Original Research Article

Impact of *Thymus vulgaris* on the embryonic development of chicken eggs

ABSTRACT

Aims/Objective: The study aims to investigate the effects of *Thymus vulgaris* (common thyme) extract on the embryonic development of chicken eggs, with a focus on its potential to enhance survival, growth, and development during incubation.
Study Design: This experimental study involved administering a standardized dose of thyme extract to chicken eggs at various stages of incubation and comparing the outcomes with untreated control eggs.
Place and Duration of Study: The study was conducted at the Institute of Ecology and Technology, University of Tetovo, located in the Polog region, over a period of six months spanning 2023 and 2024.
Methodology: Fertilized chicken eggs were assigned to either a control group or an experimental group. The experimental group received 0.5 ml of thyme infusion at predetermined stages of incubation. The eggs were incubated under controlled conditions (37.5°C, 70% humidity). Embryonic development was monitored and assessed on days 8, 14, and 21, with particular attention to organ differentiation, vascularization, and overall activity. Post-hatching, the chicks were examined for macroscopic and histopathological changes.
Results: The application of thyme extract positively influenced embryonic development at all stages of incubation. By the eighth day, treated embryos demonstrated enhanced organ differentiation, with notable improvements in vascularization and amniotic membrane development compared to the control group. On the fourteenth day, further advancements were observed in the differentiation of extremities and internal organs in treated embryos. By the twenty-first day, treated embryos exhibited increased activity and accelerated developmental milestones, achieving results comparable to or exceeding those of the control group.
Conclusions: The administration of 0.5 ml of *Thymus vulgaris* herbal extract supports and potentially enhances normal embryonic development in chicken eggs. These findings suggest that thyme extract could be a viable natural additive in poultry incubation, with potential benefits for embryo health and development. This study underscores the promising applications of thyme extract in organic poultry production.

Keywords: Thymus vulgaris, embryonic development, chicken eggs, incubation, Polog region.

1. INTRODUCTION

Embryonic development in avian species is a highly intricate process influenced by both genetic and environmental factors. The stages of embryogenesis, from initial blastoderm formation to full organogenesis, are particularly sensitive to various influences, including nutrient availability, temperature, and exposure to bioactive compounds (Hamburger & Hamilton, 1951; Romanoff, 1960; Tizard, 2017; Lambert *et al*., 2021; Muhammad *et al*., 2021). Recent research has increasingly focused on natural products and herbal extracts for their potential to enhance poultry production and improve embryonic health (Dorman & Deans, 2000; Wu *et al*., 2019; Ayalew *et al*., 2022).
Thymus vulgaris, commonly known as thyme, is a perennial herb renowned for its broad spectrum of medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory effects (Burt, 2004; Zhang *et al*., 2009; Hesabi *et al*., 2019; Pajohi-Alamoti*et al*., 2021). The essential oils of thyme, particularly thymol and carvacrol, have demonstrated significant biological activity, including effectiveness against various pathogens and potent antioxidant capabilities (Aeschbach*et al*., 1994; Pina-Vaz *et al*., 2004; Soković*et al*., 2010; Gholami-Ahangaran*et al*., 2022). These properties suggest that *Thymus*

vulgaris could confer beneficial effects when used as an additive during poultry incubation, potentially enhancing embryo health and development.

In the Sharr Mountain massif, renowned for its rich biodiversity and unique flora, *Thymus vulgaris* is one of several plant species recognized for its significant nutritional and health value. Other plants from the region, including Mint (*Mentha piperita*), St. John's wort (*Hypericum perforatum*), and Chamomile (*Matricaria chamomilla*), have long been utilized for their therapeutic benefits (Rehault-Godbert *et al*., 2019; Beadini*et al*., 2023). Traditionally used in local teas, these plants are now acknowledged for their potential as herbal supplements with healing properties.

