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 at specific stages of embryonic development during ontogenesis and to evaluate the benefits or potential consequences of its dosage on macroscopic and histopathological changes.

Study Design:This experimental study involved administering a standardized dose of thyme, a herbal supplement with healing properties, to chicken eggs at various stages of incubation, and comparing the outcomes with those of 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 1, 8, 14, and 21 days old, 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 blastomer 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). Modern broiler breeding programs aim for growth and feed efficiency, with feed additives crucial for mitigating environmental stress, improving health, and maintaining intestinal integrity (El-Baz &Khidr, 2024). 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).Burt's review highlights the antibacterial properties of essential oils and their potential applications in poultry production, demonstrating how these oils can serve as natural antimicrobials, effectively reducing pathogen loads and enhancing poultry health and productivity (Burt, 2004).*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). Thyme (*Thymus vulgaris*) essential oil, evaluated in vitro and in vivo as an antibiotic alternative in quail diets, demonstrates antimicrobial activity, reducing microbial load and improving reproductive health, which benefits the embryonic environment (Dehghani*et al*., 2019).

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 (*Matricariachamomilla*), 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 days old. We hypothesized that thyme extract would enhance embryonic growth and survival. The goal was to observe both the benefits and potential drawbacks of this treatment by examining macroscopic histopathological changes at each stage.This study underscores the potential of thyme as a beneficial natural additive for enhancing avian embryogenesis. These findings are consistent with similar research on the effects of herbal teas from Sharr Mountain on embryonic development in quail and chicken eggs (Beadini et al., 2023).

2. MATERIAL AND METHODS

2.1. Plant Material and Extraction

The thyme herb (*Thymus vulgaris*) was collected from the Sharr Mountain massif at an altitude of 1700 meters above sea level between July and September 2023/2024 (see Figures 1 and 2). The collected thyme was dried in a drying chamber. After drying, the plant material was ground using a specialized apparatus for extraction (see Figures 3 and 4). The alcoholic extraction was performed using a Rota Buchi apparatus, which yielded the thyme extract in liquid form. The extract was then filtered through a funnel with specialized sieves and stored in jars for preservation and later use (Beadini*et al*., 2023).

Fig. 1Winter Thyme -1700ma.s.l.

Fig. 2 Thyme herb tea (*Thymus Vulgaris*)

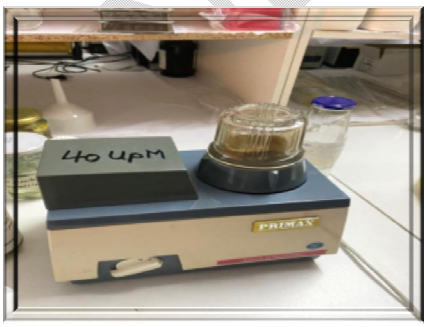

Fig. 3 Apparatus for grinding herbs

Fig. 4Apparatus for extracting herbs (Beadini*et al*., 2023)

2.2. Preparation and Incubation of Eggs

Fertilized eggs of the Brahma chicken breedwere selected using a fluorescent lamp to identify and remove non-fertilized eggs. The viable fertilized eggs were then measured and marked for identification (Fig. 5). These eggs were incubated under controlled conditions of 37.5°C and 70% humidity (Fig. 7 and 8). On the fourth day of incubation, measurements were taken to observe initial developmental changes. The *Thymus vulgaris* extract was carefully applied into the air cell of each egg to ensure optimal absorption through the egg membrane (Fig. 6). The eggs were monitored throughout the incubation period for further developmental changes.

Fig.5 Fertilized and marked eggsFig.6Dosing procedure

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

2.3. Monitoring and Analysis

Embryonic development was assessed at specific intervals (1, 8, 14, and 21 days old) to observe growth, organ differentiation, and overall activity. The developmental progress of control eggs was compared with that of eggs treated with thyme extract to evaluate the effects of the extract on various developmental stages.In the control group, eggs were not treated with the solvent. The herbal extract was administered into the air chamber of the eggs to enhance absorption through the serous membrane at specific intervals during the 21-day incubation period. Embryonic development was monitored at various stages, and changes were analyzed. Post-hatching, specimens were preserved in methanol or 70% alcohol for detailed examination of macroscopic changes, histological alterations, and signs of teratogenesis (see Figs. 9 and 10).

