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*Review Paper*

# *In ovo* technique—a new promising tool for improving growth production results, yield and meat quality of broilers

*Stamen Radulović[1], Dragan Šefer[1], Jelena Nedeljković Trailović[1], Dejan Perić[1], Mirjana Lukić[2], Bojan Stojanović[3] and Aleksandra Ivetić[4]*

[1] *University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, Belgrade, Serbia*
[2] *Institute of Meat Hygiene and Technology, Kaćanskog 13, Belgrade, Serbia*
[3] *University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun - Belgrade, Serbia*
[4] *The Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia*

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ABSTRACT

The perinatal period (the last few days prior and after hatching) is currently recognized as the most crucial time in the development of a young chick. During this time, the chicks undergo metabolic and physiological shifts from the utilization of egg nutrients to exogenous feed. Unlike mammals, embryonic development in avian species is an external process that occurs in the absence of continuous maternal energy supply. Especially in the final third of incubation, embryo development in birds becomes limited by the nutrient content present in the egg. *In ovo* technology is defined as the direct application of various feed additives (amino acids, hormones, vitamins, carbohydrates, minerals, prebiotics, probiotics), vaccines and nanoparticles into different internal parts of the egg during the incubation period. This technique has been recognized for its potential to improve digestive capacity, increase growth rate and feed efficiency, reduce post-hatch mortality and morbidity, improve immune response to enteric antigens, reduce incidence of developmental skeletal disorders, and increase muscle development and breast meat yield. Nutrients like carbohydrates, amino acids, vitamins and trace minerals were found to be most effective for the purpose of *in ovo* feeding.

## 1. Introduction

Large selection centres dealing with the genetic improvement of broilers have created fast-growing hybrids (provenances) that reach their final slaughter weight at the age of 35-39 days, with minimal feed consumption per unit of growth. It is an interesting fact that the body weight of the boilers at the end of fattening (42nd day after hatching) in the period from 1957 to 2014 grew by 3.30% every year, while at the same time, the consumption of food per unit of growth (food conversion) decreased by 2.55% (*Kucharska-Gaca et al.*, 2017). However, the intensive growth of genetically improved hybrids caused the appearance of numerous health disorders, such as ascites, sudden death syndrome, improper development of the skeletal system (leg problems), and increased sensitivity to pathogenic microorganisms (*Radulovic et al.*, 2023). Genetic progress has put

***Corresponding author:** Stamen Radulović, *stamen.radulovic@gmail.com*

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more stress on the fast growing and high meatiness bird, which resulted in histological and biochemical modifications of the muscle tissue by impairing some meat quality traits (*Petracci and Cavani*, 2012).

In addition to all these previously mentioned problems, it is considered that the amount of nutrients contained in the egg is no longer sufficient for the optimal development of embryonic tissues. Unlike in mammals, embryonic development (inside the egg) in poultry is an "external process", during which there is no possibility of supplying energy and other nutrients from the mother, and which makes the nutritional status of the embryo crucial for the success in the production of chicks. In relation to the total lifespan of modern broiler hybrids (35-39 days), the embryonic phase (21 days) represents about 33-38%. Bearing in mind that the formation and early development of tissues (muscles and digestive tract) takes place precisely in this phase, there is a strong connection between embryonic development and the production results that broilers can achieve during the fattening period. Physiological and metabolic imbalances at the end of the embryonic phase and the initial period of development after incubation of chicks negatively affect their subsequent growth and carcass characteristics (*Retes et al*., 2018).

At the same time, it should be noted that in industrial production, after hatching, chicks generally remain without food and water for 24-72 hours. This occurs due to different hatching times, handling of chicks (separation by sex, vaccination, packaging) and the time required to transport them to the farm (*Willemsen et al*., 2010). As a result, the starvation of chickens during this period has a negative effect on the morphometric characteristics of the mucous membrane of the digestive tract (shortening of the height of the intestinal villi and reduction of the depth of the crypts). In this way, the ability to digest and absorb nutrients (from the complete mixtures that the broilers will receive upon arrival at the farm) is reduced and the time required to reach slaughter weight is increased by 1-2 days. Due to the impossibility of food intake, peristaltic movements (which are activated by exogenous feeding) are absent, which results in slower transport of the contents of the yolk sac into the intestines. The impossibility of access to food additionally limits the use of nutrients that are present in the yolk, which leads to the consumption of energy reserves in the body. Under these conditions, the proliferation of satellite cells is reduced and muscle growth is inhibited, which further reduces the initial performance of broilers (*Zhao et al*., 2017, *Noy and Slan*, 1999). For all these reasons, embryonic nutrition is a key factor for muscle and digestive tract development during late embryonic development and in the first days after hatching.

