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Garlic Supplement Improves Intestinal Mucosa Morphology in Broiler Chickens with Developmental Pulmonary Hypertension

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Abstract

The aim of this study was to investigate the effects of dietary garlic on the growth performance, intestinal mucosa morphology, and pulmonary hypertensive response in broiler chickens with pulmonary hypertension induced by 3, 5, 3′-l triiodothyronine. Chicks were reared for 42 days and treated with triiodothyronine (1.5 mg/kg diet) and 0 (control), 0.2, 0.6 or 1% garlic powder. Intestinal segments (duodenum, jejunum and ileum) were dissected to assess villus variables. The proportion of chickens with RV/TV ≥ 0.29 was lower in all garlic treatments than control ($P < 0.05$). Body weight of chickens fed 0.2 and 0.6% garlic increased compared to control ($P < 0.05$). The duodenal and jejunal villus length, width (at 42 days), and surface area (at 28 and 42 days) were significantly higher in most garlic-fed groups than control ($P < 0.05$). Ileal villus length, surface area (in 0.6% garlic group), and width (in 0.6 and 1% garlic groups) also increased in chickens fed garlic supplement at 42 days compared to control ($P < 0.05$). It is concluded that supplementation of garlic (especially concentrations of 0.6 and 1%) in broiler chickens with developmental pulmonary hypertension could modulate pulmonary hypertensive response and improve intestinal mucosa morphology.

Introduction

Garlic (*Allium sativum* L.) has been widely used as a nutritional substance since antiquity. It has been also identified as a medicinal plant in the prevention and treatment of many heart diseases, intestinal disorders, and metabolic diseases, such as atherosclerosis, thrombosis, dementia, cancer, and diabetes (Fleischauer and Arab, 2001; Rahman and Lowe, 2006; Kim *et al*., 2009). Antimicrobial, antioxidative, antithrombotic, anti–platelet aggregatory, and antihypertensive properties of garlic have been reported by several studies (Londhe, 2011; Stanaćev *et al*., 2011). Garlic is also effective in the regulation and modulation of body lipid profile through inhibition of the key enzymes contributing in the lipid metabolism (e.g., glucose-6-phosphatase dehydrogenase, cholesterol 7α-hydroxylase, fatty acid synthetase, and 3-hydroxyl-3-methyl-glutaryl-CoA reductase) (Chowdhury and Smith, 2002; Kim *et al*., 2009; Prasad *et al*., 2009). There are reports in the literature regarding the bioactive components of garlic responsible for its functions. The most important components are sulphuric compounds including allin, diallyl sulfide, allyl disulfide, and allicin. Of course,

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there are also reports regarding biological activities (antifungal and antitumor) of nonsulfur compounds of garlic (steroid saponins) (Amagase, 2006).

In many experiments, garlic has been supplemented to chicken diets to understand the impacts on and growth, feed conversion and meat quality of chickens. Many results showed positive effects of garlic on these parameters (Onibi *et al*., 2009; Mansoub and Nezhady, 2011) while other reports concluded that garlic had no influence on growth and feed conversion (Horton *et al*., 1991; Ali *et al*., 2000; Choi *et al*., 2010; Elagib *et al*., 2013).

Because of the beneficial effects of garlic on cardiovascular and metabolic diseases, it would be permissible that this medicinal plant influences one of the most important metabolic disorders in broiler chickens – pulmonary hypertension syndrome (PHS; ascites). In this syndrome, the fast growth of the chicken increases tissue demand of oxygen and cardiac output. The pulmonary vascular capacity is anatomically inadequate to support this increased cardiac output. This causes a high resistance in the pulmonary vasculature, resulting in an imbalance between pulmonary vasoconstrictors and vasodilators (Wideman *et al*., 2013). Hypoxemia, right-sided congestive heart failure, central venous congestion, cirrhosis of the liver, and accumulation of ascitic fluid into the abdominal cavity are symptoms of PHS (Balog, 2003). One of the most important pathophysiologic factors in PHS is free radicals. It has been reported that broilers prone to PHS have an elevated production of free radicals which may encourage the development of the disease or worsen the disease as it occurs (Baghbanzadeh and Decuypere, 2008). This harmful effect of free radicals on the cardiovascular and gastrointestinal system of pulmonary hypertensive chickens has been demonstrated (Baghbanzadeh and Decuypere, 2008). We hypothesize that the antioxidant effects of garlic modulates the pulmonary hypertensive response induced by thyroid hormone. We investigate whether different concentrations of garlic powder could improve growth performance and intestinal morphology.

