

Influence of *In-Ovo* B-group Vitamin Administration on Postnatal Growth and Immunity in Replacement Pullets of the Hisex Brown Cross

IF Gorlov^{1*}, ZB Komarova¹, AV Rudkovskaya¹, MI Slozhenkina¹, AA Mosolov¹, OYu Drobyazko¹, EA Struk¹, DA Mosolova¹, EYu Anisimova¹ and VN Nikulin²

Received: 26th February 2024 / Accepted: 04th July 2025

ABSTRACT

Purpose: *In ovo* nutrient administration is a promising strategy for poultry improvement. In this study, the effects of B vitamins in ovo injected into the pocket of air of the eggs of the hybrid breed Hisex Brown on the 14th day of incubation were evaluated.

Research Method: Fertilized eggs ($n = 810$, $m = 63.8 \pm 0.4$) were divided into six groups: a control group (no vitamin, only sterile water injection) and groups treated with thiamine (B1), riboflavin (B2), pyridoxine (B6), Cyanocobalamin (B12), and folic acid (Bc). The age of the hens whose eggs were used for the experiment was 42 weeks. The hatchability of eggs, growth traits of birds, reproductive organs (mass and length of oviduct, weight of ovary), immune system organs (bursa of Fabricius, spleen, thymus), and natural resistance indices (bactericidal, lysozyme, and phagocytic activities) of pullets (17 weeks of age) were assessed.

Findings and Values: The hatchability of eggs was 3.70% higher in the B12 group, 2.96% higher in the B6 group, and 2.22% higher in the B1 and B2 groups than in the control group. The weight of day-old chicks ($T1 - p < 0.01$) and body weight of birds at 17 weeks ($T1, T3, T4, T5 - p < 0.01$; $T2 - p < 0.05$) in the experimental groups reliably exceeded those in the control. The formation of reproductive organs in replacement pullets differed among groups. The B1, B2, and Bc groups exhibited the most efficient immune system organ formation ($p < 0.05$). The mass of the bursa of Fabricius in these groups significantly exceeded that of the control group ($p < 0.01$). Treatment with B6 and Bc had the most significant effect on the thymus weight ($p < 0.05$). In all experimental groups, natural resistance was improved, as evidenced by increases in bactericidal, lysozyme, and phagocytic activity. *In ovo* injection with B-group vitamins increased the hatchability, growth indices, reproductive organ development, immunity, and natural resistance of pullets.

Keywords: Body weight, Embryonic development, Hatchability, In-Ovo injection, Poultry farming, Vitamin B.

INTRODUCTION

Extensive research has focused on improving embryonic nutrition by in ovo injections to increase the rate of hatching, productivity, immune status, and health of chickens (Tufarelli *et al.* 2021). Nutrient utilization begins on the first day of incubation, when both the egg albumen and yolk of the egg are food sources for

¹ Povolzhsky Research Institute of Manufacture &

Processing of Meat and Dairy Product, 6 Rokossovskogo Street, Volgograd 400131, Russian Federation.

² Orenburg State Agrarian University, 18 Chelyuskintsev street, 460014 Orenburg, Russian Federation.

*nimmmp@mail.ru

 <https://orcid.org/0000-0002-8683-8159>

the developing embryo. As a closed system, the proper supply of nutrients to the developing embryo is of paramount importance. Maternal nutrition is the only source of vitamins for eggs, and a vitamin deficiency leads to the death of embryos in the middle or end of the incubation period (Goel *et al.* 2013, Ghane *et al.* 2021, Dolgorukova *et al.* 2021). An insufficiency in water-soluble B vitamins, which function as coenzymes, in the diet of chickens leads to high embryonic mortality. In addition, post-hatching food shortages, which often occur in chicks delayed by 24–48 hours, indicate the need to store energy and nutrients prior to hatching in order to maintain metabolic processes and regulate body temperature (Uni and Ferket 2004).