## 2. *In ovo* technique

In order to overcome all the mentioned problems, and primarily to increase the availability of nutrients to the embryo, the *in ovo* nutrition technique was developed. With this technique, nutrients can be delivered (inoculated) to different internal parts of the egg. *In ovo* technology (Lat. in the egg) is defined as the direct application of feed additives (amino acids, hormones, vitamins, carbohydrates, minerals, prebiotics, probiotics, vaccines and nanoparticles) during the egg incubation period. By timely supply to the embryo of nutrients, positive effects on its growth, d evelopment, and resistance are achieved, but at the same time, long-term effects are accomplished, which become visible only during the next period, i.e., rearing and fattening of chicks (*Saeed et al.*, 2019). The main goals of this technique are reflected in treated individuals by the following:

1) Lower embryo mortality (by strengthening immunity and defence against pathogenic microorganisms),
2) Higher body mass at hatching,
3) Stimulated development of the gastrointestinal tract before and after hatching,
4) Growth stimulation,
5) Better utilization of feed, and
6) Higher meat yield (*Bhanja et al*., 2013).

The entire process of this technique can be applied manually or automatically. Both types of application have proven to be effective, but their choice depends primarily on the number of eggs that need to be treated, the age of the embryo, and types of inoculants that are used. This technique was first applied on a larger scale in the 1990s in the United States during the automatic vaccination of poultry against Marek's disease and Gumboro disease. After that, the possibility of its much wider application was recognized, primarily in the stimulation of embryonic development, immune response and the development of beneficial types of bacteria in the digestive tract of the embryo (*Kadam et al*., 2013). *Jokić et al*. (2023) described in detail the entire procedure of the *in ovo* technique, where the first step in the practical work is the selection of a specific hybrid of laying hens that will be examined or the collection of eggs from local producers. It is necessary to first examine all eggs, then discard the damaged ones and weigh only the ones that will be further used. The eggs are then laid in an incubator with controlled temperature and humidity (37.5°C and 60.0%, respectively, for Cobb 500). After a certain period (most often on the 6th day of incubation), the eggs are lamped and the non-fertile ones are discarded, while the fertile eggs are distributed into the appropriate groups. After a few days (generally on the 18th day of life) the following procedure is applied to evaluate the success of this technique:

1) The first group of eggs is treated with the prepared inoculum,
2) The second group is not treated and serves as a control, while
3) The third group is treated with normal (physiological) solution.

In this way, it is possible to compare the third with the first group, considering that handling eggs and piercing the egg shell with a needle can have negative effects on the development of the embryo. The solutions used for *in ovo* treatment must be heated to a temperature of 30°C before use. At the same time, it is very important to use a new sterile needle for each egg, which can be of different dimensions (most often 13 × 0.3 mm or 18.4 × 1.27 mm) and a syringe with a volume of 1 ml. The injection site on the egg must be disinfected with iodine, alcohol or another disinfectant, after which the contents of the syringe are applied to the desired part of the egg, usually the amnion. In order to ensure success in the work, a certain number of eggs must be broken immediately after the treatment in order to check the accuracy (precision) of the application of the inoculum in the desired region. The needle penetration site is sealed with paraffin, and all eggs are then returned to the incubator. The room where the incubator is located must be regularly disinfected and a constant temperature and air humidity must be maintained. After hatching, the time elapsed from the beginning of incubation, percentage of hatchlings, and the body mass of chicks are measured. The hatched chicks can be sacrificed immediately or they are assigned further to rearing, during which they can be subjected to histological, biochemical and microbiological tests. At the end of the 25th day from the start of incubation, all unhatched eggs are opened to assess the cause of embryonic death and then record their number. Various factors, such as the site and rate of injection, the volume of the inoculum, and even the dimensions of the needle used, can have a significant impact on the success of the *in ovo* feeding technique.