Materials and Methods

This trial was approved by Institutional Animal Care and Use Committee of Shahrekord University, Shahrekord, Iran.

Animals, management and treatments

180 one-day-old broiler chickens (Ross 308) were randomized across 12 floor pens measuring 1.5m^2 each. Chicks were assigned to one of four groups (one control and three treatments) with 3 replicates pens per group and 15 chickens per pen. Chickens were housed in a deep litter system with wood shaving. Chicks were reared in standard conditions (temperature, ventilation and light) for 42 days and had free access to water and feed. A standard basal diet in mash form was formulated for the starting (1–14 days), growing (15–29 days) and finishing (30–42 days) growth stages which were mainly composed of corn and soybean meal (Table 1; NRC, 1994). For the treatments, garlic powder was included in the starter, grower and finisher basal diets at a concentration of 0.2, 0.6 and 1% of the diet, respectively. Triiodothyronine (T_3) was also added at a concentration of 1.5 mg T_3/kg in the grower and finisher basal diets (Decuypere *et al*., 1994; Hassanpour *et al*., 2013a). In each pen, the amount of feed offered and refused was weighed and recorded every morning to calculate intake. Feed consumption (FC) and body weight (BW) were recorded per pen and then feed conversion rate (FCR) was computed. The mortalities of broilers due to ascites were recorded in each group throughout the rearing period.

Assessment of pulmonary hypertension index

At the end of the feeding trial (42 days of age), all chickens from each group were euthanized by decapitation. The heart and then the ventricles were dissected and weighed to calculate the right ventricle to total ventricle ratio (RV/TV ratio). Right ventricle hypertrophy and pulmonary hypertension were estimated by this index as described by previous studies (Wideman, 2001; Khajali and Fahimi, 2010; Hassanpour *et al*., 2011). According to this index, chickens with an RV/TV ratio >0.25 were classified as developmental pulmonary hypertensive chickens (Khajali and Fahimi, 2010). RV/TV ratio ≥ 0.29 shows clinical pulmonary hypertension syndrome i.e., ascites with considerable right ventricular dilation (Wideman, 2001). In this experiment, RV/TV index was considered as 0.29 < > 0.25, and ≥ 0.29.

Table 1. Composition of the basal diet

Feedstuff (g/kg)	Starter (1-14 d)	Grower (15-29 d)	Finisher (30-42 d)
Corn	556.9	592.4	647.6
Soybean meal (440 g/kg CP)	389.3	345.7	287.2
Soybean oil	10	22.2	27.3
Limestone	12.2	11.2	11
Dicalcium phosphate	19.3	16.9	15.8
Vitamin premix[1]	3.0	2.5	2.5
Mineral premix[2]	3.0	2.5	2.5
Salt	3.1	3.1	3.1
DL-Methionine	2.2	2.5	2
L-Lysine	1	1	1
Calculated composition			
ME (Kcal/kg)	2900	2950	3050
Crude protein (%)	21.5	20.0	18.0
Calcium (%)	1.0	0.90	0.85
Available P (%)	0.5	0.45	0.42
Sodium (%)	0.15	0.15	0.15
Arginine (%)	1.55	1.43	1.27
Methionine (%)	0.58	0.58	0.51
Lysine (%)	1.37	1.27	1.1
Methionine + Cystine (%)	0.93	0.91	0.81
Threonine (%)	0.92	0.85	0.77
Tryptophan (%)	0.29	0.26	0.24

[1]Supplied per kg diet: vitamin A, 9000 IU; cholecalciferol, 1500 IU; vitamin E, 18 IU; vitamin K, 2 mg; cobalamin, 0.015 mg; thiamin 1.8 mg; riboflavin, 6.6 mg; folic acid, 1mg; biotin, 0.1 mg; pantothenic acid 3 mg; niacin, 30 mg; pyridoxine, 3 mg; cholin chloride, 500 mg. [2]Supplied per kg diet: Mn, 100 mg; Cu, 10 mg; Zn, 85 mg; I, 1 mg; Se, 0.2 mg; Fe, 50 mg.