Several strategies have been proposed to increase efficiency in the early stages of development, such as incubator feeding immediately after hatching (Momenh & Torki 2018) and *in ovo* injection feeding, a novel method of providing nutrients for fetal growth until hatching (Chen *et al.* 2009). For example, the *in ovo* feeding technique is used to deliver nutrients, amino acids (Ohta *et al.* 1999, Bhanja & Mandal 2005, Konashi *et al.* 2000), and carbohydrates (Dolgorukova *et al.* 2019) directly to the developing embryo to improve postnatal growth, immune responses, and gastrointestinal development.

To address inconsistent results of previous studies and a general lack of comprehensive studies of multiple B vitamins in a single experiment, we studied the effects of thiamine (B1), riboflavin (B2), pyridoxine (B6), cobalamin (B12), and folic acid (Bc) provided *in ovo* on the embryonic development of chickens as well as the growth, development, and immune status of replacement pullets of the hybrid “Hisex Brown.”

MATERIALS AND METHODS

The study was conducted in accordance with the European Convention for the Protection of Animals Used for Scientific Purposes (2003) and the ethical standards of Directive 2010/63/EU of the European Parliament and of the Council of

September 22, 2010, on the protection of animals used for scientific purposes. Blood sampling and poultry handling were practiced in accordance with the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry, guidelines established at the farm, and local poultry care laws and policies. The farm owner provided consent for the use of eggs and chicks in this study, and the ethical approval of the Povolzhsky Research Institute of Manufacture and Processing of Meat and Dairy Product Institutional Animal Care and Use Committee (EA NIIMMP # 2-2024-15-01) was received.

The effects of B vitamins injected in eggs by the *in ovo* method on hatchability, the rearing of replacement young stock of the hybrid breed Hisex Brown, and productivity were evaluated under the breeding conditions of the II order of the Svetly Joint Venture of the JSC Agrofirma Vostok Volgograd region (Table 1). The age of the hens and roosters of the parent flock was 42 weeks.

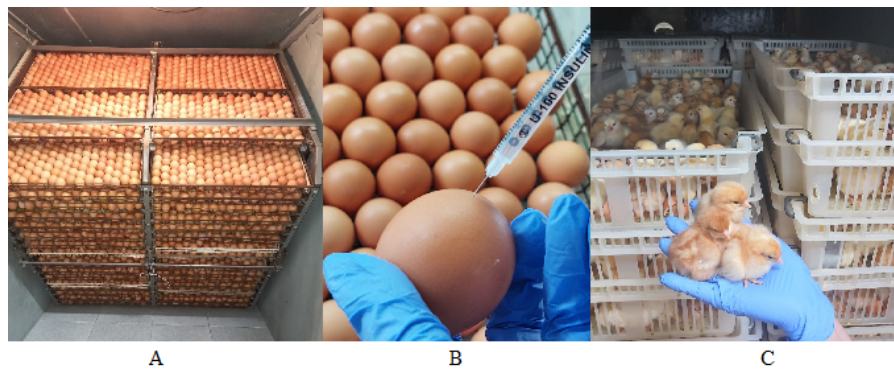
Before injecting the B vitamins, eggs are treated with a disinfectant. Then, using a small drill, a hole was made on the blunt end of the egg, through which the vitamin was injected (Fig. 1). The hole was then filled with warm wax. The manual injection of eggs during the incubation period is a labour-intensive process and is used only for research; in industrial conditions, machines are used for the vaccination of embryos (Khodorovich 2021).

In our studies, injection was performed on the 14th day of incubation. Fertilized eggs ($n = 810$, $m = 63.8 \pm 0.4$) were divided into six groups having three replicates with 45 eggs as follows: control (only sterile water injection, no vitamin), group I (vitamin B₁), group II (vitamin B₂), group III (vitamin B₆), group IV (vitamin B₁₂), and group V (vitamin B_c). All eggs were incubated immediately after collection. The eggs were disinfected with alcohol (30°C) and scanned under an ovoscope. A hole of no more than 1 mm in diameter was made.

