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THE INFLUENCE OF IN OVO GARLIC AND TOMATO EXTRACT INJECTIONS ON EMBRYOGENESIS AND NEUROLOGICAL TRAITS IN CHICKEN EMBRYOS

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Abstract

This study aimed to determine the effect of in ovo injections of garlic and tomato extracts on the embryogenesis and neurological traits of chicken embryos with a focus on growth parameters and neurological development during the embryonic stage. A total of 300 eggs were placed in five distinct groups: Group 1 (control negative, CO, without injection); Group 2 (control positive, distilled water, DW, 0.1 mL/egg); Group 3 (garlic extract, GAR, 0.1 mg/egg); Group 4 (tomato extract, TOM, 0.1 mg/egg); and Group 5 (garlic and tomato extract mix, ATM, 0.1 mL/egg). The narrow ends of the albumins were injected with the extracts on the fifth day of incubation using a 1 mL syringe with a 2-gauge needle. The results show a significant increase ($p<0.01$) in embryogenesis for embryonic mass, chick body weight, hatchability, and embryo index (EI) for ATM, TOM, and GAR groups compared to CO and DW. There was also a significant increase ($p<0.01$) in the neurological traits of neurons, brain mass, and brain index (BI) for the ATM, TOM, and GAR groups compared to the CO and DW ones. In conclusion, adding garlic and tomato extracts into the eggs improves embryonic growth and development, increases nerve

formation, and enhances nerve signal transmission within the embryo, leading to improvements in biological processes and optimal embryonic development.

Keywords: Embryo, Embryonic Development, Garlic and Tomato, Neurophysiology.

تأثير حقن بيض التفقيس بمستخلص الثوم والطماطم في التطور الجنيني والصفات الفسيولوجية العصبية في اجنة فروج اللحم

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير حقن بيض التفقيس بمستخلصي الثوم والطماطم في البيضة على التطور الجنيني والصفات الفيسيولوجية العصبية في أجنة الدجاج. ويهدف إلى إظهار كيفية تأثير هذه المستخلصات الطبيعية على تطور النمو والتطور العصبي خلال المرحلة الجنينية من التطور الجنيني. تم تقسيم ثلاثمائة بيضة إلى خمس مجموعات: Control1 (CO؛ العلاج 1، T1) (بدون حقن)؛ التحكم 2 الماء المقطر، (DW؛ 0.1 مل/بيضة)؛ مستخلص الثوم (GAR) (0.1 مجم/بيضة)؛ مستخلص الطماطم (TOM) (0.1 مجم/بيضة)؛ ومستخلص الثوم والطماطم المختلط (ATM) (0.1 مل/بيضة). تم تحضير المحلول وحقنه في الألبومين من النهاية الرقيقة للبيض في اليوم الخامس باستخدام محقنة (1 مل) بإبرة (قياس 2). أظهرت النتائج زيادة معنوية ($p<0.01$) في التطور الجنيني لوزن الجنين ووزن جسم الافراخ الفاقسة ونسبة الفقس ودليل الجنين (EI) لـ ATM وTOM وGAR، مقارنة مع CO وDW. زيادة معنوية ($P <0.01$) في الصفات الفيسيولوجية العصبية للخلايا العصبية ووزن الدماغ ودليل الدماغ (BI) لـ ATM وTOM وGAR مقارنة CO DW. تستنتج هذه الدراسة إن حقن مستخلص الثوم والطماطم داخل البيضة يساعد على تحسين النمو الجنيني والتطور الجنيني وزيادة تكوين الأعصاب ونقل الإشارات العصبية داخل الجنين مما يؤدي إلى تحسن في العمليات البيولوجية للجنين وهذا سيؤدي إلى التطور الجنيني الامثل.

كلمات مفتاحية: جنين فروج اللحم، تطور جنيني، مستخلص الثوم والمطاطة، فسجلة الاعصاب.

Introduction

Environmental and nutritional factors have a critical influence on the embryonic development of chickens. External elements can retard or improve growth, embryonic development, and physiological characteristics, with good nutrition providing the vigor required by embryos to face the stress of the hatching process (19). Natural extracts play a vital role in improving the health and growth of biological organisms, especially in poultry farming. Among others, garlic and tomato extracts have proven effective in improving public health and disease resistance, and in supporting and developing embryo growth. These extracts contain effective compounds such as allicin in garlic and lycopene in tomatoes (4), which have strong antioxidant and anti-inflammatory properties that help protect embryo cells from oxidative damage and enhance the general health of the embryo (26). These nutrients also improve metabolic efficiency and promote tissue and nerve growth, thus improving hatchability (18). The technique of intra-egg in-ovo injection is one of the modern methods that contribute to the direct entry of natural nutrients and thus increase the development of embryos and their growth. This process enhances productive performance by improving hatching rates as it provides the embryo with the necessary nutrients and nutritional supplements to overcome hatching stress. Referred to as early nutrition, it plays a crucial role in the development of broiler embryos (17).