Morphometric analysis of the intestine

At 14, 28 and 42 days of age, 9 chicks were selected randomly from each group (3 chickens from each pen for each stage), decapitated, and their morphometric variables of intestine (villus length, width, surface area and lamina propria thickness) were evaluated according to Hassanpour *et al.* (2013b) in the duodenum, jejunum and ileum. In this method, midpoint segments (about 1 cm) of the duodenum, jejunum, and ileum were fixed in Clark fixative solution for 45 min and then placed in ethyl alcohol for long-term storage. Each segment was cut into 3 sections along its length and examined. After dissection of the sections, they were stained with periodic acid-Schiff stain for 1-2 min, and villus rows were cut in sagittal part of the sections and placed between a glass slide and cover-slip. The villus parameters were measured by a microscope with the magnification of eyepiece graticule (10×) and an objective lens (10×). The villus length was estimated from the top of the villus to the top of the lamina propria. The villus surface area was calculated as (π) × (villus width) × (villus length). The lamina propria thickness as a place of lieberkuhn glands was measured from the base of the villus and the top of the muscularis mucosa.

Data are represented as mean. Comparisons were made by using one-way ANOVA with Duncan's multiple range test between different groups of the experiment at the same times. All statistical analyses were performed with the Statistical Package for Social Sciences software version 17 (SPSS Inc, Chicago, IL, USA). *P* values less than 0.05 were considered significant.

Results

Index of pulmonary hypertension syndrome and mortality rate

RV/TV ratio as an index of PHS is shown in Table 2. The number of chickens with 0.29 < RV/TV > 0.25 (developmental PHS) decreased in 0.6%-garlic group compared to other groups ($P < 0.05$) while chickens with RV/TV ≥ 0.29 (ascites) decreased in all garlic-groups compared to control ($P < 0.05$).

Ascites mortality rate (%) in broiler chickens were 22.2, 17.7, 17.7 and 26.7% in 0.2, 0.6 and 1% garlic groups and control, respectively, with the pooled *SEM* of 3.1.

Growth performance

The initial body weight did not differ between experimental groups (data not shown). Growth performance (BW, FC and FCR) of chickens were not significantly different between garlic-groups and control at 14 and 28 days of rearing period. At the end of the trial (day 42), the BW of chickens in 0.2 and 0.6%-garlic groups was greater than control ($P < 0.05$). FC and FCR did not differ among groups ($P > 0.05$; Table 2).

Table 2. Effect of garlic on broiler growth performance and RV/TV ratio at different times

Item	No. of chickens	BW (g)	FC (g)	FCR	RV/TV (0.29 < > 0.25)	RV/TV (≥ 0.29)
d 14						
Control	45	331	362	1.28	-	-
Garlic (0.2%)	45	333	351	1.20	-	-
Garlic (0.6%)	45	351	370	1.22	-	-
Garlic (1%)	45	342	372	1.25		
SEM	-	5.12	6.40	0.05	-	-
P-value	-	0.12	0.23	0.15		
d 28						
Control	36	1046	1766	1.72	-	-
Garlic (0.2%)	36	1091	1644	1.56	-	-
Garlic (0.6%)	36	1121	1710	1.58	-	-
Garlic (1%)	36	1112	1711	1.60	-	-
SEM	-	10.1	28.4	0.09	-	-
P-value	-	0.22	0.19	0.24		
d 42						
Control	27	1991^{b}	4010	1.89	44^{a}	36^{a}
Garlic (0.2%)	27	2080^{b}	3892	1.83	44^{a}	24^{b}
Garlic (0.6%)	27	2122^{a}	3942	1.86	32^{b}	16^{c}
Garlic (1%)	27	2061^{ab}	3841	1.86	36^{a}	20^{bc}
SEM	-	13.7	11.1	0.09	0.6	0.4
P-value	-	0.01	0.12	0.09	0.02	0.01

$^{a-c}$Means with different superscripts within the same column differ ($P < 0.05$). Proportion (%) of chickens with RV/TV (0.29 <, > 0.25) or (≥ 0.29). BW, body weight; FC, feed consumption; FCR, feed conversion ratio; No. of chickens, Total number of chickens, and 15 chickens/pen at each time.