Fig.9Decapitation of the fetus
Fig.10 Preservation of the fetus in conical vials with 70% alcohol

3. RESULTS AND DISCUSSION

The research results encompass measurement parameters including marking, pre-incubation and post-incubation measurements, and comparisons between control and experimental eggs. Systematic documentation of morphological changes was performed. Fig. 11 illustrates the marking procedure and show eggs opened for examination at various stages of embryonic development. The study demonstrates the beneficial effects of *Thymus vulgaris* extract on embryonic development in chicken eggs. Administering a 0.5 ml dose of the extract at different embryonic stages led to significant improvements in developmental outcomes, suggesting that thyme positively influences embryo health and growth. Results are organized according to specific developmental parameters—such as organ differentiation, vascularization, and activity levels—across the chronological stages of Days 1, 8, 14, and 21 of incubation.

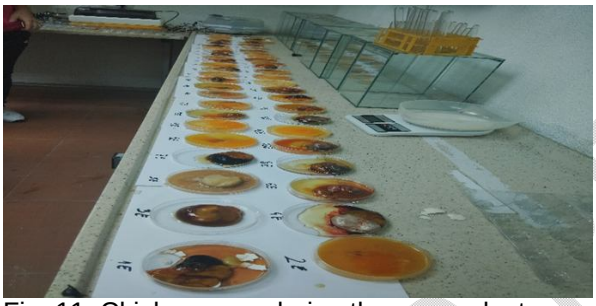

Fig. 11 .Chicken eggs during the research stage

The experimental group of eggs exhibited notable differences compared to the control group:
Day 1: Initial observations revealed that the experimental eggs, treated with thyme extract, were comparable to control eggs in terms of early embryonic development. Notably, the thyme extract did not impact the formation of early blastomeres (Fig. 12a and 12b)

Fig. 12a) First day of incubation - control egg
Fig. 12 b) First day of incubation - experimental egg

In Figure 12a, the fertilized control egg shows clear differentiation of blastomeres, a finding consistent with that observed in the experimental egg depicted in Figure 12b.

Day 8: The experimental eggs showed advanced differentiation compared to the control group, with notable improvements in heart and blood vessel development, enhanced growth of the amniotic membrane, and more pronounced embryo movements, indicating improved development. Fig.13a illustrates the differentiation of the heart, significant vascularization, and the growth of the amniotic membrane enveloping the embryo, along with the onset of embryonic movement. Fig.13b highlights that the experimental egg displays more advanced differentiation of these organs.

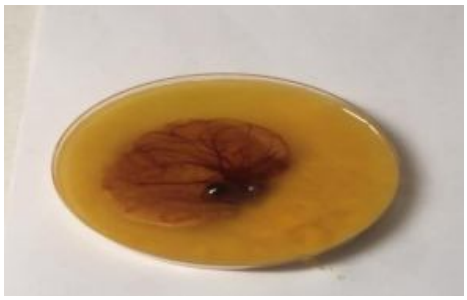

Fig. 13 a) 8 thday of incubation - control egg
Fig. 13 b) 8 thday of incubation - experimental egg

Day 14: The experimental eggs showed more advanced differentiation of extremities and other organs compared to the control group. The fetus in the experimental group exhibited further development of the head, body, and limbs, with thyme extract promoting more rapid and extensive organ growth. Fig.14a clearly depicts the advanced differentiation of the fetus, including the extremities and other organs. In contrast, Fig. 14b shows even greater differentiation in the experimental egg, particularly in the development of the head, body, and limbs.

