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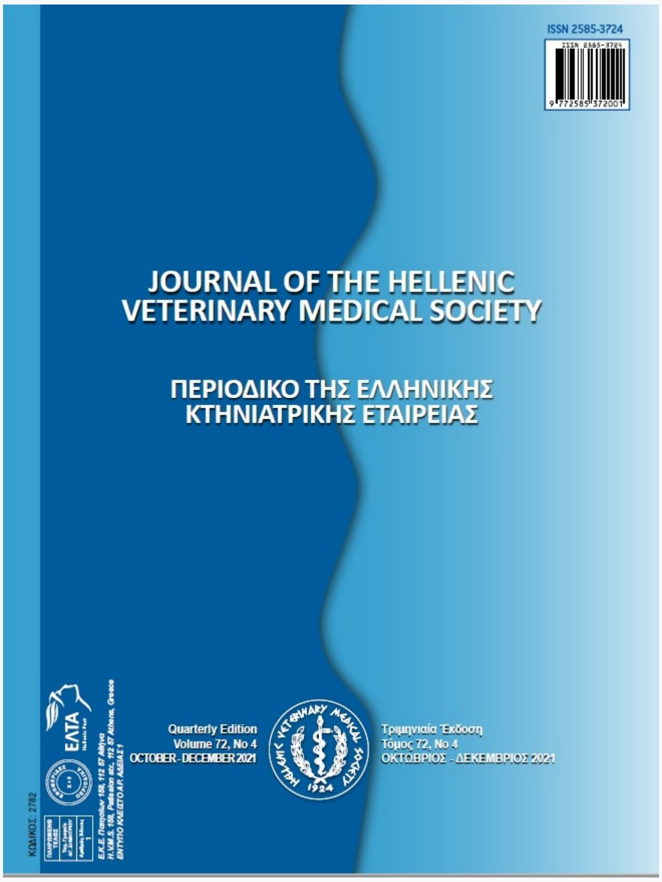

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Research article
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Garlic powder attenuates pulmonary hypertension in broiler chickens: an electrocardiographic-based study

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ABSTRACT: One of the most severe health-threatening issues in broiler chickens is believed to be pulmonary hypertension,characterized by inadequate oxygen levels in blood, elevated workload of the cardiopulmonary network, right ventricle hypertrophy, and death. A total of 180 one-day-old male chickens were dividedinto four equal groups, with 3 replicates per group (45 birds per group, 15 birds per replicate). To induce pulmonary hypertension syndrome, triiodothyronine was added to their diet. A corn-soy based feed was formulated for all treatment groups, and birds were treated with different doses of dietary garlic powder (0.2%, 0.6%, and 1%) for 49 days.Right ventricle/total ventricles (RV/TV), RV/body weight, TV/body weight ratio,and electrocardiographic records were assessed. RV to TV ratio was significantly reduced in all treatment groups at 49 days of age ($P <0.05$). The S-wave amplitude was decreased significantly (0.2% and 1% garlic powder groups, lead II; and all treatment groups, lead aVF) at 49 days of age. R-wave amplitude showeda significant reduction at 49 days of age (0.6% and 1% garlic powder receivers, lead aVR; all treatment groups, lead II) ($P<0.05$). QT interval showed a significant increase at 14 days (0.2% garlic powder group, lead III) and at 49 days (0.6% garlic powder group, lead II) ($P<0.05$). RR interval at 49 days of age (0.6% and 1% treatment groups, lead II; and1% garlic powder receivers, leads III, aVR, and aVF) showed a significant increase compared with controls ($P<0.05$).According to our study, garlic powder canimprove the electrocardiographic patterns and reversethe detrimental effects offree radicals and oxidative stress in birds withpulmonary hypertension.