In this context, the present study investigates the impact of *Thymus vulgaris* extract on the embryonic development of chicken eggs. Specifically, we aimed to evaluate the effects of a 0.5 ml dose of Thymeinfusion administered at various stages of incubation—days 1, 8, 14, and 21. Our hypothesis was that this herbal extract would positively influence embryonic growth and survival, promoting more advanced development at key stages. Our findings demonstrate that thyme application resulted in favorable outcomes, including enhanced organ differentiation and increased embryo activity, comparable to the progress observed in control eggs. By illustrating the positive effects of this natural additive, the study supports the broader use of herbal extracts in enhancing avian embryogenesis. Similar research has been conducted on teas grown on the Sharr Mountain, investigating their impact on the stages of embryonic development in quail and chicken eggs (Beadini*et al*., 2023).

2. MATERIAL AND METHODS

2.1. Plant Material and Extraction

The thyme herb was collected from the Sharr Mountain massif at an altitude of 1700 m above sea level during the period from July to September 2023/2024 (Figures 1 and 2). The collected plant material was subsequently dried in a drying chamber. Once dried, the plant was ground using a specialized apparatus for extraction (Figures 3 and 4). The extraction process was performed using a Rota Buchi apparatus to obtain Thymus vulgaris extract in liquid form. The extract was then filtered through a funnel equipped with specialized sieves and stored in jars for subsequent preparation and preservation (Beadini*et al*., 2023).

Figure 1.Winter Thyme -1700ma.s.l.Figure 2. Thyme herb tea (*Thymus Vulgaris*)

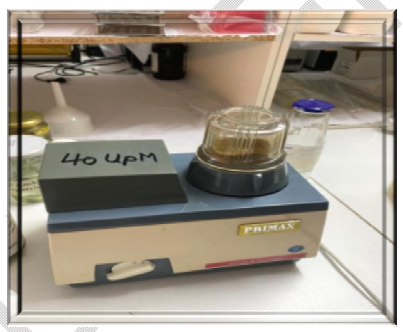

Figure 3. Apparatus for grinding herbsFigure 4. Apparatus for extracting herbs (Beadini*et al*., 2023)

2.2. Preparation and Incubation of Eggs

Fertilized chicken eggs were selected using a fluorescent lamp to identify and remove non-fertilized eggs. The viable fertilized eggs were then measured and marked for identification (Figure 5).The eggs were incubated under controlled conditions of 37.5°C and 70% humidity (Figure 7 and 8).On the fourth day of incubation, measurements were taken to observe initial developmental changes. The *Thymus vulgaris* infusion was carefully applied into the air cell of each egg to

ensure optimal absorption through the egg membrane (Figure 6). The eggs were subsequently monitored throughout the incubation period for further developmental changes.

Figure 5. Fertilized and marked eggsFigure 6. Dosing procedure

Figure 7 and 8. Incubator for incubating chickeneggs (Beadini*et al*., 2023)

2.3. Monitoring and Analysis

Embryonic development was assessed at specific intervals (Days 1, 8, 14, and 21) to observe growth, organ differentiation, and overall activity. The development of control eggs was compared with that of eggs treated with thyme infusion. This comparison was essential for evaluating the effects of the extract on various developmental stages.
After 21 days of incubation, the embryos were preserved in 70% alcohol or methanol for subsequent histopathological analysis (Figures 9 and 10). The preserved specimens were examined for macroscopic changes, histological alterations, and signs of teratogenesis (Beadini*et al*., 2023).

Figure 9. Decapitation of the fetus Figure 10. Preservation of the fetus in conical vials with 70% alcohol (Beadini*et al*., 2023)

