

Original Article

Effects of Vitamin E on Electrocardiographic Parameters of Broiler Chickens With Pulmonary Hypertension



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ABSTRACT

Background: Pulmonary hypertension in broiler chickens is a multifactorial syndrome leading to significant mortality, primarily due to hypoxemia and cardiac overload.

Objectives: This study aimed to assess the impact of vitamin E supplementation on electrocardiographic parameters in broiler chickens subjected to triiodothyronine (T₃)-induced pulmonary hypertension.

Methods: A total of 180 chicks were randomly assigned to four groups: One control and three treatment groups. The chicks in the treatment groups received vitamin E (α -tocopherol) at doses of 100, 200, and 400 IU from one day to 49 days of age. To induce pulmonary hypertension, all chicks were administered 1.5 mg/kg of T₃ from the seventh day to the end of the experiment through their diet. Electrocardiograms were recorded at 14 and 49 days of age, and right ventricular hypertrophy (RVH) was assessed post-euthanasia.

Results: Significant decreases in the right ventricle/ total ventricle weight (RV/TV) ratio were observed in the treated groups on day 49 ($P < 0.05$). Electrocardiographic analysis revealed reductions in S, T, and R wave amplitudes specifically on day 49 for all vitamin E doses ($P < 0.05$). The QRS interval significantly increased at all doses on day 49, while the RR interval increased at certain doses on days 14 and 49 compared to the control group ($P < 0.05$).

Conclusion: Vitamin E supplementation effectively modulates the induction of pulmonary hypertension and associated ventricular hypertrophy in broiler chickens.

Keywords: Broiler chickens, Electrocardiographic parameters, Pulmonary hypertension, Triiodothyronine (T₃), Vitamin E

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Introduction

Pulmonary hypertension that progresses to ascites in broiler chickens is a complex syndrome influenced by a combination of genetic, environmental, dietary, and physiological factors (Mocumbi et al., 2024).

This metabolic condition is characterized by hypoxemia, an increased workload on the cardiopulmonary system, central venous congestion, right ventricular hypertrophy (RVH), a flaccid heart, significant fluid accumulation in the abdominal cavity, and an elevated metabolic rate with high oxygen demands, all of which are critical triggers for pulmonary hypertension leading to increased cardiac output (Mandras et al., 2020). The limited vascular space within the lungs may render growing broilers more vulnerable to pulmonary hypertension (Hossain & Akter, 2017).

Various factors have been associated with the onset of pulmonary hypertension, including feed-related issues, such as feed restriction, nutrient density, diet formulation, high sodium content, and low dietary phosphorus levels. Additionally, the presence of hepatotoxins, mycotoxins, and furazolidone in the diet, along with respiratory diseases, altitude variations, lighting conditions, air quality, and ventilation practices have all been implicated in its development (Biswas, 2019). Recent studies investigating the causes of ascites in chickens have highlighted cellular damage induced by reactive oxygen species as a significant factor (Fulton et al., 2017).

The primary defense mechanism against reactive oxygen species consists of endogenous antioxidants, including tocopherols, glutathione, and ascorbic acid. Research indicates that the concentrations of glutathione, α -tocopherol, and γ -tocopherol are diminished in the mitochondria of ascitic broilers, implying that reactive oxygen species are generated at the principal site of energy conversion (Das Gupta & Suh, 2016). Evidence suggests that the application of antioxidants and omega-3 fatty acids can reduce the occurrence of this syndrome (Massaro et al., 2024). Furthermore, administering vitamin E prior to exposure to stressors significantly decreased mortality associated with pulmonary hypertension (Tian et al., 2024). In healthy broilers treated with vitamin E, levels of α -tocopherol in the liver and lungs were elevated, offering enhanced protection against reactive oxygen species (Cheng et al., 2016). It appears that vitamin E supplementation mitigates mortality related to ascites, likely by bolstering antioxidant defenses against reactive species that contribute to tissue damage and exacerbate ascite development (Miyazawa et al., 2019). Addition-

ally, incorporating vitamin E into the diets of broiler chickens under heat stress has been shown to improve both antioxidant activity within the immune system and overall growth performance (Lu et al., 2014).