A syringe (needle 0.3 mm in diameter) was used to inject a vitamin solution through the shell membrane into the air cell. The

Table 1. Experimental design

Vitamins in <i>ovo</i> (µg/egg)	Control	Experimental groups				
		I	II	III	IV	V
Thiamine (B1)	–	100	–	–	–	–
Riboflavin (B2)	–	–	100	–	–	–
Pyridoxine (B6)	–	–	–	50	–	–
Cobalamin (B12)	–	–	–	–	50	–
Folic acid (Bc)	–	–	–	–	–	150

**Figure 1. Overview of the experiment: A – incubation; B – *in ovo* injection; C – day-old chicks.**

pinhole site was sealed with sterile paraffin immediately after injection (Dolgorukova & Mikheeva 2020). The injected eggs were returned to the incubator *Universal-UIF-45* (*Pyatigorskseľ'mash*, Russia). The incubation conditions were as follows: 1st stage—37.7–37.8°C and 55–60% relative humidity from 1 to 14 days of incubation; 2nd stage—37.5°C and 45–50% relative humidity from 15 to 19 days of incubation; 3rd stage—37.0–37.2°C and 70% relative humidity from 20 days to time of hatching.

Before injection, weighed portions of vitamins were dissolved in 0.5 mL of sterile water. The body weight of day-old chicks was determined weekly using electronic scales (*Polaris PKS 0323 DL*, Polaris Co., Ltd., China; and *CAS SW-10W*, CAS Co., Ltd., Korea). For further experience, from hatched day-old chicks, only hens were selected (50 heads in each group). The reproductive (mass and length of oviduct, weight of ovary) and immune system (bursa of Fabricius, spleen, thymus) organs of the replacement pullets were examined after slaughter at the slaughterhouse of the farm: stunning plus exsanguination (10 birds per group

at 17 weeks of age). All organ samples were dried by wiping to remove excess moisture. The weights of the ovaries and oviducts were determined using an electronic scale (*EHA-501*, *Middle*, Russia). The length of the oviducts was measured with a ruler. Immune system organs of the replacement pullets (bursa of Fabricius, spleen, and thymus) were weighed on an electronic scale (*EHA-501*). The bactericidal activity in serum was evaluated by the kinetic bioluminescent method (Deryabin & Polyakov 2006), lysozyme activity by the turbidimetric method (Fogelson *et al.* 1954), and phagocytic activity of leukocytes by the bioluminescence extinction degree (Shirshev *et al.* 2014). The data were processed using Microsoft Office (Excel 2017). The Student's *t*-test was conducted at three probability levels. $P < 0.05$ was considered statistically significant compared with the control group.

RESULTS AND DISCUSSION

As summarized in Table 2, the injection of vitamin B12 (experimental group IV) during embryogenesis had the greatest effect on the development of chickens. Hatching increased

by five heads compared to the control, and the hatchability increased to 91.85%, compared with 88.15% in the control, inconsistent with the results of Teymouri *et al.* (2020), who reported that a significant difference was detected in the hatchability rate of the eggs *in ovo* injected with vitamin B12 compared to the control and sham groups ($P < 0.05$), where a significant decrease was observed in eggs *in ovo* injected with vitamin B12 (20 and 40 µg) on day 13 of incubation and 20 µg on day 15 of incubation; however, no significant difference was observed in eggs *in ovo* injected with vitamin B12 (40 µg) on day 15 of incubation compared to the control group ($P > 0.05$).

In experimental group III, in which vitamin B6 was administered *in ovo*, chicken hatching increased by four heads, and the hatchability was 91.11%, which was 2.96% higher than that in the control, consistent with the results of Bhanja *et al.* (2007).

In experimental group I (vitamin B1) and experimental group II (vitamin B2), three more chickens were obtained than the number in the control, and hatchability increased in both groups by 2.22%. Similar data were obtained by Bakyaraj *et al.* (2012). However, Goel *et al.* (2013) found no increase in egg hatchability. In experimental group V (folic acid Bc), the egg incubation results were equivalent to those in the control, with a hatchability of 88.15%. A previous study (Ismail *et al.* 2019) also reported no effect of *in ovo* folic acid injections on egg hatchability. In experimental groups I, II, III, and IV, an increase in the hatchability of eggs was achieved by reducing the number of dead embryos at the end of incubation and hatching.

The weights of day-old chickens in groups II (B2), III (B6), IV (B12), and V (folic acid) were slightly higher than the control values, and the weight in group I (B1) was significantly higher than that in the control by 0.48 g ($P < 0.01$).