***Keywords*: garlic, hypertension, electrocardiogram, broilers, waves**

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INTRODUCTION

Overthe pastthree decades, one of the most concerning issues known aspulmonary hypertension syndrome (PHS) has led to difficulties in movement, fast growth, increased metabolic processes of the cardiopulmonary system,and death in broiler chicken (Tankson et al., 2001). PHS is also known as ascites or "water belly" and is frequently imposed by insufficient oxygen to the lungs causing systemic arterial hypoxemia.High altitude is the most conspicuous environmental factor that plays an essential role in PHS formation in broilers. Stressful conditions such as cold temperature,feeding irregularities, and lighting alsoresult in PHS (Hassanpour et al., 2011). Ascites syndromes include pulmonary edema, aggregation of fluid in the pericardial sac, epicardial fibrosis, heart dilation, and right ventricular hypertrophy (Baghbanzadeh and Decuypere, 2008) which are all associated with the reactive oxygen species (ROS) accumulation. The elevated levels of ROS in the birds with PHS potentially contribute to disease development and increased mortality numbers (Tankson et al., 2001;Baghbanzadeh and Decuypere, 2008). Cellular dysfunction resulting from lipid and protein changes caused by ROS aggregation plays an important role in the deterioration of the cardiopulmonary system in ascites syndromes (Nain et al., 2008).

Nutritious foods are one of the most important and modifiable determinants in life. To mitigate the progression of various disorders such as PHS and support the metabolic requirements, appropriate nutritionalapproachesshould be applied, as mentioned by many investigations (Callejo et al., 2020;Wideman et al., 2007).Nemati and colleagues (2017) reported thata combination of vitamin C (300 mg/kg) and coenzyme Q10 in the daily diet of birds with PHS induced by cold temperature could significantly improve the body weight, feed conversion ratio and reduce the mortality rates (Nemati et al., 2017). Behroojet al. (2012) found that mortality rates would increase as a low-protein diet was added. Conversely, when high-protein meals were added to their diets, mortality rates reduced significantly (Behrooj et al., 2012).

In the challenging condition, proper nutrition, including antioxidant-based interventions, is supposed to inhibit the onset of ascites in chickens (Khajali and Wideman, 2016).Potent therapeutic effects of antioxidants have introduced them as a rightcandidate for preventing and/or treating and treating different diseases (Pham-Huy et al., 2008). Among these, Garlic (*Allium sativum* L.), a traditional medicinal plant, has gained a global reputation for treating different diseases such as cardiovascular disorders, hypertension, and lung diseases (Bayan et al., 2014). Garlic is a rich source of beneficial nutrients andcontainsbioactive constituents such as alliin, allicin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allyl-cysteine, and ajoene (Zeng et al., 2017; Shang et al., 2019).Organic sulfides, saponins, phenolic compounds, and polysaccharides are also known as other beneficial garlic compounds, which all have made it a usefulherb for many centuries (Shang et al., 2019).

Many studies have illustrated that the expressions of IL-6, IL-10, and TNF-α can be regulated by dietary garlic (Shang et al., 2019;Li et al., 2017;Rabe et al., 2015). Garlic is prepared in two forms including, solid form containing dried powders and aliquid form containing aqueous, oil, and solvent extracts. The main components of powder from crushed and dried garlic are appeared to be alliin and diallyl disulfide (DADS) (Trio et al., 2014).Due to having numerous compounds, dietary garlic has been demonstrated to have cardioprotective, antibacterial, antioxidant, antifungal, immunomodulatory, anti-carcinogenic, anti-apoptotic and anti-inflammatory properties against a wide variety of diseases (Schäfer and Kaschula, 2014;Lee et al., 2016). Taken together, we aimed at understanding the possible useful effects of garlic powder on electrographic parameters of birds with pulmonary hypertension syndrome induced by T_3.

MATERIALS AND METHODS

Animals, management and treatments

A total of 1801-day old chickens (Ross 308) were separately divided into 4 groups including 1 control and 3 treatment groups with 3 replicates per group (45 birds per group, 15 birds per replicate), as mentioned below:

Control group: Basic diet receivers over the test period; 3 Treatment groups: Receiver of basic diet + 0.2%, 0.6% and 1% garlic powder over the test period.To induce PHS, from day 7 onwards, 1.5 mg / kg triiodothyronine (T_3) (Sigma Chemical Co.) was added to the diet of birds.

Birds were reared for 49 days. From day 1 of age, all chickens were kept under a 23-h light and 1-h without light. They were fed with standard feed mixture and water *ad libitum* (starter: 3200 kcal metabolizable energy/kg of diet, 23% crude protein; grower:

3200 kcal metabolizable energy/kg of diet, 20% crude protein; finisher: 3200 kcal metabolizable energy/kg of diet, 18% crude protein) according to the National Research Council (NRC) recommendation for the broilers (NRC, 1994).