Recent studies have shown that oral supplementation of vitamin E has a beneficial effect on growth performance and improves gut morphology in growing broiler chickens under hypoxic conditions (Bautista-Ortega & Ruiz-Feria, 2010). This study aimed to assess the electrocardiographic alterations resulting from vitamin E supplementation in broiler chickens subjected to pulmonary hypertension. This evaluation is significant, as electrocardiography serves as a noninvasive technique for detecting cardiac hypertrophy, which is commonly associated with pulmonary hypertension syndrome (Finocchiaro et al., 2020).

Materials and Methods

Animals, management, and treatments

A total of 180 one-day-old Ross 308 chicks were randomly assigned to four equal groups, comprising one control group and three treatment groups. From 7 to 49 days of age, all chicks were administered 1.5 mg/kg of triiodothyronine (T3) in their diet to induce pulmonary hypertension (Sigma Aldrich, USA) (Hassanpour et al., 2014). The treatment groups received vitamin E (α -tocopherol) supplementation starting from one day of age, with three dosage levels (100, 200, and 400 IU) for 49 days (Bayraktar et al., 2011). The chicks were raised under standard conditions for seven weeks, with ad libitum access to water and a standard diet formulated to meet the nutritional requirements outlined by the National Research Council for broilers (National Research Council, 1997).

Electrocardiographic recording

On days 14 and 49, eight chicks from each group were randomly selected, and electrocardiograms were obtained using an automatic device (Cardiomax FX-2111, Fukuda, Japan), standardized at 10 mm=1 mV with a chart speed of 50 mm/s. Electrocardiographic leads I, II, III, aVR, aVL, and aVF were recorded for each chick, and measurements were taken for the amplitudes of the T, R, and S waves, as well as the QRS, QT, RR, and ST intervals, along with the mean electrical axis (MEA).

Dissection and assessment of RVH

Chicks were weighed and subsequently euthanized by decapitation immediately after electrocardiographic recording. A dissection was performed to evaluate gross pathological lesions. RVH was assessed using the following protocol: The heart was dissected, and the atria were removed at the level of the atrioventricular valves, after which the total ventricular mass (TV) was recorded. The right ventricular (RV) wall was then carefully separated from the left ventricle (LV) and septum, and the RV was weighed. The ratios of RV to TV and TV to body weight (TV/BW) were calculated. Pulmonary hypertension syndrome was identified as an RV/TV ratio exceeding 0.28 during the experimental procedures (Wideman, 2001).

Statistical analysis

All results are expressed as Mean±SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) via the SPSS software, version 18 with a P threshold of <0.05 deemed statistically significant.

Results

Assessment of RVH

The RV/TV ratio in the treated groups showed a significant decrease at various ages, with a notable reduction (P<0.05) observed at 49 days of age across all vitamin E dosage levels. However, the differences in the TV/BW

ratio between the treated groups and the control group were not statistically significant (P≤0.05) (Table 1).

Electrocardiographic parameters and MEA

At 49 days of age, S wave amplitudes exhibited a significant reduction across all three doses of vitamin E (leads II and aVF) and specifically for the 400 IU dose in lead III compared to the control group. While decreases in S wave amplitude were also observed at both 14 and 49 days of age for all three doses of vitamin E (leads II, III, and aVF), the variations among other leads did not reach statistical significance. A notable decrease in T wave amplitude (P<0.05) was recorded exclusively at 49 days of age, with significant reductions identified in lead II for the 400 IU dose, lead III for both the 200 and 400 IU doses, and lead aVF for the 100, 200, and 400 IU doses within the treated groups. Additionally, R wave amplitude showed a significant decrease (P<0.05) at 49 days of age in leads aVR and II for the 100 IU, 200, and 400 IU doses, as well as in lead aVF for the 200 and 400 IU doses. No significant differences were observed among S, T, and R wave amplitudes at 14 days of age when comparing the treated groups to the control group (P≤0.05) (Table 2).