Although the body weight of day-old chicks reliably exceeded that of the control only in the first experimental group ($P < 0.01$), in the process of growing replacement pullets,

higher weights were observed in all experimental groups than in the control group (T1, T3, T4, T5: $p < 0.01$; T2: $p < 0.05$). By the onset of physiological maturity (17 weeks), the body weights of replacement pullets were 82.2 g higher in experimental group I (5.50%; $P < 0.01$), 50.6 g higher in group II (3.38%; $P < 0.05$), 67.8 g higher in group III (4.53%; $P < 0.01$), 74.0 g higher in group IV (4.95%; $P < 0.01$), and 69.9 g higher in group V (4.67%; $P < 0.01$) than in the control group.

When growing replacement pullets, the formation of reproductive organs is a key determinant of further productivity. The state of the reproductive organs of replacement pullets at the age of 17 weeks is shown in Fig. 2. The length of the oviduct in pullets from experimental group I (B1) was significantly higher than that of the control group by 2.27 cm (6.28%; $P < 0.01$). Group III (B6) exhibited an increase of 2.07 cm (5.77%; $P < 0.01$), group IV (B12) by 2.39 cm (6.66%; $P < 0.01$), and group V (folic acid) by 2.24 cm (6.24%; $P < 0.01$). In group II (B2), the oviduct length exceeded that of the control by 1.42 cm (3.96%; $P < 0.05$). The mass of the oviduct was also increased in all experimental groups compared to the control. Specifically, the increase was 12.04% in group I ($P < 0.01$), 7.56% in group II ($P < 0.05$), 8.57% in group III ($P < 0.05$), 10.07% in group IV ($P < 0.01$), and 9.88% in group V ($P < 0.01$).

Ovary mass during physiological maturation also varied across experimental groups. The most pronounced increases were observed in groups I (B1) and IV (B12), with mean masses of 22.69 g and 22.63 g, respectively—representing increases of 19.29% and 18.97% over the control ($P < 0.01$ for both). In group III (B6) and group V (folic acid), ovary masses were 16.61% ($P < 0.01$) and 17.61% ($P < 0.05$) higher, respectively. In group II (B2), ovary mass increased by 14.14% compared to control ($P < 0.05$). The masses of central (thymus and bursa of Fabricius) and peripheral (spleen) immune organs in 17-week-old pullets across experimental and control groups are presented in Table 3.

Results of our studies have also shown that the treatment of embryos *in ovo* with vitamins B1

Table 2. Egg incubation results

Values	Control	B1	B2	B6	B12	Bc
Eggs with live embryos						
Pieces	135	135	135	135	135	135
"Blood ring" – death of embryos 1–7 days						
Pieces	3	2	3	2	2	3
%	2.22	1.49	1.49	1.49	1.49	2.22
Dead-in-shell – death of embryos 8–18 days						
Pieces	7	6	6	5	5	7
%	5.19	4.44	4.44	3.70	3.70	5.19
Addled egg – death on hatching, 19–21 days						
Pieces	6	5	5	5	4	6
%	4.44	3.70	3.70	3.70	2.96	4.44
Young stock bred						
Goals	119	122	122	123	124	119
Hatchability of eggs						
%	88.15	90.37	90.37	91.11	91.85	88.15
Weight of chickens						
g	36.73± 0.11	37.21± 0.13**	36.77± 0.10	36.79± 0.12	36.75± 0.09	36.81± 0.12