## 3. *In ovo* administration of the inoculum

There are five basic egg regions into which inoculation can be conducted:

1) The body of the chicken embryo,
2) Amniotic fluid,
3) Allantoin membrane,
4) Air sac, and
5) Yolk sac (*Saeed et al.*, 2019).

In the initial stage of embryonic development, the inoculum is injected into the egg whites at a depth of 12 mm, as close as possible to the germinal disc. Only at a later stage of development are embryos inoculated into the yolk sac. This site is suitable for injection, primarily due to its large surface area and ability to digest nutrients. After the 17th day of incubation, the yolk sac is resorbed (but not completely), so the application is made into the air sac or amnion. It is considered that in the late stage of embryo development, *in ovo* administration is best carried out into the amniotic fluid, which then comes into contact with the cells of the digestive tract and serves the embryo as a source of nutrients. Therefore, the material injected into this area will be used together with the amniotic fluid (*Kucharska-Gaca et al.*, 2017). It is interesting to note that when vaccine is administered into the amniotic fluid, regardless of the day of vaccination, more than 90% protection is achieved, while injecting the same vaccine into the allantoin fluid or the air sac creates less than 50% protection. Special attention must be paid to the depth of injection. If the needle is not applied deep enough into the egg, dispersion of the inoculum into the allantoic fluid or air sac may occur, while inserting the needle too deeply can result in trauma to the embryo. The injection site is determined based on the characteristics of the injected substance, the procedure of its introduction, the age of the embryo, etc. (Roto et al., 2016).

## 4. Nutrients with highest potential for in ovo administration

### Carbohydrates

Among the nutrients most often examined in the *in ovo* technique, carbohydrates are in first place, because inside the egg they occur only in very low concentrations (amounting to < 1% of total nutrients, while only 0.3% is free glucose). An additional reason for the use of carbohydrates in this diet is the fact that increasing the amount of available carbohydrates can reduce the use of amino acids in gluconeogenesis and, thus, increase protein synthesis in the muscles of the embryo (*Retes et al*., 2017). In this way, by using carbohydrates and stimulating the proliferation of satellite cells, long-term effects on muscle growth can be achieved (increased myofibril diameter and muscle weight on the 35th day after hatching). By injecting carbohydrates (sucrose + maltose), it is possible to increase glycogen reserves in the liver and muscles of the embryo, thus creating favourable conditions for overcoming the energy deficit in the last days of incubation (*Neves et al.*, 2016; *Saeed et al*., 2019). An important effect of *in ovo* application of carbohydrates is the achievement of a higher body mass of chicks at hatching. This can be the result of an increase in the mass of the yolk sac, considering that with an additional supply of carbohydrates, the embryo has less need to use nutrients from the yolk. However, an increase in the mass of the yolk sac can also be a consequence of an increase in water, i.e., the amount of water present in the yolk, which has a negative effect on the transport of nutrients from the yolk sac to the body of the embryo. In order to avoid this effect, it is necessary to use adequate amounts of inoculum (using too little inoculum does not induce the desired effect, while using too much inoculum reduces the percentage of hatching) (*Kucharska-Gaca et al*., 2017). Using carbohydrates with the *in ovo* technique ensures:

1) Greater presence of these nutrients in the intestinal lumen,
2) Stimulation of intestinal villi development,
3) Stimulation of the activity of brush coat enzymes, and consequently
4) Improved intestinal capacity for digestion and absorption after hatching (*Tako et al*., 2004).

Today, there is an opinion that with *in ovo* nutrition, the digestive tract of the treated individuals, in terms of their functionality on hatching day, approaches the digestive tract of a two-day-old chicken. The digestive tract does not have the same ability to absorb all nutrients, nor is it always the same (constant). Thus, the absorption of carbohydrates and proteins is low at the time when chicks hatch, and gradually increases during the first four days of life. On the other hand, fatty acid absorption is high at hatching and remains at this level during the first four days after hatching. Numerous studies have shown that when eggs are inoculated with carbohydrates, this additional source of energy increases the development of goblet cells (the presence of carbohydrates in the lumen of the intestine is a trigger for the proliferation of goblet cells) and increases the surface of the intestinal villi, which creates better conditions for the absorption of nutrients (*Jokić et al*., 2023).