Nevertheless, despite the vast developments in the poultry farming industry, there is a need for additional improvements in growth and embryogenesis to increase hatching rates as well as strengthen the immunity of hatched chicks. As such, this study aimed to establish that injecting eggs with natural nutrients such as garlic and tomato extract contributes to enhancing embryonic development and neurological traits, as these extracts contain effective compounds for promoting health and increasing immunity. Also, their prominent role as antioxidants works to increase metabolism thus contributing to healthy embryo development and growth, and ultimately to higher hatchability rates (26).

Garlic (*Allium sativum*) is known for its antibacterial and antiviral properties and has several effective compounds such as allicin, which is an important antioxidant. The study by (6) used garlic to determine if there was a significant increase in the weight of broilers as well as development of their immune systems by stimulating T and natural killer cells. It also sought to establish if there was increased production of cytokines such as interferon, which is important for immune response, thus leading to improved growth and reduced rates of injury (13). Garlic extract is known to increase the levels of glutathione, an antioxidant that protects cells from free radicals, and at the same time, activates the enzyme superoxide dismutase (SOD) and catalase, which contribute to reducing oxidative stress and thus promote embryonic development and hatching rates (31).

Tomatoes (*Solanum lycopersicum*) are essential for biological processes, especially in increasing nerve growth and development and the activity of antioxidant enzymes in the brain such as the enzyme glutathione peroxidase (GPx) (22). Tomato extract contains phenolic compounds and vitamins, such as vitamins C and E (5 and

39) which stimulate the activity of GPx. This enzyme protects nerve cells in the brain from oxidative damage when it converts hydrogen peroxide into water (9). In addition, tomato extract contains lycopene, a carotenoid that promotes mitochondrial formation, and increases the production of neurotransmitters such as dopamine and serotonin, thus improving neural communication, the formation of neural circuits, and enhancing brain functions (38).

This study investigated the effects of in-ovo garlic and tomato extract injections on the growth and neurological traits of broiler embryos in developing improved and innovative approaches in poultry farming.

Materials and Methods

Animal study: The study followed the protocols approved by the University of Anbar/Ethical Approval Committee, Iraq. Hatching eggs (Ross 308) were procured from a local hatchery.

Garlic material: The garlic extract was created using steam distillation. Matured garlic tubers (*Allium sativum L.*) were obtained from a domestic market. The garlic tubers were cleaned, exfoliated, and rinsed. They were then dried and minced with a grinder and 200 mL of distilled water added to prepare it. The extract was kept in the distillation bottle of a steam distillation apparatus with a capacity of 1000 mL under steam for 3 h at 100°C (24). Then, 0.1 mL aqueous G. extract was injected into each egg (17).

Tomato material: A system SFE-SFC (10AVP Shimadzu, Japan) extracted tomatoes (21) using 25 g samples at 50°C and 32 MPa, then reduced to 15 MPa and 80°C. The extract, recovered with 5 mL of dichloromethane and diluted with 10 mL of distilled water, was injected at 0.1 mL per egg (17).

Tomato and garlic mixed extract: Using an electric mixer, 5 mL of each extract was combined after the tomato and garlic were extracted. Ten milliliters of distilled water were used to dilute the entire extracted material, and 0.1 milliliters of this combination were then injected into each egg (17).

In ovo injection: The 300 eggs were divided into 5 groups: Group 1 (Control negative, CO, without injection); Group 2 (Control positive, distilled water, DW, 0.1 mL/egg); Group 3 (garlic extract, GAR, 0.1 mg/egg); Group 4 (tomato extract, TOM, 0.1 mg/egg); and Group 5 (garlic and tomato extract mix, ATM, 0.1 mL/egg). The extract was injected into the albumin from the narrow end on the fifth day of incubation using a 1 mL syringe with a 2-gauge needle. The ingredients were prepared and injected into the albumin on five different days from the thin end of the eggs (6). Due to the growth of molds, the injection sites on the eggs were cleansed with 70% ethylic alcohol before being sealed with dye-treated nails rather than melted Merck paraffin. Similar injections of 0.1 mL/egg of distilled water were made into the eggs in the control 2 group. The eggs were incubated at 37.5°C in a commercial hatchery at a relative humidity of 56%. Once every hour, the eggs were automatically rotated. On the tenth day of incubation, candling was used to verify that the eggs had not developed into embryos (18).