Electrocardiographic recordings

At 14 and 49 days of age, 8 chickens from each group were randomly selected, and electrocardiograms were recorded with an automatic recorder (Cardiomax FX- 2111, Fukuda, Japan) and standardized at 10 mm = 1 mV with a chart speed of 50 mm/s. Leads I, II, III, aVR, aVL, and aVF recorded for each bird. The amplitude of T, R, and S waves, the intervals of QRS, QT, RR, and ST, and the mean electrical axis (MEA) were measured.

Dissection and assessment of right ventricular hypertrophy

After the recording of electrocardiograms, the chickens were weighed and euthanized. Then, they were killed by decapitation, and right ventricle hypertrophy was evaluated, as previously described by Cueva et al.(1974).

The heart was dissected, and to the plane of the atrial ventricular valves, the atria were removed, and the total ventricles (TV) were weighed. The right ventricular (RV) wall was dissected from the left ventricle (LV) and septum. The RV was weighed, and the RV-to-TV ratio was calculated. Over our trial, pulmonary hypertension syndrome was defined as RV-to-TV ratio greater than 0.28 (Wideman, 2001).

Statistical analysis

All results were represented as mean ±SEM. Comparisons were made by one-way ANOVA using SPSS 22.0 (Chicago, IL, USA), with $P < 0.05$ accepted as significant.

RESULTS

Compared with controls, asignificant reduction of R-wave amplitude at 49 days of agein 0.6% and 1% garlic powder group (lead aVR) was observed ($P<0.05$). Besides, lead II in all three treatment groups (0.2%, 0.6% and 1% garlic powder), at 49 daysof age, showed a significant decrease ($P <0.05$) compared with the controls (Table 1, Figure1).

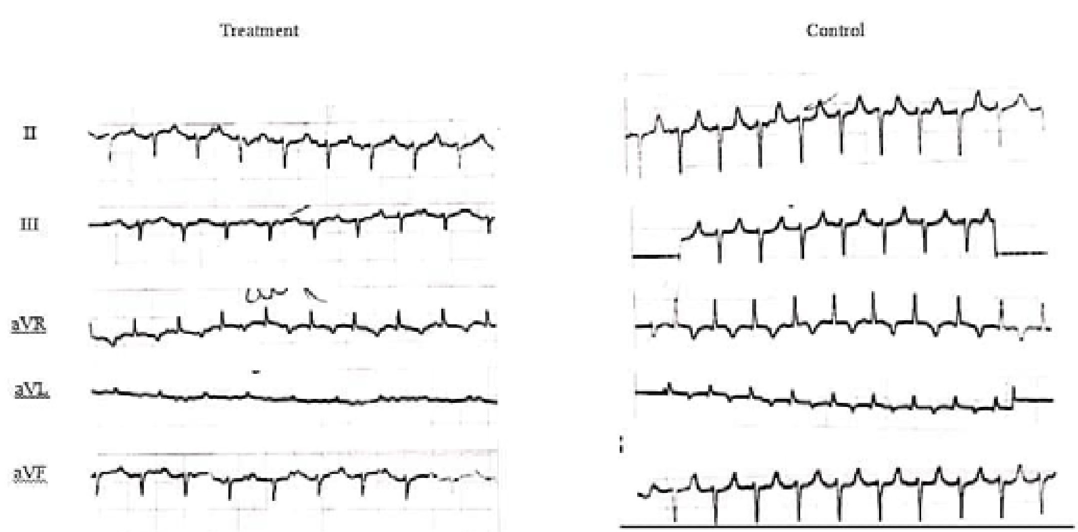

Figure 1: Samples of different electrocardiographs in 2 groups of hypertensive broilers (control and 1% garlic powder groups) at 49 days. Standardization, 10 mm = 1 mV; chart speed, 50 mm/s. Garlic powder in treatment group decreased the high amplitudes of R and S waves seen in control group (chickens with PHS).