The QRS interval was significantly elevated only at 49 days of age in all treated groups (leads II and aVF: 100, 200, and 400 IU). Additionally, the RR interval showed a significant increase (P<0.05) at 14 days of age (lead III and aVF: 400 IU) and at 49 days of age (lead II: 100 IU; lead aVR: 200 and 400 IU; leads III and aVL: 100,

Table 1. Cardiac indices in the different groups

Age (d)	Group	Mean±SEM	
		RV/TV	%TV/BW
14	Control	0.19±0.01	0.43±0.01
	T, 100 IU	0.18±0	0.38±0.02
	T, 200 IU	0.19±0.01	0.42±0
	T, 400 IU	0.18±0	0.42±0.03
49	Control	0.31±0.02	0.35±0.02
	T, 100 IU	0.24±0.01*	0.32±0.01
	T, 200 IU	0.25±0*	0.34±0.03
	T, 400 IU	0.25±0*	0.32±0.01

T: Treated.

*Significantly different compared to the control group (P<0.05).

Table 2. Amplitude of the electrocardiographic waves in the different groups

Age (d)	Groups	Mean±SEM								
		S(mV)			T(mV)			R(mV)		
		Lead II	Lead III	Lead aVF	Lead II	Lead III	Lead aVF	Lead II	Lead aVF	Lead aVR
14	Control	0.23±0.04	0.23±0.03	0.15±0.05	0.09±0	0.08±0.01	0.12±0.02	0.17±0.07	0.18±0.02	0.14±0.03
	T, 100 IU	0.18±0.04	0.13±0.02	0.14±0.04	0.1±0.01	0.06±0.01	0.1±0.04	0.08±0.04	0.14±0.02	0.12±0.02
	T, 200 IU	0.22±0.03	0.21±0.03	0.17±0.04	0.1±0.01	0.04±0	0.08±0.04	0.09±0.03	0.11±0.04	0.11±0.02
	T, 400 IU	0.15±0.05	0.13±0.03	0.11±0.03	0.17±0.05	0.06±0	0.11±0.03	0.09±0.03	0.17±0.04	0.09±0.02
49	Control	0.31±0.03	0.27±0.04	0.36±0.03	0.15±0.01	0.13±0.01	0.12±0.01	0.51±0.05	0.43±0.04	0.45±0.03
	T, 100 IU	0.16±0.03*	0.2±0.03	0.15±0.03*	0.13±0.03	0.09±0.01	0.07±0*	0.25±0.08*	0.33±0.03	0.19±0.07*
	T, 200 IU	0.16±0.03*	0.15±0.02	0.16±0.02*	0.09±0.01	0.05±0*	0.05±0*	0.27±0.06*	0.21±0.07*	0.21±0.04*
	T, 400 IU	0.13±0.02*	0.13±0.03*	0.19±0.02*	0.06±0.01*	0.07±0.02	0.05±0.01*	0.16±0.04*	0.15±0.04*	0.15±0.04*

T: Treated.

*Significantly different compared to the control group (P<0.05).

200, and 400 IU) in the treated groups compared to the control group. No significant differences were observed in the QT and ST intervals or the MEA across different ages in the treated groups compared to the control group (P<0.05) (Table 3).

Discussion

Previous research indicates a potential relationship between thyroid function and the development of pulmonary hypertension. In ascitic chickens, levels of thyroid hormones in relation to cardiopulmonary parameters are elevated. It has been demonstrated that T3 supplementation leads to an increased metabolic rate, which

Table 3. Intervals of the electrocardiographic waves and MEA in different groups

Age (d)	Group	Mean±SEM								MEA (0)
		QRS (s)				RR (s)				
		Lead aVF	Lead	Lead	Lead aVL	Lead aVR	Lead aVF	Lead II	Lead	
14	Control	0.01±0	0.02±0	0.03±0.01	0.11±0	0.11±0	0.11±0	0.1±0	0.1±0	157.28±46.02
	T, 100 IU	0.02±0	0.02±0	0.02±0	0.1±0	0.12±0	0.12±0	0.11±0	0.11±0	254.5±25.37
	T, 200 IU	0.02±0	0.02±0	0.03±0	0.11±0	0.12±0	0.11±0	0.12±0.01	0.11±0	118.37±37.8
	T, 400 IU	0.02±0.03	0.03±0	0.02±0	0.11±0	0.11±0	0.13±0*	0.12±0	0.13±0*	201±36.49
49	Control	0.02±0	0.01±0	0.02±0	0.06±0	0.11±0.01	0.12±0.02	0.12±0.01	0.12±0.01	173.62±25.99
	T, 100 IU	0.03±0*	0.02±0*	0.02±0	0.11±0*	0.1±0.02	0.11±0.01	0.16±0.01*	0.15±0*	185.5±33.9
	T, 200 IU	0.05±0.03*	0.02±0*	0.02±0	0.12±0.02*	0.15±0*	0.11±0.02	0.15±0	0.15±0*	204.25±43.66
	T, 400 IU	0.04±0*	0.03±0*	0.02±0	0.1±0*	0.15±0.01*	0.1±0	0.14±0.02	0.16±0*	141.62±35.46

T: Treated.

