E. Tako, O. Gal-Garber, and D. Sklan, "Morphological, Molecular, and Functional Changes in the Chicken Small Intestine of the Late-Term Embryo," Poultry Science, vol. 82, pp. 1747–1754, 2003.
- [19] S. Bakyaraj, S. K. Bhanja, S. Majumdar, and B. Dash, "Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens," J Sci Food Agric, vol. 92, no. 2, pp. 313–320, Jan. 2012, doi: 10.1002/jsfa.4577.
- [20] Z. Uni and R. Ferket, "Enhancement Of Development Of Oviparous Species By In Ovo Feeding," US 6,592,878 B2, 2003
- [21] C. A. Ricks et al. , "In ovo vaccination technology," Adv Vet Med, vol. 41, p. 495–515, 1999, [Online]. Available: http://europepmc.org/abstract/MED/9890038
- [22] C. J. Williams and A. S. Zedek, "Comparative field evaluations of in ovo applied technology," Poult Sci, vol. 89, no. 1, pp. 189–193, 2010, doi: 10.3382/ps.2009-00093.
- [23] C. J. Williams and B. A. Hopkins, "Field evaluation of the accuracy of vaccine deposition by two different commercially available in ovo injection systems," Poult Sci, vol. 90, no. 1, pp. 223–226, 2011, doi: 10.3382/ps.2010-00759.
- [24] K. G. Joanna, K. Emilia, and D. Michalina, "In ovo feeding Technology of the future A review," Annals of Animal Science, vol. 17, no. 4. Sciendo, pp. 797–992, Oct. 01, 2017. doi: 10.1515/aoas-2017-0004.
- [25] M. R. Ebrahimi, Y. Jafari Ahangari, M. J. Zamiri, A. Akhlaghi, and H. Atashi, "Does preincubation in ovo injection of buffers or antioxidants improve the quality and hatchability in long-term stored eggs?," Poult Sci, vol. 91, no. 11, pp. 2970–2976, 2012, doi: 10.3382/ps.2012-02246.
- [26] L. Kannan Silva Alves et al., "In ovo feeding: a review," Veterinaria Noticias, vol. 26, no. 1, pp. 50–67, 2020.
- [27] T. M. Shafey, A. H. Mahmoud, A. A. Alsobayel, and M. A. Abouheif, "Effects of in ovo administration of amino acids on hatchability and performance of meat chickens," South African Journal of Animal Sciences, vol. 44, no. 2, pp. 123–130, 2014, doi: 10.4314/sajas.v44i2.4.
- [28] G. Han et al., "Effects of in ovo feeding of L-leucine on amino acids metabolism and heatshock protein-70, and -90 mRNA expression in heat-exposed chicks," Poult Sci, vol. 98, no. 3, pp. 1243–1253, Mar. 2019, doi: 10.3382/ps/pey444.
- [29] P. Groff-Urayama et al., "Performance, intestinal morphometry, and incubation parameters of broiler chickens submitted to in ovo feeding with different techniques and amino acids," Can J Anim Sci, vol. 99, no. 4, pp. 732–740, 2019, doi: 10.1139/cjas-2018-0131.
- [30] A. L. B. de Moreira Filho et al., "Intra-amnionic threonine administered to chicken embryos reduces salmonella enteritidis cecal counts and improves posthatch intestinal development," J Immunol Res, vol. 2018, 2018, doi: 10.1155/2018/9795829.
- [31] S. H. Lee et al., "Effects of in ovo injection with selenium on immune and antioxidant responses during experimental necrotic enteritis in broiler chickens," Poult Sci, vol. 93, no. 5, pp. 1113–1121, 2014, doi: 10.3382/ps.2013-03770.

- [32] R. Yair, R. Shahar, and Z. Uni, "Prenatal nutritional manipulation by in ovo enrichment influences bone structure, composition, and mechanical properties," J. Anim. Sci. , vol. 91:, pp. 2784–2793, 2013.
- [33] Y. Geng et al. , "Effect of in ovo manganese injection on the embryonic development, antioxidation, hatchability, and performances of offspring broilers under normal and high temperatures," Poult Sci, vol. 101, no. 8, Aug. 2022, doi: 10.1016/j.psj.2022.101936.