Table 1: Amplitude of the electrocardiographic waves in the different groups

Age (days)	Lead Group	R (mV)				S (mV)			T (mV)			
		Lead II	Lead III	Lead aVR	Lead aVF	Lead II	Lead III	Lead aVF	Lead II	Lead III	Lead aVR	Lead aVF
14	Control	0.17±0.07	0.13±0.05	0.14±0.03	0.18±0.02	0.24±0.05	0.21±0.05	0.18±0.05	0.09±0.00	0.08±0.01	0.08±0.01	0.12±0.02
	T (0.2%)	0.14±0.03	0.09±0.01	0.17±0.02	0.19±0.08	0.16±0.03	0.18±0.02	0.17±0.04	0.08±0.03	0.08±0.02	0.10±0.02	0.09±0.06
	T (0.6%)	0.19±0.03	0.12±0.02	0.18±0.05	0.17±0.02	0.25±0.01	0.23±0.02	0.23±0.04	0.08±0.03	0.07±0.02	0.08±0.06	0.13±0.02
	T (1%)	0.22±0.02	0.16±0.03	0.15±0.02	0.21±0.04	0.21±0.05	0.26±0.01	0.21±0.06	0.09±0.02	0.09±0.03	0.10±0.02	0.08±0.04
49	Control	0.51±0.05	0.38±0.05	0.45±0.03	0.28±0.04	0.42±0.05	0.25±0.05	0.37±0.04	0.15±0.01	0.13±0.01	0.13±0.02	0.12±0.01
	T (0.2%)	0.17±0.04*	0.25±0.04	0.29±0.02	0.21±0.03	0.21±0.04*	0.16±0.04	0.15±0.03*	0.17±0.04	0.13±0.04	0.12±0.02	0.10±0.03
	T (0.6%)	0.21±0.03*	0.28±0.02	0.12±0.02*	0.24±0.03	0.26±0.03	0.23±0.03	0.18±0.03*	0.12±0.03	0.12±0.02	0.12±0.02	0.09±0.03
	T (1%)	0.18±0.04*	0.21±0.03	0.17±0.06*	0.23±0.03	0.18±0.04*	0.20±0.03	0.19±0.03*	0.11±0.04	0.13±0.01	0.10±0.04	0.11±0.03

T = treated; *significantly different vs. control ($P < 0.05$);

Table 2: Intervals of the electrocardiographic waves in the different groups

Age (days)	Lead Group	QT (seconds)				ST (seconds)		
		Lead II	Lead III	Lead aVR	Lead aVF	Lead II	Lead III	Lead aVF
14	Control	0.09±0.00	0.08±0.01	0.10±0.00	0.09±0.01	0.03±0.00	0.02±0.00	0.03±0.00
	T (0.2%)	0.10±0.00	0.11±0.00*	0.10±0.00	0.11±0.00	0.03±0.00	0.03±0.01	0.03±0.00
	T (0.6%)	0.10±0.00	0.10±0.00	0.09±0.01	0.09±0.00	0.03±0.00	0.03±0.00	0.02±0.00
	T (1%)	0.10±0.00	0.10±0.00	0.09±0.00	0.10±0.00	0.03±0.00	0.02±0.01	0.03±0.00
49	Control	0.09±0.00	0.10±0.01	0.11±0.00	0.09±0.00	0.04±0.00	0.03±0.00	0.04±0.00
	T (0.2%)	0.11±0.00	0.12±0.00	0.10±0.00	0.12±0.00	0.03±0.01	0.04±0.00	0.05±0.01
	T (0.6%)	0.13±0.01*	0.12±0.00	0.12±0.00	0.13±0.00	0.04±0.00	0.04±0.00	0.05±0.00
	T (1%)	0.12±0.00	0.12±0.01	0.11±0.00	0.12±0.00	0.03±0.00	0.05±0.01	0.05±0.00

T = treated; *Significantly different vs. control ($P < 0.05$)

Table 3: Intervals of the electrocardiographic waves in the different groups

Age (days)	Group	QRS (seconds)			RR (seconds)			
		Lead II	Lead III	Lead aVF	Lead II	Lead III	Lead aVR	Lead aVF
14	Control	0.03±0.00	0.02±0.00	0.03±0.00	0.11±0.00	0.11±0.00	0.11±0.00	0.11±0.01
	T (0.2%)	0.03±0.00	0.03±0.01	0.03±0.00	0.11±0.00	0.12±0.00	0.11±0.00	0.11±0.00
	T (0.6%)	0.03±0.00	0.04±0.00	0.03±0.00	0.11±0.00	0.11±0.00	0.11±0.01	0.11±0.01
	T (1%)	0.03±0.00	0.03±0.01	0.03±0.01	0.12±0.01	0.11±0.00	0.12±0.01	0.11±0.00
49	Control	0.04±0.00	0.05±0.01	0.04±0.00	0.13±0.00	0.13±0.00	0.13±0.00	0.14±0.00
	T (0.2%)	0.04±0.01	0.04±0.00	0.04±0.01	0.14±0.00	0.14±0.00	0.15±0.00	0.15±0.00
	T (0.6%)	0.03±0.00	0.04±0.00	0.04±0.00	0.16±0.00*	0.15±0.00	0.16±0.00	0.16±0.00
	T (1%)	0.04±0.01	0.04±0.00	0.04±0.00	0.16±0.00*	0.17±0.01*	0.16±0.00*	0.17±0.00*