Studied Traits:

Embryonic mass, chick body weight, and hatchability: The weights of the eggs used in the experiment ranged between 45 and 55 g. Embryos were extracted on the 19th day of incubation and weighed, following the method of Abdulateef (1). After the hatching process, the hatching rate was determined by dividing the hatched chicks with the fertilized eggs.

Embryo index (EI): After the experiment, each egg in the different groups was weighed individually. The embryos were then isolated, cleaned, and their weights recorded. The embryo index (EI) was calculated for all groups following the method of Dhinakar (11).

$$EI = \left[\frac{Embryo\ Weight\ (gr)}{Egg\ Weight\ (gr)}\right] \times 100$$

Tissue collection and processing: The young birds were sedated with ether and humanely put to rest. Their brains were carefully removed and weighed. The brain samples were preserved in 4% paraformaldehyde at 4°C for a fortnight, dehydrated, treated, and encased in paraffin wax. Coronal sections of 7 µm thickness were cut and stained to visualize the Nissl substance. Brain sections from the test and CO groups were compared at a 2 mm distance from the end. The nuclei sizes were measured using an AXIO ZEISS image analysis system at ×100 magnification, measuring 0.51 µm (2).

Brain index (BI): At the end of the experiment, chicks from both the control and experimental groups were weighed on the day of hatching. The brain was then extracted as described previously and weighed. The Brain Index (BI) was determined using the following method:

$$BI = \left[\frac{Brain\ Weight\ (gr)}{Body\ Weight\ (gr)}\right] \times 100$$

This method allows for a comparison of relative brain size with the body weight of the chicks (2).

Statistical analysis: This experiment was evaluated using a Completely Randomized Design (CRD). Data analysis was performed using the SAS statistical software (33). Mean values for each treatment were compared using Duncan's multiple range test at various significance levels to identify significant differences between the averages (13).

Results and Discussion

Table 1 shows the influence of in-ovo injections of garlic and tomato extract on the embryogenesis and hatchability of the embryo samples. Significant superiority ($P \leq 0.05$) was noted in embryo weights at age 19 days for ATM, reaching 35.8 gm on CO and DW, not different from GAR and TOM, which outperformed the CO and DW and the latter two did not differ between them. Chick weight after hatching (chick weight) outperformed ($P \leq 0.05$) ATM, at 40.33 gm, higher than CO and DW as well as GAR and TOM which outperformed CO and DW, and the latter two did not differ between them. There was a significant superiority in hatchability, as it outperformed ($P \leq 0.05$) ATM at 70.92 gm, above CO and DW, as well as on GAR and TOM, which outperformed CO. and DW. The latter two did not differ between them.

Table 1: The influence of in ovo injections of garlic and tomato extract on the embryogenesis and hatchability of chick embryos.

Treatments	Embryo Weight* (gm.)	Chick Weight (gm.)	Hatchability (%)
CO	32 c	35.13c	62.61 c
DW	34.51 c	37.5 c	68.2 c
GAR	34.13 ab	38.23 b	74.3 b
TOM	33.61 ab	38.13 b	73.1 b
ATM	35.8 a	40.33 a	76.4 a
Mean	34.01	37.864	70.92
****SEM**	0.61	0.83	2.47
Significance	0.0001	0.0001	0.0001

* Embryonic mass at 19 days
** SEM: Standard Error Mean
a, b, c: indicate significant differences within the same column.