- [34] P. Joshua, C. Valli, and V. Balakrishnan, "Effect of in ovo supplementation of nano forms of zinc, copper, and selenium on post-hatch performance of broiler chicken," Vet World, vol. 9, no. 3, pp. 287–294, Mar. 2016, doi: 10.14202/vetworld.2016.287-294.
- [35] M. Alizadeh et al., "In ovo co-administration of vitamins (A and D) and probiotic lactobacilli modulates immune responses in broiler chickens," Poult Sci, vol. 101, no. 4, Apr. 2022, doi: 10.1016/j.psj.2022.101717.
- [36] D. L. P. Iglesias et al., "Ascorbic Acid in Egg Injection Minimizes the Effects of Fasting Between Hatching and Housing of Broiler Chicks," Int J Poult Sci, vol. 14, no. 7, pp. 387–393, 2015.
- [37] H. Zhang, K. E. C. Elliott, O. A. Durojaye, S. A. Fatemi, M. W. Schilling, and E. D. Peebles, "Effects of in ovo injection of L-ascorbic acid on growth performance, carcass composition, plasma antioxidant capacity, and meat quality in broiler chickens," Poult Sci, vol. 98, no. 9, pp. 3617–3625, Sep. 2019, doi: 10.3382/ps/pez173.
- [38] S. A. Fatemi, K. E. C. Elliott, A. Bello, and E. D. Peebles, "Effects of the in ovo injection of vitamin D3 and 25-hydroxyvitamin D3 in Ross 708 broilers subsequently challenged with coccidiosis. I. performance, meat yield and intestinal lesion incidence1,2,3," Poult Sci, vol. 100, no. 10, Oct. 2021, doi: 10.1016/j.psj.2021.101382.
- [39] I. C. S. Araújo et al., "Effect of vitamin E in ovo feeding to broiler embryos on hatchability, chick quality, oxidative state, and performance," Poult Sci, vol. 98, no. 9, pp. 3652–3661, Sep. 2019, doi: 10.3382/ps/pey439.
- [40] T. Abbasi, M. Shakeri, M. Zaghari, and H. Kohram, "Growth performance parameters, bone calcification and immune response of in ovo injection of 25-hydroxycholecalciferol and vitamin K3 in male ross 308 broilers," Theriogenology, vol. 90, pp. 260–265, Mar. 2017, doi: 10.1016/j.theriogenology.2016.12.016.
- [41] C. M. Pender, S. Kim, T. D. Potter, M. M. Ritzi, M. Young, and R. A. Dalloul, "In ovo supplementation of probiotics and its effects on performance and immune-related gene expression in broiler chicks," Poult Sci, vol. 96, no. 5, pp. 1052–1062, May 2017, doi: 10.3382/ps/pew381.
- [42] J. E. de Oliveira, E. van der Hoeven-Hangoor, I. B. van de Linde, R. C. Montijn, and J. M. B. M. van der Vossen, "In ovo inoculation of chicken embryos with probiotic bacteria and its effect on posthatch Salmonella susceptibility," Poult Sci, vol. 93, no. 4, pp. 818–829, 2014, doi: 10.3382/ps.2013-03409.
- [43] X. Y. Dong, Y. J. Jiang, M. Q. Wang, Y. M. Wang, and X. T. Zou, "Effects of in ovo feeding of carbohydrates on hatchability, body weight, and energy status in domestic pigeons (Columba livia)," Poult Sci, vol. 92, no. 8, pp. 2118–2123, 2013, doi: 10.3382/ps.2013-03091.
- [44] H. H. Liu et al., "In ovo administration of rhIGF-1 to duck eggs affects the expression of myogenic transcription factors and muscle mass during late embryo development," J Appl Physiol, vol. 111, pp. 1789–1797, 2011, doi: 10.1152/japplphysiol.00551.2011.-In.
- [45] Y. Ohta and M. T. Kidd, "Optimum Site for In Ovo Amino Acid Injection in Broiler Breeder Eggs," Poultry Science, vol. 80, pp. 1425–1429, 2001.
- [46] A. O. Sokale, C. J. Williams, T. S. Cummings, P. D. Gerard, A. Bello, and E. D. Peebles, "Effects of in ovo injection of different doses of coccidiosis vaccine and turn-out times on broiler performance," Poult Sci, vol. 97, no. 6, pp. 1891–1898, Jun. 2018, doi: 10.3382/ps/pey028.