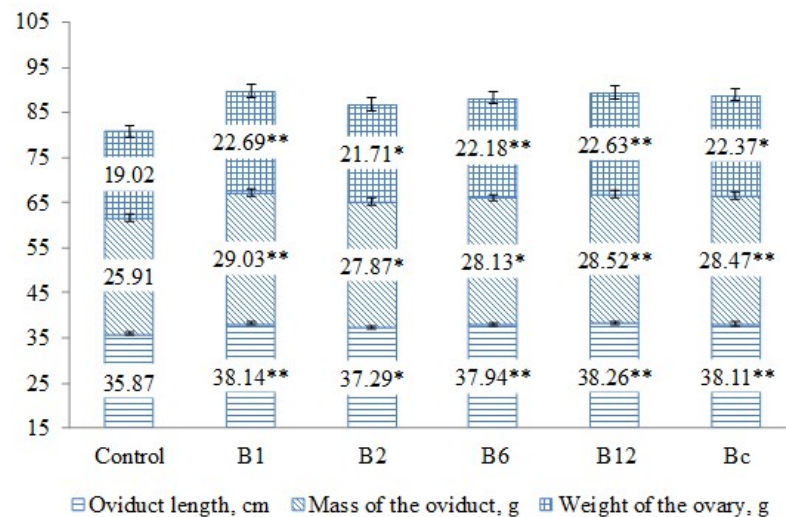
**P<0.01 compared with control group. M±m indicates mean ± standard error of mean.

(thiamine), B2 (riboflavin), and Bc (folic acid) effectively promotes the formation of immune system organs. The masses of the bursa of Fabricius in experimental groups I, II, and V were significantly higher than that in the control by 34.97% (P<0.01), 32.34% (P<0.01), and 28.99% (P<0.01), and the mass of the spleen was higher by 22.18% (P<0.05), 19.72% (P<0.05), and 15.85% (P<0.05).

In groups III (V6) and IV (V12), increases in the masses of these organs were observed; however,

the differences were not significant (P>0.05). The thymus mass was 20.57% (P<0.05) and 16.71% (P<0.05) higher after vitamin B6 (pyridoxine, group III) and Bc (folic acid, group V) than in the control group. In experimental groups I (B1), II (B2), and IV (B12), the differences in thymus mass compared with that in the control group were 9.51%, 3.34%, and 13.11% (P>0.05).

The injection of embryos with various B vitamins contributed to the activation of natural



Note: ** $p < 0.01$; * $p < 0.05$ compared with data for the control group

Figure 2. State of the reproductive organs.

Table 3. Metric indicators of the immune system organs (n = 10)

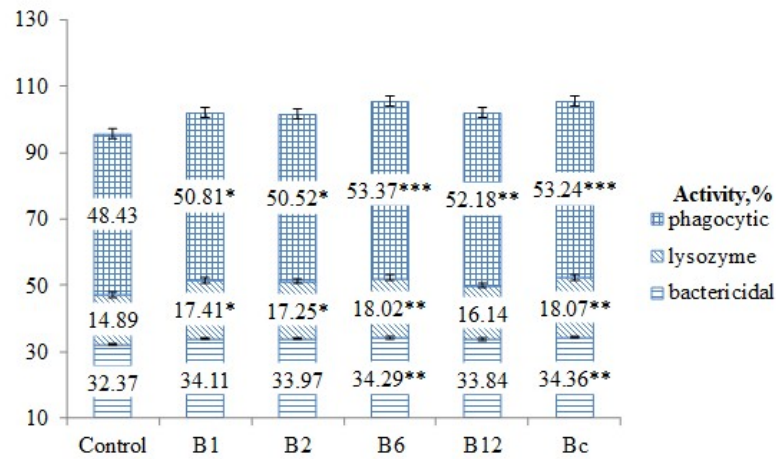
Values	Body weight of pullets (g)	Weight					
		Bursa Fabricius		Spleen		Thymus	
		g	%	g	%	g	%
Control	1495.9±16.14	2.69±0.18	0.18	2.84±0.17	0.19	3.89±0.19	0.26
B1	1578.1±17.39**	3.63±0.21**	0.23	3.47±0.19*	0.22	4.26±0.21	0.27
B2	1546.5±13.27*	3.56±0.19**	0.23	3.40±0.16*	0.22	4.02±0.15	0.26
B6	1563.7±15.32**	2.97±0.15	0.19	3.28±0.15	0.21	4.69±0.22*	0.30
B12	1569.9±17.99**	2.98±0.17	0.19	3.14±0.16	0.20	4.40±0.17	0.28
Bc (Folic Acid)	1565.8±11.69**	3.47±0.16**	0.22	3.29±0.13*	0.21	4.54±0.20*	0.29

* $P < 0.05$; ** $P < 0.01$ compared with the control group. Values are presented as mean ± standard error (M±m).

defense factors (Fig.3).