### Amino acids

Amino acids play a significant role in the body's immune response, growth and development of embryonic tissues, while, in the absence of carbohydrates, they can also be used as sources of energy. In this case, the application of amino acids can improve embryonic survival and increase embryo resistance to pathogenic microorganisms. It was established that during the early period of its development, the embryo uses the amino acids glycine, proline, lysine and arginine to a great extent. The needs for proline and glycine increase during embryonic development, so that in the last third of incubation, the contents of these two amino acids are not sufficient for normal embryo growth (*Cardeal et al*., 2015; *Saeed et al*., 2019). In overcoming this problem, exogenous (*in ovo* technique) addition of the amino acids in this phase is useful. By adding the amino acids proline and glycine in the last third of embryonic development, it is possible to limit antibodies from using them as a source of energy or as a source of protein. In this way, the basic (immunological) purpose of antibodies can be preserved, and thus, the conditions for improving production results during the rearing period can be provided. In addition to the amino acids proline and glycine, numerous studies have examined the effects of using arginine and threonine, either individually or in combination with other amino acids. Arginine is considered an essential amino acid for embryo growth, with its primary function in protein synthesis, although it can also be used for the synthesis of glucose (glucogenic amino acid). Also, this amino acid is used to create biologically active components that stimulate the secretion of growth hormones. Arginine also has the role of an immunomodulator, because it participates in the production of nitric oxide and in the increasing the function of the thymus over time. The amino acid threonine is the main component of intestinal mucin and participates in the synthesis and control of the secretion of pancreatic enzymes, which promotes the development of the digestive tract and the intake of food after hatching. In addition, threonine plays an important role in the immune protection of the body, given that it is part of gamma globulin (*Kucharska-Gaca et al*., 2017). After *in ovo* application (20–30 mg) of threonine into the yolk sac of embryos, the titre of antibodies against sheep red blood cells increases in broilers. Similar results can be achieved if other amino acids, such as glycine and serine, are used simultaneously with threonine (*Toghyani et al.*, 2018).

**Vitamins**

The only source of vitamins for the development of the chicken embryo is a well-balanced diet of the mother hen. Mistakes made when formulating the ration intended for the parent flock often result in deficits and inadequate embryo development, as well as increased embryonic mortality (most often in the middle or later stages of incubation). In this way, the application of vitamins and minerals is aimed at:

1) Improving the antioxidant status of the embryo,
2) Increasing embryo immune response,
3) Improving the bone system, and
4) Improving production results of chicks after hatching (Jokić et al., 2023).

During the utilization of lipids from the yolk sac, long-chain polyunsaturated fatty acids (PUFA) are formed in the embryonic tissues. A high concentration of PUFA in cell membranes increases the sensitivity of the embryo to the action of free radicals, which results in tissue breakdown and cell death (*Selim et al*., 2012). Antioxidants present in the egg yolk control this process by reducing or deactivating free radicals. However, a special problem is caused by the fact that when hatching, chicks switch from the so-called chorioallantoic respiration to the pulmonary type of respiration. This switch causes the concentration of oxygen in the tissues to increase, and due to intensive oxidative processes, the production of free radicals increases significantly (*Araujo et al*., 2019). Therefore, *in ovo* inoculation of antioxidants can significantly improve the antioxidant capacity of embryonic tissues, hatching, quality of chicks, as well as the production results that they will achieve during fattening. The effects of the use of vitamin E have been mostly studied in numerous *in ovo* studies. Its use can provide adequate protection of intestinal cells in the fight against free radicals and, thus, increase cell renewal and stimulate the growth of intestinal villi in the duodenum (*Bhanja et al*., 2012). At the same time, by increasing the antioxidant capacity (with the use of vitamin E) in the yolk sac, both the embryo and the chick in the first days after hatching are given the opportunity to more fully use the available nutrients. In addition to vitamin E, positive effects have been noted with the use of other vitamins, such as vitamins B1 and B2 in stimulating growth, as well as vitamins A, B1 and B6 in improving the immune response of broilers (*Saeed et al*., 2019).

**Mineral substances**

The role of minerals in the development of the skeletal, muscular, immune and cardiovascular systems is well known. Their optimal deposition in different parts of the egg is crucial for the proper development of the embryo. The hen (mother) deposits minerals in the egg in two ways:

1) Through the ovary to the yolk, and
2) Through the oviduct to the albumen, shell and shell membrane (Yair et al., 2015).