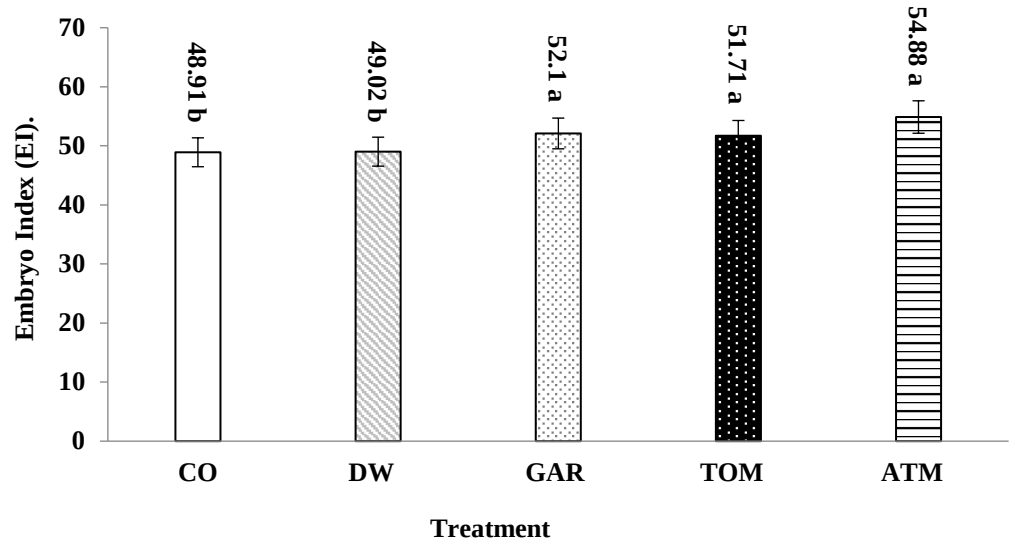
Table 2 shows the influence of in-ovo garlic and tomato extract injections on neurological traits in embryos. Significant advantage (P≤0.05) was derived in neuron traits for ATM at 44.13 µm, above CO and DW, but not different from GAR and TOM, which outperformed CO and DW and the latter two did not differ between them. Also, brain weight significantly outperformed (P≤0.05) for ATM at 0.93 g on CO and DW as well as on GAR and did not differ from TOM and the latter did not differ from GAR, which outperformed CO and DW with the latter two not differing among them.

Table 2: The influence of in ovo injections of garlic and tomato extract on the neurological traits of chick embryos.

Treatments	Neurons Micron (µm)	Brain weight (gm.)
CO	33.2 c	0.65 c
DW	41.1 c	0.73 c
GAR	42.01 ab	0.82 b
TOM	42.13 ab	0.84 ab
ATM	44.13 a	0.93 a
Mean	40.51	0.79
***SEM**	1.89	0.048
Significance	0.0001	0.0001

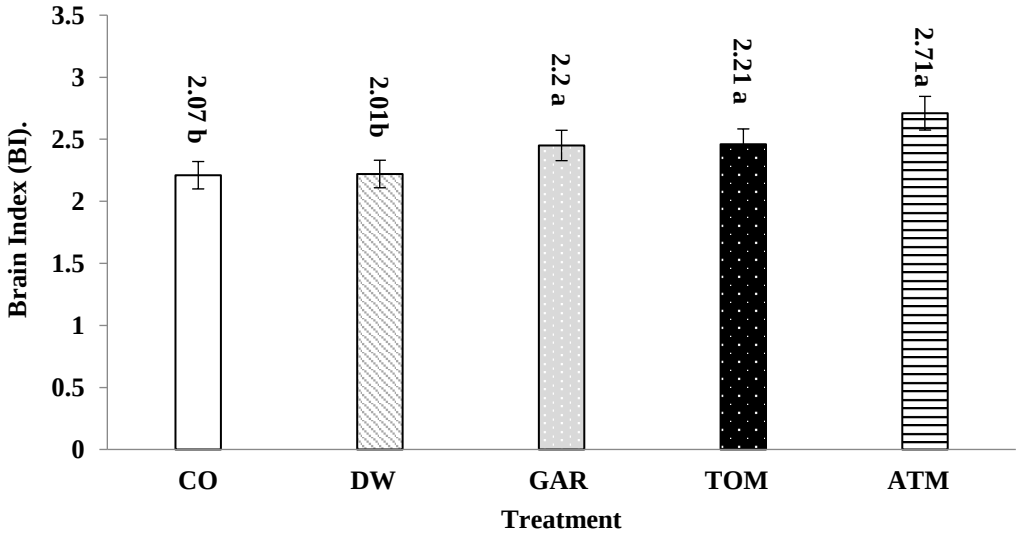
* Embryonic mass at 19 days
** SEM: Standard Error Mean
a, b, c: indicate significant differences within the same column.

Figure 1 shows the influence of in ovo injection of garlic and tomato extract on the embryo index. There was significant advantage (P≤0.05) for ATM, TOM, and GAR amounting to 54.88, 51.71 and 52.1%, respectively, and on DW and CO at 49.02 and 48.91%, respectively, though there was no significant difference between ATM, TOM and GAR, as well as effects between DW and CO.



Embryonic mass at 19 days.
a, b, c: indicate significant differences.

Figure 1: The influence of in ovo garlic and tomato extract injections on the embryo index.



Embryonic mass at 19 days.
a, b, c: indicate significant differences.

Figure 2: The influence of in ovo garlic and tomato extract injections on the brain index.