Morphometric assessment of intestine

The duodenal villus length and width were greater in chickens fed diets supplemented with garlic (0.6 and 1%, day 42) than their controls ($P < 0.05$; Table 3). The duodenal surface area was also greater in chickens fed garlic supplement (0.2, 0.6 and 1%, day 42; 0.6 and 1%, day 28) than control ($P < 0.05$; Table 3). The duodenal length and width at 14 and 28 days as well as lamina propria thickness at all times were similar between experimental groups.

The jejunal villus length was greater in chickens fed diets supplemented with garlic (0.6%, day 28; 0.6 and 1%, day 42) compared to control groups ($P < 0.05$; Table 4). The jejunal villus width was also greater in all garlic-groups at 42 days ($P < 0.05$; Table 4). The jejunal villus surface area was higher in chickens fed garlic supplement (all concentrations, day 28; 0.6 and 1%, day 42) than controls ($P < 0.05$; Table 4). The jejunal length in 14 days, width in 14 and 28 days, and lamina propria thickness in all times were similar between experimental groups. The ileal villus length, surface area (in 0.6% garlic group) and width (in 0.6 and 1% garlic groups) were also higher in chickens fed garlic supplement than control at day 42 ($P < 0.05$; Table 5). The ileal variables did not significantly change in 14 and 28 days of rearing.

Table 3. Effect of garlic on morphologic parameters of duodenal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	1.06	0.66	0.37	2.25
Garlic (0.2%)	9	1.12	0.80	0.35	2.77
Garlic (0.6%)	9	1.32	0.77	0.43	3.36
Garlic (1%)	9	1.36	0.78	0.41	3.45
SEM	-	0.20	0.06	0.04	0.64
P-value	-	0.11	0.26	0.11	0.09
d 28					
Control	9	0.93	1.02	0.44	2.97^{b}
Garlic (0.2%)	9	1.08	1.13	0.54	3.89^{ab}
Garlic (0.6%)	9	1.32	1.24	0.43	5.06^{a}
Garlic (1%)	9	1.36	1.11	0.38	4.72^{a}
SEM	-	0.36	0.71	0.03	0.32
P-value	-	0.22	0.18	0.08	0.03
d 42					
Control	9	0.95^{b}	0.97^{b}	0.40	2.88^{b}
Garlic (0.2%)	9	1.28^{ab}	1.19^{ab}	0.54	4.81^{a}
Garlic (0.6%)	9	1.47^{a}	1.33^{a}	0.49	6.35^{a}
Garlic (1%)	9	1.40^{a}	1.30^{a}	0.52	5.71^{a}
SEM	-	0.12	0.31	0.05	0.81
P-value	-	0.01	0.03	0.33	0.01

[a,b] Means with different superscripts within the same column in each day differ significantly ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Table 4. Effect of garlic on morphologic parameters of jejunal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	0.70	0.65	0.39	1.45
Garlic (0.2%)	9	0.71	0.64	0.40	1.46
Garlic (0.6%)	9	0.80	0.77	0.38	1.91
Garlic (1%)	9	0.72	0.71	0.36	1.60
SEM	-	0.07	0.07	0.05	0.54
P-value	-	0.09	0.11	0.32	0.13
d 28					
Control	9	0.82^{b}	0.78	0.44	1.97^{b}
Garlic (0.2%)	9	0.99^{ab}	0.99	0.33	3.02^{a}
Garlic (0.6%)	9	1.16^{a}	1.02	0.36	3.73^{a}
Garlic (1%)	9	0.96^{ab}	1.03	0.40	3.11^{a}
SEM	-	0.05	0.07	0.04	0.29
P-value	-	0.01	0.24	0.31	0.03
d 42					
Control	9	1.08^{b}	0.89^{b}	0.34	3.01^{b}
Garlic (0.2%)	9	1.15^{ab}	1.18^{a}	0.35	4.17^{b}
Garlic (0.6%)	9	1.39^{a}	1.41^{a}	0.56	6.10^{a}
Garlic (1%)	9	1.42^{a}	1.32^{a}	0.50	5.86^{a}
SEM	-	0.11	0.07	0.04	0.51
P-value	-	0.02	0.04	0.29	0.03