During the last days of incubation, the amount of Cu, Mn, P and Zn in the yolk is very low, so the embryo uses these minerals very little or not at all during this period (Yair and Uni, 2011). *In ovo* use of organic forms of Cu, Fe, Mn and Zn can increase their contents in the yolk and, thus, their use by the embryo. In this way, a long-term positive effect on mineralization and bone strength of broilers is achieved. Likewise, when using the aforementioned microelements, but in inorganic forms, their concentration in the yolk increases, which indicates that the form of the used minerals is not crucial for this transport (*Oliveira et al*., 2015). In addition to the abovementioned forms, in recent years, nanoparticles have been attracting more attention, i.e., nano forms of minerals are interesting considering the possibility that they bypass normal transport and undergo facilitated passage through the cell membrane. The use of nano forms of Zn, Cu and Se through *in ovo* feeding improves the feed conversion in broilers during fattening, as well as increasing the yield of breast muscle (*Joshua et al*., 2016). Using sodium selenite ($Na_2SeO_3$) as a source of Se improves the immune response and antioxidant capacity of chickens, especially if they are previously exposed to *Eimeria maxima* and *Clostridium perfringens*—the causative agents of necrotic enteritis (Lee et al., 2014).

**Other substances suitable for *in ovo* feeding**

In addition to all the listed and described nutrients, specific potential is also shown by other supplements, such as:

1) Growth hormone (IGF I)—stimulation of growth and development of muscle tissue (Bhanja et al., 2014),
2) Grapefruit seed extract—improved degree of hatching, improved daily gain and reduction of the population of *Escherichia coli* in the ileum (Hayati et al., 2014),
3) L-carnitine—improvement of the transport of fatty acid acyl residues from the yolk sac to embryonic tissues (Cardeal et al., 2015), and
4) In recent years, the possibility of early management of the microbial composition of the digestive tract in chickens has been highlighted as a special segment of *in ovo* nutrition. The concept itself does not represent anything new in poultry production, but the possibility that the management of microbiota, instead of in chicks during the rearing and fattening period, can already be started during embryonic development, represents a great innovative progress. The aforementioned concept of nutrition in modern literature is referred to as “early programming” or “early programmed development”, and implies the use of feed supplements such as probiotics, prebiotics, symbiotics and phytogenic preparations. Their common goal in the body of the treated individual is the establishment of a desirable and stable relationship between beneficial and harmful bacteria in the digestive tract. In this way, a eubiotic effect and a positive impact on the health of the digestive tract, as well as the health of the whole organism, is achieved, which is a basic prerequisite for achieving high production results (*Pruszynska-Oszmalek et al*., 2015; *Tavaniello et al*., 2018).

## 5. Future approaches regarding the in ovo technique

With the introduction of the *in ovo* feeding technique in poultry production, a new chapter has opened in the development and nutrition of broilers, and farmers are given the opportunity to manage this process before the birds arrive on the farm. The *in ovo* technique offers great potential for improving the animal’s immune system and production results. Given that *in ovo* vaccination has already become a common practice in modern poultry production, it is also desirable to enable the simultaneous application of *in ovo* nutrition and vaccination. In this way, the negative impact of additional egg handling is reduced and its practical application is facilitated, with lower overall costs. New trends in the application of *in ovo* nutrition are mainly aimed at examining the influence of the used inoculum on the expression of genes that control the creation of hormones and enzymes (as part of the modern concept of nutrigenomics). A number of substances used in the *in ovo* technique have shown potential to improve embryo growth and development, but the results obtained have often been inconsistent. For these reasons, further research is necessary in which only those substances with a constant effect in application will be singled out, and the mechanisms underlying the obtained results will be explained. Taking into account the fact that in a large number of *in ovo* feeding experiments, an unsuitably low percentage of hatched chicks resulted, it is necessary to define and establish less aggressive procedures which will avoid or at least reduce this effect and keep the hatching rate above 90%. In order to motivate producers for the wider application of *in ovo* nutrition, more detailed assessment of the long-term effects that this technique provides, primarily through the improvement of production results during the exploitation of individuals, will be necessary. A new aspect of this diet, which is developing intensively, points to the possibility of early programming, i.e., management of the microbiota of the embryo with the aim of establishing a desirable bacterial population, which will be maintained after hatching and during the first contact with pathogenic microorganisms from the environment. It is important to keep in mind that the development of the *in ovo* diet should not be any obstacle for the continuation of the development of a precisely formulated diet for chicks during the first week after hatching (the so-called pre-starter mixture). In fact, both mentioned feeding techniques should be developed in a synchronized manner to enable the best possible results during both production phases (incubation and fattening periods).