The bactericidal activity in the blood serum of replacement pullets in all experimental groups differed significantly from that of the control, with the greatest difference observed in groups III (B6) and V (folic acid) (i.e., 5.93 and 6.15%; both $P < 0.01$). Lysozyme activity levels in experimental groups I, II, III, and V were higher than that in the control group by 16.92% ($P < 0.05$), 15.85% ($P < 0.05$), 21.02% ($P < 0.01$), and 21.36% ($P < 0.01$); in experimental group

IV, the difference was negligible ($P > 0.05$). The phagocytic activity of leukocytes differed substantially in response to treatment in ovo with B vitamins. In groups III (B6), IV (B12), and V (folic acid), the phagocytic activity of leukocytes relative was 10.20% ($P < 0.001$), 7.74% ($P < 0.01$), and 9.93% ($P < 0.001$) higher than that in the control group. In experimental groups I (B1) and II (B2), this indicator also exceeded levels in the control by 4.91% ($P < 0.05$) and 4.32% ($P < 0.05$), consistent with the results of Goel *et al.* (2013) and Ghane *et al.* (2021).



Note: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ compared with data for the control group

Figure 3. State of natural resistance against infections in the blood.

CONCLUSIONS

The results of this study revealed that B group vitamins administered in ovo affect the hatchability, growth, reproductive organ formation, immune system activation, and natural resistance of the organism. Amongst the different vitamin B's administered, the most influence on hatchability and growth traits as well as on reproductive and immune organ

development has been shown by B1 and B12, although B6 and Bc have had a higher effect on natural resistance indices of birds.

Acknowledgements

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation. The authors are grateful to the owners and employees of farm where the investigation was conducted.

REFERENCES

- Bakayaraj S, Bhanja SK, Majumdar S, Dash B (2012) Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens. *Journal of the Science of Food and Agriculture*, 92(2), 313–320. <https://doi.org/10.1002/jsfa.4577>.
- Bhanja SK, Mandal AB (2005) Effect of in ovo injection of critical amino acids on pre and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens. *Asian Australasian Journal of Animal Sciences*, 18, 524–531. <https://doi.org/10.5713/AJAS.2005.524>.
- Bhanja S, Mandal A, Agarwal S, Majumdar S, Bhattacharyya A (2007) Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. *World Poultry Science Association*, Proceedings of the 16th European Symposium on Poultry Nutrition, France. <https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-france-2007/46.pdf> [Access date: 14.06.2025].
- Chen W, Wang R, Wan H, Xiong X, Peng P, Peng J (2009) Influence of in ovo injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. *British Poultry Science*, 50(4), 436–442. <https://doi.org/10.1080/00071660903114341>.