Means with different superscripts within the same column in each day differ significantly ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Table 5. Effect of garlic on morphologic parameters of ileal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	0.42	0.46	0.31	0.61
Garlic (0.2%)	9	0.42	0.47	0.28	0.63
Garlic (0.6%)	9	0.47	0.51	0.28	0.76
Garlic (1%)	9	0.51	0.46	0.23	0.75
SEM	-	0.06	0.07	0.06	0.54
P-value	-	0.22	0.23	0.09	0.12
d 28					
Control	9	0.53	0.62	0.29	1.05
Garlic (0.2%)	9	0.62	0.73	0.30	1.42
Garlic (0.6%)	9	0.58	0.74	0.24	1.37
Garlic (1%)	9	0.64	0.78	0.28	1.58
SEM	-	0.05	0.08	0.05	0.48
P-value	-	0.08	0.08	0.10	0.07
d 42					
Control	9	0.64^{b}	0.57^{b}	0.47	1.16^{b}
Garlic (0.2%)	9	0.75^{ab}	0.67^{ab}	0.46	1.62^{ab}
Garlic (0.6%)	9	0.84^{a}	0.73^{a}	0.54	1.97^{a}
Garlic (1%)	9	0.78^{ab}	0.78^{a}	0.56	1.89^{ab}
SEM	-	0.04	0.05	0.08	0.51
P-value	-	0.02	0.01	0.07	0.02

[a,b] Means with different superscripts within the same column differ ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Discussion

The results showed that garlic could modulate pulmonary hypertensive response and decrease developmental hypertrophy and dilation of the heart in broilers. Sun and Ku (2006) also confirmed that allicin in garlic inhibits or hinders coronary endothelial dysfunction and right heart hypertrophy due to pulmonary hypertension in rats. Sobenin *et al.* (2009) showed that garlic can reduce systolic and diastolic blood pressure in arterial hypertension which may protect heart from hypertrophy. The hypotensive effect of garlic has numerous mechanisms. Garlic constituents are able to produce and release vasodilation factors such as nitric oxide in the arterial wall (Kim-Park and Ku, 2000). Allicin in garlic could act similarly to sodium nitroprusside as a nitric oxide donor (100-fold less potent) (Al-Qattan *et al.*, 2006; Sobenin *et al.*, 2009). Garlic can also elicit a direct relaxing effect on the muscles of the aorta and heart (Aqel *et al.*, 1991). Another mechanism is the blocking action of garlic on the heart β-adrenoreceptor, leading to the depression of heart automaticity and tension (Martin *et al.*, 1992). Garlic also shows its hypotensive effect through inhibition of Na, K-ATPase in the kidney which offers biphasic diuretic and natriuretic responses (Pantoja *et al.*, 2000). Finally, it has been reported that garlic components may cause membrane hyperpolarization through potassium ion channels that cause closing of Ca^{2+} channels, which result in vasodilatation (Siegel *et al.*, 1991; Sobenin *et al.*, 2009).

Although many reports have shown that garlic improves body weight, feed conversion ratio, and carcass yield percentage in broiler chickens (Mansoub and Nezhady, 2011; Elagib *et al.*, 2013), there are conflicting reports (Horton *et al.*, 1991; Onibi *et al.*, 2009; Choi *et al.*, 2010). In studies on garlic, different products of this plant with different concentrations have been used. The usual garlic products are raw garlic, garlic powder, garlic essential oil, garlic oil macerate, and aged garlic. The main compounds and characteristics of garlic are different in these products and may considerably change the garlic's function in the studies (Amagase *et al.*, 2001). In the present study, garlic powder was