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## References

**Ara´ujo, C. S., Caf´e, B. M., Noleto, R. A., Martins, J. M. S., Ulhoa, C. J., Guareshi, G., & Reis, M., Leandro, N. S. M. (2019).** Effect of vitamin E in ovo feeding to broiler embryos on hatchability,chick quality, oxidative state, and performance. *Poultry Science,* 98, 3652–3661.

**Bhanja, S. K., Mandal, A. B., Agarwal, S. K., Majumdar, S., & Bhattacharyya, A. (2012).** Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. *Indian Journal of Poultry Science*, 16th European Symposium on Poultry Nutrition, 143–146.

**Bhanja, S. K., Sudhagar, M., Goel, A., Pandey, N., Mehra, M., Agarwal, S. K., & Mandal, A. (2014).** Differential expression of growth and immunity related genes influenced by in ovo supplementation of amino acids in broiler chickens. *Czech Journal of Animal Science*, 59(9), 399–408.

**Cardeal, P. C., Caldas, E. O. L., Lara, L. J. C., Rocha, J. S. R., Baião, N. C., Vaz, D. P., & Silva Martins, N. R. (2015).** In ovo feeding and its effects on performance of newly-hatched chicks. *World's Poultry Science Journal*, 71, 655–662.

**Hajati, H., Hassanabadi, A., Golian, A., Nassiri-Moghaddam, H., & Nassiri, M. R. (2014).** The effect of in ovo injection of grape seed extract and vitamin C on hatchability, antioxidant activity, yolk sac absorption, performance and ileal micro flora of broiler chickens. *Research Opinions in Animal & Veterinary Sciences,* 4(12), 633-638.

**Jokić, Ž., Radulović, S., & Šefer, D. (2023).** Ishrana živine, Univerzitet u Beogradu – Poljoprivredni fakultet.

**Joshua, P. P., Valli, C., & Balakrishnan, V. (2016).** Effect of in ovo supplementation of nano forms of zinc, copper, and selenium on post-hatch performance of broiler chicken. *Veterinary World,* 9(3), 287–294.

**Kadam, M. M., Barekatain, M. R., Bhanja, S. K., Iji, & P. A. (2013).** Prospects of in ovo feeding and nutrient supplementation for poultry: The science and commercial applications – a review. Journal of the Science of Food and Agriculture, 93(15), 3654-3661.

**Kucharska-Gaca J., Kowalska E., & Dębowska M. (2017).** In ovo feeding – technology of the future – a review. *Annals of Animal Science*, 17(4), 979–992.

**Lee, S. H., Lillehoj, H. S., Jang, S. I., Jeong, M. S., Xu, S. Z., Kim, J. B., Park, H. J., Kim, H. R., Lillehoj, E. P., & Bravo, DM. (2014).** Effects of in ovo injection with selenium on immune and antioxidant responses during experimental necrotic enteritis in broiler chickens Poultry Science, 93, 1113–1121.

**Neves, D. G., Retes, P. L., Rocha, R. R., Ferreira, L. G., Naves, L. P., & Alvarenga, R. R. (2016).** Effects of in ovo feeding with glycerol for broilers. *Journal of Animal Physiology and Animal Nutrition*, 101, 434–440.

**Noy, Y., & Sklan D. (1999).** Energy utilization in newly hatched chicks. *Poultry Science,* 78, 1750–1756.

**Oliveira, T. F. B., Bertechini, A. G., Bricka, R. M., Kim, E. J., Gerard, P. D., & Peebles, E. D. (2015).** Effects of in ovo injection of organic zinc, manganese, and copper on the hatchability and bone parameters of broiler hatchlings. *Poultry Science*, 94, 2488–2494.