*Significantly different compared to the control group (P<0.05).

significantly raises oxygen demand and cardiac output, consequently elevating pulmonary arterial pressure and imposing a pressure overload on the right ventricle (Naeije et al., 2022). Furthermore, a significant correlation exists between thyroid hormone activity and the concentrations of carbon dioxide and oxygen in venous blood concerning susceptibility to ascites (Moayyedian et al., 2011), suggesting that T3 administration may serve as a method for inducing ascites.

Research has demonstrated that systemic hypoxia is the primary contributor to pulmonary hypertension in broiler chickens, leading to cellular hypoxia and an increase in free radicals and superoxide anions, which are significant factors in the development of pulmonary hypertension (Rawat et al., 2022). Additionally, Arab et al. reported elevated levels of hydroxyl radicals in ascitic chickens (Arab et al., 2006). This condition results in tissue hypoxia, which can enhance the production of reactive oxygen species, ultimately causing damage to the heart, lungs, kidneys, and intestines of growing chickens (De los et al., 2005). Cytotoxic effects of hydrogen peroxide have been observed in the mitochondrial matrices of myocardial cells in ascitic birds. Hypoxic mitochondria generate excess superoxide, which can activate signaling pathways or interact with proteins and lipid membranes, leading to alterations in molecular chaperones or growth factors and subsequent cellular damage (Clanton, 2007). Furthermore, mitochondrial dysfunction in the lungs of broilers with pulmonary hypertension has been linked to oxidative stress. Genetic resistance to pulmonary hypertension syndrome correlates with more efficient oxidative phosphorylation in lung mitochondria and a lower inherent level of oxidative stress (Zhang et al., 2024).

Vitamin E supplementation is typically advised for broilers located in areas facing health challenges, as it may enhance animal performance. As a biological antioxidant, vitamin E scavenges lipid radicals present in cell membranes and sub-cellular organelles. It interacts with primary products of lipid peroxidation, specifically fatty acid peroxy radicals, thereby interrupting the chain reaction and preventing further radical formation (Rebolé et al., 2006). During this antioxidant process, vitamin E is converted into a stable radical, which helps mitigate oxidative stress and cellular damage (Delles et al., 2014). Research indicates that elevated levels of vitamin E in chickens can diminish oxidative deterioration by safeguarding polyunsaturated fatty acids (Li et al., 2009). Furthermore, plasma lipid peroxide levels are reduced in healthy birds treated with vitamin E, highlighting the enhanced protection against lipid peroxida-

tion that this vitamin provides. In conditions of low temperature, the addition of organic selenium (Se), either alone or in conjunction with inorganic Se and vitamin E, has been shown to increase glutathione peroxidase (GSHPx) activity and glutathione (GSH) concentration in the liver of broilers. This suggests an improved antioxidant defense mechanism in birds exposed to sub-optimal environments (Ozkan et al., 2007). Additionally, Bautista-Ortega and Ruiz-Feria demonstrated that the combination of arginine, vitamin E, and vitamin C could protect nitric oxide (NO) and enhance its bioavailability, thereby improving cardiovascular function and increasing xanthine oxidase (XO) activity near the pulmonary endothelium in hypoxemic broilers (Bautista-Ortega & Ruiz-Feria, 2010). These enzymes are upregulated in cases of idiopathic pulmonary arterial hypertension.

Vitamin E may play a role in immune system regulation by stabilizing fatty acids that function as immunoregulatory molecules, facilitating cellular communication, maintaining membrane fluidity, and influencing the production of second messengers. Additionally, vitamin E affects the metabolism of arachidonic acid, resulting in the synthesis of prostaglandins and leukotrienes (Jiang et al., 2022). Furthermore, vitamin E modulates gene expression through its interactions with transcription factors. Notably, it regulates the expression of cytosolic phospholipase A2, a key enzyme involved in phospholipid oxidation (Azzi et al., 2004).