Table 3. Effect of garlic on morphologic parameters of duodenal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	1.06	0.66	0.37	2.25
Garlic (0.2%)	9	1.12	0.80	0.35	2.77
Garlic (0.6%)	9	1.32	0.77	0.43	3.36
Garlic (1%)	9	1.36	0.78	0.41	3.45
SEM	-	0.20	0.06	0.04	0.64
P-value	-	0.11	0.26	0.11	0.09
d 28					
Control	9	0.93	1.02	0.44	2.97^{b}
Garlic (0.2%)	9	1.08	1.13	0.54	3.89^{ab}
Garlic (0.6%)	9	1.32	1.24	0.43	5.06^{a}
Garlic (1%)	9	1.36	1.11	0.38	4.72^{a}
SEM	-	0.36	0.71	0.03	0.32
P-value	-	0.22	0.18	0.08	0.03
d 42					
Control	9	0.95^{b}	0.97^{b}	0.40	2.88^{b}
Garlic (0.2%)	9	1.28^{ab}	1.19^{ab}	0.54	4.81^{a}
Garlic (0.6%)	9	1.47^{a}	1.33^{a}	0.49	6.35^{a}
Garlic (1%)	9	1.40^{a}	1.30^{a}	0.52	5.71^{a}
SEM	-	0.12	0.31	0.05	0.81
P-value	-	0.01	0.03	0.33	0.01

[a,b] Means with different superscripts within the same column in each day differ significantly ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Table 4. Effect of garlic on morphologic parameters of jejunal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	0.70	0.65	0.39	1.45
Garlic (0.2%)	9	0.71	0.64	0.40	1.46
Garlic (0.6%)	9	0.80	0.77	0.38	1.91
Garlic (1%)	9	0.72	0.71	0.36	1.60
SEM	-	0.07	0.07	0.05	0.54
P-value	-	0.09	0.11	0.32	0.13
d 28					
Control	9	0.82^{b}	0.78	0.44	1.97^{b}
Garlic (0.2%)	9	0.99^{ab}	0.99	0.33	3.02^{a}
Garlic (0.6%)	9	1.16^{a}	1.02	0.36	3.73^{a}
Garlic (1%)	9	0.96^{ab}	1.03	0.40	3.11^{a}
SEM	-	0.05	0.07	0.04	0.29
P-value	-	0.01	0.24	0.31	0.03
d 42					
Control	9	1.08^{b}	0.89^{b}	0.34	3.01^{b}
Garlic (0.2%)	9	1.15^{ab}	1.18^{a}	0.35	4.17^{b}
Garlic (0.6%)	9	1.39^{a}	1.41^{a}	0.56	6.10^{a}
Garlic (1%)	9	1.42^{a}	1.32^{a}	0.50	5.86^{a}
SEM	-	0.11	0.07	0.04	0.51
P-value	-	0.02	0.04	0.29	0.03

Means with different superscripts within the same column in each day differ significantly ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Table 5. Effect of garlic on morphologic parameters of ileal villi in the broiler chickens at different times

Item	No. of chicken	Length (mm)	Width (mm)	Lamina propria (mm)	Surface area (mm^2)
d 14					
Control	9	0.42	0.46	0.31	0.61
Garlic (0.2%)	9	0.42	0.47	0.28	0.63
Garlic (0.6%)	9	0.47	0.51	0.28	0.76
Garlic (1%)	9	0.51	0.46	0.23	0.75
SEM	-	0.06	0.07	0.06	0.54
P-value	-	0.22	0.23	0.09	0.12
d 28					
Control	9	0.53	0.62	0.29	1.05
Garlic (0.2%)	9	0.62	0.73	0.30	1.42
Garlic (0.6%)	9	0.58	0.74	0.24	1.37
Garlic (1%)	9	0.64	0.78	0.28	1.58
SEM	-	0.05	0.08	0.05	0.48
P-value	-	0.08	0.08	0.10	0.07
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Control	9	0.64^{b}	0.57^{b}	0.47	1.16^{b}
Garlic (0.2%)	9	0.75^{ab}	0.67^{ab}	0.46	1.62^{ab}
Garlic (0.6%)	9	0.84^{a}	0.73^{a}	0.54	1.97^{a}
Garlic (1%)	9	0.78^{ab}	0.78^{a}	0.56	1.89^{ab}
SEM	-	0.04	0.05	0.08	0.51
P-value	-	0.02	0.01	0.07	0.02

[a,b] Means with different superscripts within the same column differ ($P < 0.05$).
Length, from top of the villus to top of the lamina propria; Width, the width of villus at the base; The lamina propria, the space between base of the villus and top of the muscularis mucosa; and Villus surface area, calculated as (π) × (height) × (width); No. of chicken, Total number of chickens.