Figure 2 shows how in ovo injections of garlic and tomato extract affect the brain index. There was significant advantage (P≤0.05) for ATM, TOM, and GAR, at 2.71, 2.21 and 2.2%, respectively, and on DW and CO at 2.01 and 2.07%, respectively. No significant differences were noted between ATM, TOM, and GAR, as well as between DW and CO.

In ovo garlic and tomato extracts in the egg contribute to embryonic growth and development, promote nerve formation, and increase nerve signal transmission within the embryos, leading to developments in their biological processes. These extracts were selected for this study as they contain bioactive ingredients that can positively

affect the growth and development of the embryonic system as well as develop the nervous system of embryos (4).

Garlic has effective compounds such as allicin, which has antibacterial and antiviral properties and plays an important role in resisting damage caused by free radicals, which cause cell destruction and affect embryonic development (15). Free radicals, which are unstable molecules that cause oxidative damage to cells, are produced naturally in the body during metabolic processes, but higher amounts in the system have a deleterious impact on body health (35). Thus, allicin works as an effective antioxidant by donating an electron to free radicals, stabilizing them and reducing their ability to interact with important cellular components such as proteins, fats, and nucleic acids (12). Antioxidant enzymes such as GPx, SOD, and catalase are involved in this biological process, all of which have a critical role in the destruction and breakdown of free radicals and their transformation into less harmful molecules (8 and 29).

In addition, allicin has a biological role in reducing oxidative stress by creating a more stable environment for protecting embryonic cells from damage and destruction. This enhances their development by reducing levels of reactive oxygen species (ROS), thus improving cell function and maintaining the integrity of DNA, proteins, and cell membranes (10). Allicin regulates the levels of glucocorticoids, which play a biological role in regulating the body's response to stress through the HPA axis, thereby enhancing the embryo's response to stressful external stimuli that put its life at risk (19). Tomatoes contain an effective compound, lycopene, which is a natural carotenoid. It has effective antioxidant properties and plays a biological role in protecting cells and tissues from oxidative damage in bird embryos (30 and 32). Embryos suffer in the last stages of hatching due to strong stress and severe fatigue and need biological nutrients to help them overcome such trauma (34), as well as activate and maintain lycopene for the general health of their nerves and in their development. Lycopene is a powerful antioxidant that activates some important enzymes mentioned earlier, namely GPx, SOD, and catalase. All of these play a crucial role in the destruction and breakdown of free radicals and their transformation into harmless molecules (16).

Lycopene contributes to promoting cardiovascular health, which facilitates the faster delivery of oxygen and nutrients to the embryo, and thus works to effectively increase embryo growth (36). Several possible elements, including lycopene, may help maintain the elasticity of vessels and prevent atherosclerosis by reducing harmful levels of LDL and the accumulation of plaque in arteries. This occurs with the participation of the enzyme paraoxonase which has an important role in preventing fat oxidation (37).

Another potential biological mechanism of lycopene is its ability to promote the production of red blood cells by improving the effectiveness of erythropoietin (25), the hormone responsible for the production of red blood cells. Such cells are sensitive to oxidative stress as high levels of ROS hinder the function of red progenitor bone marrow cells. Lycopene increases the RBC needed by the bird embryos, especially in the last stages of hatching when the abundance of cells cause most embryos to perish due to the lack of oxygen or hypoxia (28).

Several other theories allude to the role of lycopene in increasing nerve growth and development. This study found that lycopene affects cell signaling pathways that promote the growth and differentiation of neurons as well as the Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF). Stimulation of these factors leads to the growth of axons and dendrites which, in turn, increase synapses (3) that enhance neural communication and promote faster and better embryo reactions (23). In addition, lycopene has a key role in resisting and reducing inflammation by producing cytokines such as -6 (IL-6) and (TNF-α) that help support and develop the immune system (26).

Another possible theory is that lycopene enhances cell signaling to the Insulin-like Growth Factor (IGF) by improving cell response and reducing oxidative stress, thereby increasing IGF receptor expression on the cell surface and enhancing cell proliferation and cell differentiation. This stimulates PI3K/Akt pathways, while MAPK/ERK promotes the creation and growth of proteins in the body as well as increases gene expression leading to the healthy growth and development of nostalgic cells (27).

Conclusions

The in ovo injection of hatching eggs with garlic and tomato extract led to improved biological processes in the embryo, the development of nerves and nerve signals, the growth of neurons, and an increase in the weight of the brain mass and the brain index. This consequently led to an increase in embryogenesis and weights of hatched chicks, and an improved embryo index as well as chick hatchability.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author S. M. Abdulateef; methodology, writing—original draft preparation, Author Z. J. M. Saed, Th. T. Mohammed, M. M. Awad, N. A. Mirzan and R. D. Mustafa writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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