T = treated; *Significantly different vs. control (P < 0.05)

Table 4: Cardiac indices and mean electrical axis (MEA) in the different groups

Age	Groups	MEA (°)	RV/TV	%TV/BW
14	Control	-53.25±42.96	0.19±0.00	0.43±0.00
	T (0.2%)	-6.60±23.53	0.17±0.00	0.36±0.00
	T (0.6%)	-31.11±25.69	0.19±0.00	0.38±0.01
	T (1%)	-54.00±36.00	0.18±0.00	0.41±0.01
49	Control	77.66±12.57	0.31±0.00	0.35±0.00
	T (0.2%)	-17.60±35.41	0.23±0.00*	0.33±0.00
	T (0.6%)	51.40±57.70	0.21±0.00*	0.30±0.00
	T (1%)	45.60±50.33	0.21±0.01*	0.31±0.00

T = Treated; *Significantly different vs. control (P < 0.05)

However, there were no significant differences-between treatmentand control groups in leads III and aVF (Table 1).At 49 days of age, a significant reduction of S wave (leads II, 0.2% and 1% treatment groups) and (lead aVF, all three treatment groups)was observed (P <0.05), while there were no significant differences between control and treatment groups in T wave amplitude (Table 1).

According to table 2, in treatment groups, mean ST intervals did not show any significant differences compared with control group.The mean QT interval (leads III) at 14 days of age in the treatment group receiving 0.2% garlic powder, and also at day 49 of age in the 0.6% garlic powder group (lead II) showed a significant increase (P <0.05) compared with the control group (Table 2, Figure 1).

By comparing control with treatment groups, RR and QRS intervals showed no significant differences at 14 days of age, while RR intervals in treatment groups (0.6%; Lead II, and 1%; all leads) at 49 days of age showed a significant increase (Table 3, Figure 1).

Compared to the control group, the mean RR interval at day 49 of age in the treatment group (1% garlic powder, all leads) and in the treatment group (0.6% garlic diet, lead II) showed a significant difference (P

<0.05) (Table 3). Our results also showed no significant differences in MEA (Table 4).

Evaluation of right ventricle hypertrophy

Compared to the controls, the mean RV/ TV ratios in the treatment groups were decreased in both ages, but it was significant for birds at 49 days of age($P<0.05$). There was no significant difference between the treatments than the control group in the mean TV/BW ratio. Our results showed no significant differences in electrocardiographic patterns among 3 treatment groups.

DISCUSSION

Environmental factors such as high altitude leading to oxygen loss mainly contribute to the PHS progression (Hassanpour et al., 2011). Cold, growth rate, and consumption of high-energy diets that increase the incidence of ascites via promoting the metabolic activity of chickens directly affect thyroid hormones, especially T_3. It has been revealed that 1.5 mg / kg T_3 can result in right ventricular hypertrophy, ascites, and increased mortality (Hassanzadeh et al., 2000).In the current study, the aim of adding T_3 to the broilers diet was to accelerate metabolism and consequently increase oxygen consumption and metabolic activities.

High demand for oxygen causes tissue hypoxia, which increases cardiac output to meet tissue requirements.Increased pulmonary blood flow leads to accelerated pulmonary blood pressure and eventually ascites (Julian, 2000). Increased oxygen consumption induced by T_3 causes cardiovascular disorders and reduced systematic vascular resistance (Hafe et al., 2019).