3. RESULTS AND DISCUSSION

The results of this study provide convincing evidence of the beneficial effects of Thymus vulgaris extract on embryonic development in chicken eggs. Administering a 0.5 ml dose of the extract at various stages of embryogenesis led to significant improvements in developmental outcomes, suggesting that thyme may positively influence embryo health and growth. The results are categorized according to specific stages of embryonic development: Days 1, 8, 14, and 21 of incubation.The experimental group of eggs exhibited notable differences compared to the control group:
Day 1: Initial observations indicated that the experimental eggs were comparable to the control eggs, showing early signs of embryonic development. The thyme extract did not affect the early blastoderm formation.
Day 8: The experimental eggs exhibited advanced differentiation compared to the control group. The heart and blood vessels in the treated eggs were more developed. The amniotic membrane showed improved growth and enveloped the foetus more effectively. Additionally, the embryos' movements were more pronounced, indicating enhanced development.
Day 14: Differentiation of extremities and other organs in the experimental eggs was more advanced than in the control eggs. The embryos in the experimental group demonstrated further development of the head, body, and limbs. The extract appeared to promote more rapid and extensive organ development.
Day 21: By the final stage of incubation, the activity of the foetuses in the experimental eggs was comparable to, or even exceeded, that of the control group. The chicks from the experimental eggs displayed accelerated movements and higher activity levels, suggesting that the thyme extract positively influenced the overall development and vitality of the embryos.

Figure 11. Twenty-first day of incubation - experimental egg

From Day 1 to Day 21, the treated eggs consistently demonstrated more advanced stages of development compared to the control group. On Day 8, the experimental eggs exhibited pronounced differentiation of the heart and vascularization. This finding aligns with the research of Shabani *et al*. (2022) andBeadini*et al*. (2023), who reported that herbal extracts can enhance organ differentiation and development in avian embryos. The more advanced organ differentiation observed in the experimental group suggests that *Thymus vulgaris* may stimulate cellular processes involved in organogenesis and vascular development.
By Day 14, the experimental eggs showed further advanced differentiation of extremities and other organs. This observation supports the work of Labaque*et al*. (2013), which demonstrated that herbal extracts could positively influence the growth and differentiation of fetal tissues. The enhanced development in the experimental group indicates that *Thymus vulgaris* promotes more rapid and effective embryonic growth, likely due to its rich content of bioactive compounds, including thymol and carvacrol (Pina-Vaz *et al*., 2004; De Martino *et al*., 2009; Gholami-Ahangaran*et al*., 2022).
By Day 21, the experimental eggs displayed increased fetal activity and accelerated movement compared to the control group. This observation is consistent with the research of Ruberto and Baratta (2005), who found that natural antioxidants could enhance embryonic activity and vitality. The improved activity in the experimental chicks suggests that *Thymus vulgaris* not only supports normal developmental processes but may also contribute to overall embryo vigor and readiness for hatching (Surai, 2020; Ricardo-Rodrigues *et al*., 2024).
Importantly, the study did not observe any adverse effects or teratogenic changes in the embryos exposed to thyme extract. This finding aligns with previous studies by Adámez*et al*. (2012) and Nostro *et al*. (2004), which reported that appropriate concentrations of herbal extracts did not compromise developmental integrity. The absence of teratogenic effects indicates that the dosage used in this study was well-tolerated and beneficial for embryonic development (Valenzuela-Grijalva *et al*., 2017).
The positive effects observed can be attributed to the various biological activities of *Thymus vulgaris*. Its antimicrobial, antioxidant, and anti-inflammatory properties (Shapiro & Guggenheim, 1995; Burt, 2004; Ebrahimabadi*et al*., 2010) likely contribute to creating a more favorable environment for embryonic development. In particular, the antioxidant activity of

Thymus vulgaris may help mitigate oxidative stress, which is known to negatively impact embryo viability (Surai, 2007; Halliwell & Gutteridge, 2015).

4. CONCLUSION

The results of this study underscore the potential of *Thymus vulgaris* as a beneficial additive in poultry production, particularly during the incubation period. The extract's ability to enhance embryonic development, stimulate organ differentiation, and increase fetal activity -without causing adverse effects - highlights its potential as a natural supplement in avian embryology. This research could have significant applications in alternative therapy and the production of bio-products within the food industry, contributing to the development of natural foods and overall health. Future research should aim to elucidate the mechanisms underlying these effects and assess the long-term implications of using thyme in poultry production.

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