- Deryabin DG, Polyakov EG (2006) On-line determination of serum bactericidal activity using recombinant luminescent bacteria. *Bulletin of Experimental Biology and Medicine*, 142, 234–238. <https://doi.org/10.1007/s10517-006-0336-4>.
- Dolgorukova AM, Mikheeva MS (2020) Influence of prenatal feeding of embryos on the development of the organs of the gastrointestinal tract and the growth rate of chickens. *Achievements of Science and Technology of the Agro-industrial Complex*, 34(3), 62–65. <https://doi.org/10.24411/0235-2451-2020-10312>.
- Dolgorukova AM, Tishenkova MS, Zotov AA, Gupalo IM (2021) Influence of the use of L-carnitine and ubiquinone in ovo on the embryonic development of meat chickens. *Journal of Agriculture and Environment*, 4(20), 1–5. https://jae.cifra.science/media/legacy_articles/jae_16704.pdf?ysclid=lrqcl6nhj5739794665 [Access date: 14.06.2025].
- Dolgorukova AM, Titov VY, Zotov AA, Mikhaleva MS, Sidorov LI (2019) A method for increasing the growth rate of chickens. *Official bulletin "Inventions. Utility models"*, RF, RU 2700095, Bull. No. 26, 9p. <https://www1.fips.ru/iiss/document.xhtml?faces-redirect=true&id=4319fbbf1d91eb44402163d2c81df998> [Access date: 14.06.2025].
- Fogelson SJ, Ross A, Lobstein OE (1954) A method for the quantitative determination of lysozyme activity in blood. *American Journal of Digestive Diseases*, 21, 327–331. <https://doi.org/10.1007/BF02883952>.
- Ghane F, Qotbi AAA, Slozhenkina M, Mosolov AA, Gorlov I, Seidavi A, Colonna MA, Laudadio V, Tufarelli V (2021) Effects of in ovo feeding of vitamin E or vitamin C on egg hatchability, performance, carcass traits and immunity in broiler chickens. *Animal Biotechnology*, Jul 19, 1–6. <https://doi.org/10.1080/10495398.2021.1950744>.
- Goel A, Bhanja SK, Pande V, Mehra M, Mandal A (2013) Effects of in ovo administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. *Indian Journal of Animal Science*, 83(9), 916–921. https://www.researchgate.net/publication/258255366_Effects_of_in_ovo_administration_of_vitamins_on_post_hatch-growth-immunocompetence_and_blood_biochemical_profiles_of_broiler_chickens [Access date: 14.06.2025].
- Ismail F, Beshara M, El-Gayar M (2019) Effect of in-ovo injection of ascorbic, folic acids and their combination on hatchability and subsequent growth performance of broiler chicks. *Journal of Animal and Poultry Production*, 10(9), 289–295. <https://doi.org/10.21608/jappmu.2019.54813>.
- Khodorovich V (2021) Vaccination and stimulation with biological products. The main aspects of immunization of chickens in ovo. *Animal Husbandry of Russia*, 4, 18–20. <https://zsr.ru/zsr-2021-04-005?ysclid=lrqczhv5i8624239241> [Access date: 14.06.2025].
- Konashi S, Takahashi K, Akiba Y (2000) Effects of dietary essential amino acid deficiencies on immunologic variables in broiler chickens. *British Journal of Nutrition*, 83, 449–456. <https://doi.org/10.1017/S0007114500000556>.
- Momeneh T, Torki M (2018) Effects of in ovo injection of vitamins B6 and B12 in fertile eggs subjected to ethanol stress on hatching traits, performance and visceral organs of broiler chicks reared under cold stress condition. *Iranian Journal of Applied Animal Science*, 8(3), 491–498. https://www.researchgate.net/publication/327618912_Effects_of_in_ovo_injection_of_vitamins_B6_and_B12_in_fertile_eggs_subjected_to_ethanol_stress_on_hatching_traits_performance_and_visceral_organs_of_broiler_chicks_reared_under_cold_stress_condition [Access date: 14.06.2025].

- Ohta YN, Tisushima K, Koide K, Kidd MT, Ishibashi T (1999) Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poultry Science*, 78, 1493–1498. <https://doi.org/10.1093/ps/78.11.1493>.
- Shirshev SV, Kuklina EM, Zamorina SA, Nikitina NM, Nekrasova IV (2014) Method for determining phagocytic activity of human peripheral blood neutrophils from bioluminescence extinction degree data. *Immunologiya*, 30(6), 312–317. https://www.elibrary.ru/download/elibrary_22629211_72889535.pdf [Access date: 14.06.2025].
- Teymouri B, Ghalehkandi JG, Hassanpour S, Aghdam-Shahryar H (2020) Effect of in ovo feeding of the vitamin B₁₂ on hatchability, performance and blood constituents in broiler chicken. *International Journal of Peptide Research and Therapeutics*, 26(1), 381–387. <https://doi.org/10.1007/s10989-019-09844-0>.
- Tufarelli V, Baghban-Kanani P, Azimi-Youvalari S, Hosseintabar-Ghasemabad B, Slozhenkina M, Gorlov I, Frolova MV, Seidavi A, Laudadio V (2021) Effect of dietary flaxseed meal supplemented with dried tomato and grape pomace on performance traits and antioxidant status of laying hens. *Animal Biotechnology*, May 6, 1–8. <https://doi.org/10.1080/10495398.2021.1914070>.
- Uni Z, Ferket R (2004) Methods for early nutrition and their potential. *World's Poultry Science Journal*, 60(1), 101–111. <https://doi.org/10.1079/WPS20038>.