The results demonstrate that the observed benefits lead to a reduction in the occurrence of pulmonary hypertension in broiler chickens, as indicated by a lower RV/TV ratio, which serves as a marker for pulmonary hypertension in the treated groups. Previous studies investigating electrocardiographic parameters in cold-induced pulmonary hypertensive broilers reported notable increases in the amplitudes of T and S waves, aligning with the findings of Martinez-Lemus et al. (1995). These observations may further substantiate the hypothesis that ventricular dilation and hypertrophy are significant factors contributing to the increased S wave amplitude, which is indicative of prolonged ventricular depolarization (Chen et al., 2018). In this study, a decrease in the amplitudes of S, T, and R waves was noted during vitamin E administration at 49 days of age across all treated groups. This finding implies that broilers receiving vitamin E supplementation are less likely to experience ventricular hypertrophy and dilation. Furthermore, both QRS and RR intervals showed an increase at 49 days of age in all treated groups, suggesting a reduction in tachycardia among vitamin E-supplemented chickens exposed to pulmonary hypertension.

Conclusion

Vitamin E supplementation may influence the development of pulmonary hypertension and ventricular hypertrophy linked to T3 administration. These modulatory effects are evidenced by significant changes in electrocardiographic parameters, particularly the amplitudes of the T, R, and S waves, along with alterations in the QRS complex and RR intervals.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the local Ethics Committee for animal experiments of [Garmsar Branch, Islamic Azad University](#), Garmsar, Iran (Code: IAUGB 2022.11.14).

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Authors' contributions

All authors contributed equally to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

اثرات ویتامین E بر پارامترهای الکتروکاردیوگرافی جوجه های گوشتی مبتلا به افزایش فشار خون ریوی

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چکیده

زمینه مطالعه: افزایش فشار خون ریوی در جوجه های گوشتی یک سندرم چندعاملی است که عمدتاً به دلیل هیپوکسمی و اضافه بار قلبی منجر به مرگ و میر قابل توجهی می شود.

هدف: هدف این مطالعه ارزیابی تأثیر مکمل ویتامین E بر پارامترهای الکتروکاردیوگرافی در جوجه های گوشتی دچار افزایش فشار خون ریوی القا شده توسط تری پدوتیرونین (T₃) بود.

روش کار: در مجموع ۱۸۰ جوجه به طور تصادفی به چهار گروه تقسیم شدند: یک گروه کنترل و سه گروه درمانی. جوجه های گروه های درمانی ویتامین α (E-توکوفرول) را با دوزهای ۱۰۰، ۲۰۰ IU و ۴۰۰ IU از روز اول تا ۴۹ روزگی دریافت کردند. برای القای افزایش فشار خون ریوی، به تمامی جوجه ها از روز هفتم تا پایان آزمایش از طریق رژیم غذایی ۱.۵ میلی گرم بر کیلوگرم T₃ داده شد. الکتروکاردیوگرامها در روزهای ۱۴ و ۴۹ ثبت شدند و هایپر تروفی بطن راست (RVH) پس از یوتانازیز ارزیابی شد.

نتایج: کاهش های معناداری در نسبت وزن بطن راست به وزن کل بطن (RV/TV) در گروه های درمانی در روز ۴۹ مشاهده شد ($P < 0.05$). تحلیل الکتروکاردیوگرافیک کاهش را در دامنه های امواج T، S و R به طور خاص در روز ۴۹ برای تمامی دوزهای ویتامین E نشان داد ($P < 0.05$). فاصله QRS به طور قابل توجهی در تمام دوزها در روز ۴۹ افزایش یافت، در حالی که فاصله RR در دوزهای مشخصی در روزهای ۱۴ و ۴۹ نسبت به گروه کنترل افزایش یافت ($P < 0.05$).

نتیجه گیری نهایی: بر اساس نتایج مطالعه حاضر، به نظر می رسد که مکمل ویتامین E به طور مؤثری القای افزایش فشار خون ریوی و هایپر تروفی بطنی مرتبط را در جوجه های گوشتی تعدیل می کند.

کلیدواژه ها: جوجه های گوشتی، پارامترهای الکتروکاردیوگرافی، افزایش فشار خون ریوی، تری پدوتیرونین (T₃)، ویتامین E

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