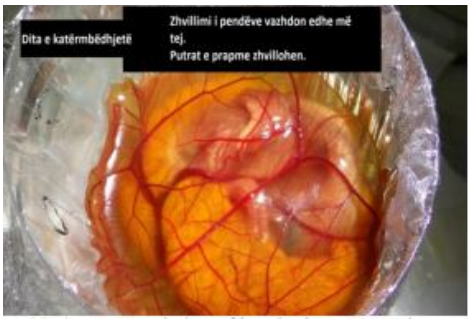

Fig.14a) 14thday of incubation-control egg
Fig.14b)14thday of incubation-experimental egg

Day 21: By the final stage of incubation, the activity of the fetuses in the experimental eggs was comparable to, or even exceeded, that of the control group. The chicks from the experimental eggs exhibited increased movement and higher activity levels, indicating that thyme extract had a positive influence on overall embryonic development and vitality. Fig. 15a clearly shows the increase in fetal activity and the onset of accelerated movements, which is similarly observed in the experimental egg in Fig. 15b.

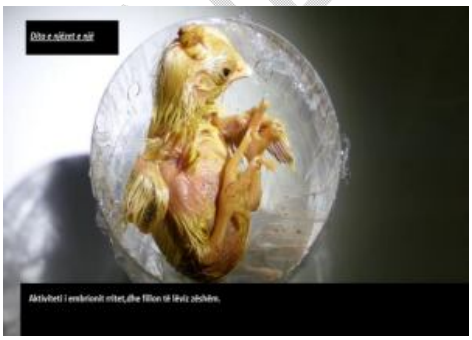

Fig.15a) 21st day of incubation-control egg
Fig.15b) 21st day of incubation-experimental egg

Fig.16a) 22nd day of incubation-control egg
Fig.16b) 22nd 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. The improved organ differentiation observed on Day 8 aligns with the findings of Shabani et al. (2022) and Beadini et al. (2023), who reported that herbal extracts can enhance organ differentiation and development in avian embryos. The more advanced differentiation in the experimental group suggests that *Thymus* and *Chamomile* extracts may stimulate cellular processes involved in organogenesis and vascular development, supporting their potential role in promoting more rapid and efficient embryonic growth.
By Day 14, the observed advanced differentiation of extremities and other organs supports the work of Labaque et al. (2013), which demonstrated that herbal extracts can positively influence fetal tissue growth and differentiation. The enhanced development in the experimental group indicates that *Thymus vulgaris* promotes more rapid and effective embryonic growth, likely due to its bioactive compounds, including thymol and carvacrol (Pina-Vaz et al., 2004; De Martino et al., 2009; Gholami-Ahangaran et al., 2022). Thymol and carvacrol's antioxidant properties help reduce oxidative stress, thereby supporting healthier cell differentiation and overall embryo viability (Hashemipour, 2013).
Thyme's anti-inflammatory properties, particularly through thymol and carvacrol's regulation of cytokine production and inhibition of the NF-κB pathway, help protect developing embryos from inflammation during critical stages of organ development (Daglya, 2012).
By Day 21, the increased fetal activity and accelerated movement in the experimental eggs can be attributed to the antimicrobial, antioxidant, and anti-inflammatory properties of *Thymus vulgaris* (Shapiro & Guggenheim, 1995; Burt, 2004; Ebrahimabadi et al., 2010). These properties likely create a more favorable environment for embryonic development. The antioxidant activity of *Thymus vulgaris* mitigates oxidative stress, which negatively impacts embryo viability (Surai, 2007; Halliwell&Gutteridge, 2015). This finding aligns with Ruberto and Baratta (2005), who reported that natural antioxidants enhance embryonic activity and vitality. The improved activity in the experimental chicks indicates that *Thymus vulgaris* supports normal developmental processes and contributes to overall embryo vigor and readiness for hatching (Surai, 2020; Ricardo-Rodrigues et al., 2024).Moustafa et al. (2020) also reported that thymol enhances nutrient digestion and absorption by stimulating digestive enzymes, which indirectly supports embryonic development by providing adequate nutrients during gestation, aligning with our research findings.
Importantly, the study observed no adverse effects or teratogenic changes in embryos exposed to thyme extract. This finding is consistent with Adámez et al. (2012) and Nostro et al. (2004), who reported that appropriate concentrations of herbal extracts do 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).

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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Author(s) hereby declare that Ginger Online Proofreading Service https://www.gingersoftware.com/proofreading, have been used during writing or editing of manuscripts.

Ethical approval: All procedures were conducted in compliance with standard ethical guidelines.

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