Discussion

The results showed that garlic could modulate pulmonary hypertensive response and decrease developmental hypertrophy and dilation of the heart in broilers. Sun and Ku (2006) also confirmed that allicin in garlic inhibits or hinders coronary endothelial dysfunction and right heart hypertrophy due to pulmonary hypertension in rats. Sobenin *et al.* (2009) showed that garlic can reduce systolic and diastolic blood pressure in arterial hypertension which may protect heart from hypertrophy. The hypotensive effect of garlic has numerous mechanisms. Garlic constituents are able to produce and release vasodilation factors such as nitric oxide in the arterial wall (Kim-Park and Ku, 2000). Allicin in garlic could act similarly to sodium nitroprusside as a nitric oxide donor (100-fold less potent) (Al-Qattan *et al.*, 2006; Sobenin *et al.*, 2009). Garlic can also elicit a direct relaxing effect on the muscles of the aorta and heart (Aqel *et al.*, 1991). Another mechanism is the blocking action of garlic on the heart β-adrenoreceptor, leading to the depression of heart automaticity and tension (Martin *et al.*, 1992). Garlic also shows its hypotensive effect through inhibition of Na, K-ATPase in the kidney which offers biphasic diuretic and natriuretic responses (Pantoja *et al.*, 2000). Finally, it has been reported that garlic components may cause membrane hyperpolarization through potassium ion channels that cause closing of Ca^{2+} channels, which result in vasodilatation (Siegel *et al.*, 1991; Sobenin *et al.*, 2009).

Although many reports have shown that garlic improves body weight, feed conversion ratio, and carcass yield percentage in broiler chickens (Mansoub and Nezhady, 2011; Elagib *et al.*, 2013), there are conflicting reports (Horton *et al.*, 1991; Onibi *et al.*, 2009; Choi *et al.*, 2010). In studies on garlic, different products of this plant with different concentrations have been used. The usual garlic products are raw garlic, garlic powder, garlic essential oil, garlic oil macerate, and aged garlic. The main compounds and characteristics of garlic are different in these products and may considerably change the garlic's function in the studies (Amagase *et al.*, 2001). In the present study, garlic powder was

used in the developmental pulmonary hypertension condition and led to improved body weight at the end of experiment. This could be due to the direct effect of garlic or a consequence of its beneficial effect on the cardiovascular system. It must be noted that in our study, all concentrations of garlic did not influence the performance at all sampling times of rearing period which is agreed upon with many studies reporting no effect of garlic on body performance.

In the studies of Solis de los Santos *et al.* (2005a) and Zamani Moghaddam *et al*. (2009), it was demonstrated that developmental pulmonary hypertension was associated with a progressive impairment of gut architecture and function. Many studies have examined different additives and supplements (e.g., vitamin C, canola, prebiotic) in the diets of pulmonary hypertensive chickens to modulate PHS and improve gut function (Solis De Los Santos *et al*., 2005b; Zamani Moghaddam *et al.*, 2009; Khajali *et al.*, 2011). In our study, we determined that oral supplementation of garlic affected intestinal morphology and improved villus dimensions in pulmonary hypertensive broilers. This effect of garlic on villus length, width, and surface area was considerable in all parts of the intestine by the end of the experiment. These data probably provide evidence of improved nutrient absorption in the intestine. This beneficial effect of garlic on the intestine may be associated with its antioxidant and anti-apoptotic effects (Borek, 2001) as it has previously been shown that dietary antioxidants such as vitamin C protect epithelial cells of intestine against pro-apoptotic oxidant stress, which increase epithelial cell growth and villus dimensions (Miller *et al*., 2001; Zamani Moghaddam *et al.*, 2009). Therefore, the antioxidant effect of garlic could be a crucial factor to protect the intestine against oxidative damage in PHS. Certainly, this effect of garlic is not limited to the intestine.

Conclusion

It is concluded that supplementation of garlic (especially concentrations of 0.6 and 1%) in broiler chickens with developmental pulmonary hypertension could modulate pulmonary hypertensive response and improve intestinal mucosa morphology.

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