Previously it has been revealed that nutritional interventions can attenuate the ascites-related cardiovascular disorders and improve different electrocardiographic parameters in broilers. Ahmadipour et al. (2019) found that flavonoids extracted from hawthorn due to having potent antioxidant and free radical scavenging activities would effectively alleviate the NO serum levels in the numbers of PHS birds. Flavonoids (0.1 ml/L and 0.2ml/L) could significantly improve the amplitudes of S and T waves. Heart weight, RV:TV, RV:BW, and TV:BW ratios were also significantly reduced at receivers of 0.1 and 0.2 ml/L flavonoids (Ahmadipour et al., 2019). In a trial by Hassanpour and colleagues (2011), it was reported that 0.4g/L acetic acid would significantly alleviate S amplitudesat the age of 36 (leads II and III) and 45 days (lead aVF) in the birds with PHS. Acetic acid also reduced T amplitudes at 28 (leads aVR and aVL) and 36 days (lead aVL) of age. Besides, they reported a significant elevation of QRS and QT intervals in these broilers (Hassanpour et al., 2011).

In a similar trial by Hassanpouret al. (2009), 1.5 g/l citric acid significantly decreased S amplitude (leads II, III, aVF at 45 days), and T amplitude (lead aVR, at 28 days and lead aVL at 36 days). A significant reduction of R-wave amplitude was also reported. A significant increase in QRS, QT and RR intervals in some leads in all citric acid groups was observed (Hassanpour et al., 2009). Furthermore, in a different investigation on birds with PHS, they also reported that at 3 different doses (400, 800 and 1200ppm) of ascorbic acid after 36 and 45 days, RV/RT ratio was significantly decreased. S amplitude reduction in all 3 treatment groups was also reported and QRS, QT and RR intervals were increased significantly (Hassanpour et al., 2008). These studies are all consistent with our results on reducing wave amplitude and increase of wave intervals by antioxidant properties of garlic powder.

Garlic has been used as a herbal medicine for more than 4 thousand years, and the potentantioxidant traits of garlic have been represented by accumulating evidence (Shang et al., 2019; Locatelli et al., 2017). Bioactive substances of garlic such as ethyl linoleate have been demonstrated to have anti-inflammatory responses. Through the NO synthase (iNOS) and cyclooxygenase-2 (COX2) gene suppression, they can inhibit the generation of nitric oxide (NO) and prostaglandin E-2 (Park et al., 2014). It has been revealed that 14-kDa protein isolated from garlic prevents NO, tumor necrosis factor-α (TNF-α), and interleukin (IL) -1β, and enhances the adenosine monophosphate-activated protein kinase (AMPK). Another useful compound of garlic, allicin, cantackle potently with inflammatory responses (Shang et al., 2019;Rabe et al., 2015;Metwally et al., 2018).

Dietary garlic by scavenging free radicals can cope effectively with the pathogenesis of vascular diseases. They canimpede oxidative stress, systolic blood pressure, aortic NAD(P)H oxidase activity, and vascular disorders (Vazquez-Prieto et al., 2010). Varmaghany and colleagues (2015) found that after 42 days, 5 g/kg dietary garlic bulb reduced the incidence of ascites in broilers. The anti-hypertensive property of the garlic bulb also decreased PHS- associated mortality (Varmaghany et al., 2015). Ogbuewuet al. (2019) showed that garlic-active components strongly affected livestock and poultry and reduced their blood lipid and cholesterol content. Garlic could stimulate the gastrointestinal defense system, promote glutathione concentration, and reduce the free radical attack (Ogbuewu et al., 2019).

Javandel et al. (2008) showed that 2% garlic meal for birds at 42 days of age would significantly reduce their daily weight and improve feed conversion ratios (Javandel et al., 2008).Elagibet al. (2013) reported that after 42 days,3% garlic powder in broilersimproved feed consumption ratios and growth performance without any side effects on blood biomarkers (Elagib et al., 2013).Bahadoran et al. (2016) showed that after 42 days, 2 different doses of garlic supplement (0.6% and 1%)could significantly improve the duodenal, jejunal and ileal villus length and width in chickens with PHS (Bahadoran et al., 2016).In this study, the beneficial effects of garlic on electrocardiographic parameters of broilers with PHS have been approved.

CONCLUSION

Our results showed that after 49 days of treatment with dietary garlic,R and S waves were significantly decreased, and RR and QT intervals were significantly increased in some treated broilers showing improved ventricular hypertrophy.RV-to-TV and RV-to-BW ratios were improved in treated birds.We also had low-mortality rates among the birds that consumed dietary garlic compared to controls. It is deduced that garlic powder can modulate pulmonary hypertension, hypertrophy, and arrhythmia of ventricles induced by T_3.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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