**Petracci M., & Cavani C. (2012).** Muscle growth and poultry meat quality issues. *Nutrients*, 4, 1–12.

**Pruszynska-Oszmalek, E., Kolodziejski, P. A., Stadnicka, K., Sassek, M., Chalupka, D., Kuston, B., Nogowski, L., Mackowiak, P., Maiorano, G., Jankowski, J., & Bednarczyk M. (2015).** In ovo injection of prebiotics and synbiotics affects the digestive potency of the pancreas in growing chickens. *Poultry Science*, 94, 1909–1916.

**Radulović, S., Jokić, Ž., Šefer, D., Marković, R., Petrujkić, B., Perić, D., & Ivetić A. (2023).** Restriktivna ishrana brojlera- uticaj na proizvodne rezultate i kvalitet mesa Savetovanje veterinara Srbije Zlatibor 07 - 10. septembar 2023, 114–123.

**Retes, P. L., Clemente, A. H. S., Neves, D. G., Espósito, M., Makiyama, L., Alvarenga, R. R., Pereira, L. J., & Zangeronimo, M. G. (2018).** In ovo feeding of carbohydrates for broilers—a systematic review. *Journal of Animal Physiology and Animal Nutrition*, 102, 361–369.

**Roto, S. M., Kwon, Y.M., & Ricke, S. C. (2016).** Applications of in ovo technique for the optimal development of the gastrointestinal tract and the potential influence on the establishment of its microbiome in poultry. *Frontiers: Veterinary Science*, 3, 63.

**Saeed, M., Babazadeh, D., Naveed, M., Alagawany, M., El-Hack, M., Arain, M., & Tiwari, R. (2019).** In ovo delivery of various biological supplements, vaccines and drugs in poultry: current knowledge. *Journal of the Science of Food and Agriculture*, 99, 3727–3739.

**Selim, S. A., Gaafar, K. M., & El-ballal, S.S. (2012).** Influence of in-ovo administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. *Emirates Journal of Food and Agriculture*, 24, 264–271.

**Tako, E., Ferket, P. R., & Uni Z. (2004).** Effects of in ovo feeding of carbohydrates and beta hydroxy-beta-methylbutyrate on the development of chicken intestine. *Poultry Science*, 83, 2023–2028.

**Tavaniello, S., Mucci, R., Stadnicka, K., Acaye, O., Bednarczyk, M., & Maiorano, G. (2018).** Effect of in ovo administration of different synbiotics on carcass and meat quality traits in broiler chickens. Poultry Science, 1–9.

**Toghyani, M., Tahmasebi, S,. Modaresi, M., & Fosoul, S. (2018).** Effect of arginine and threonine in ovo supplementation on immune responses and some serum biochemical attributes in broiler chickens. *Italian Journal of Animal Science*, DOI: 10.1080/1828051X.2018.1529545.

**Willemsen, H., Debonne, M., Swennen, Q., Everaert, N., Careghi, C., Han, H., Bruggeman, V., Tona, K., & Decuypere, E. (2010).** Delay in feed access and spread of hatch: importance of early nutrition. *World's Poultry Science Journal*, 66, 177–188.

**Yair, R., & Uni Z. (2011).** Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment. Poultry Science, 90,1523–1531.

**Yair, R., Shahar, R., & Uni, Z. (2015).** In ovo feeding with minerals and vitamin $D_3$ improves bone properties in hatchlings and mature broilers. *Poultry Science*, 94, 2695–2707.

**Zhao, M. M., Gao, T., Zhang, L., Li, J. L., Lv, P. A., Yu, L. L., Gao, F., & Zhou, G. H. (2017).** In ovo feeding of creatine pyruvate alters energy reserves, satellite cell mitotic activity and myogenic gene expression of breast muscle in embryos and neonatal broilers. *Poultry Science*, 96, 3314–3323.

## Authors info

**Stamen Radulović,** https://orcid.org/0000-0002-7250-537X
**Dragan Šefer,** https://orcid.org/0000-0002-4394-6336
**Jelena Nedeljković Trailović,** https://orcid.org/0000-0002-9813-8176
**Dejan Perić,** https://orcid.org/0000-0002-4752-7489
**Mirjana Lukić,** https://orcid.org/0009-0001-7749-864X
**Bojan Stojanović,** https://orcid.org/0000-0001-5057-1790
**Aleksandra Ivetić,** https://orcid.org/0000-0003-2762-1870