



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

Bahadoran S<sup>1</sup>, Hasanpour H<sup>2</sup> & Shafigh Z<sup>1</sup>

<sup>1</sup>Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

<sup>2</sup>Department of Basic Sciences, Physiology Division, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Poultry Science Journal 2016, 4(2): 117-125

### Keywords

Villi  
Garlic  
Intestine  
Pulmonary hypertension

### Corresponding author

Shahab Bahadoran  
Bahadoran4@yahoo.com

### Article history

Received: February 3, 2016  
Revised: May 14, 2016  
Accepted: October 3, 2016

### 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'-I 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  $\geq$  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; Stanačev *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 7 $\alpha$ -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,

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<sup>2</sup> 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<sub>3</sub>) was also added at a concentration of 1.5 mg T<sub>3</sub>/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  $\geq$  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  $\geq$  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 <sup>1</sup>	3.0	2.5	2.5
Mineral premix <sup>2</sup>	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
<i>Calculated composition</i>			
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

<sup>1</sup>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. <sup>2</sup>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  $(\pi) \times (\text{villus width}) \times (\text{villus length})$ . 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  $0.29 < \text{RV/TV} < 0.25$  (developmental PHS) decreased in 0.6%-garlic group compared to other groups ( $P < 0.05$ ) while chickens with  $\text{RV/TV} \geq 0.29$  (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 ( $\geq 0.29$ )
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 <sup>b</sup>	4010	1.89	44 <sup>a</sup>	36 <sup>a</sup>
Garlic (0.2%)	27	2080 <sup>b</sup>	3892	1.83	44 <sup>a</sup>	24 <sup>b</sup>
Garlic (0.6%)	27	2122 <sup>a</sup>	3942	1.86	32 <sup>b</sup>	16 <sup>c</sup>
Garlic (1%)	27	2061 <sup>ab</sup>	3841	1.86	36 <sup>a</sup>	20 <sup>bc</sup>
SEM	-	13.7	11.1	0.09	0.6	0.4
P-value	-	0.01	0.12	0.09	0.02	0.01

<sup>a-c</sup>Means with different superscripts within the same column differ ( $P < 0.05$ ). Proportion (%) of chickens with RV/TV (0.29 < > 0.25) or ( $\geq 0.29$ ). 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 <sup>2</sup> )
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 <sup>b</sup>
Garlic (0.2%)	9	1.08	1.13	0.54	3.89 <sup>ab</sup>
Garlic (0.6%)	9	1.32	1.24	0.43	5.06 <sup>a</sup>
Garlic (1%)	9	1.36	1.11	0.38	4.72 <sup>a</sup>
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 <sup>b</sup>	0.97 <sup>b</sup>	0.40	2.88 <sup>b</sup>
Garlic (0.2%)	9	1.28 <sup>ab</sup>	1.19 <sup>ab</sup>	0.54	4.81 <sup>a</sup>
Garlic (0.6%)	9	1.47 <sup>a</sup>	1.33 <sup>a</sup>	0.49	6.35 <sup>a</sup>
Garlic (1%)	9	1.40 <sup>a</sup>	1.30 <sup>a</sup>	0.52	5.71 <sup>a</sup>
SEM	-	0.12	0.31	0.05	0.81
P-value	-	0.01	0.03	0.33	0.01

<sup>a,b</sup> 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 ( $\pi$ )  $\times$  (height)  $\times$  (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 <sup>2</sup> )
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 <sup>b</sup>	0.78	0.44	1.97 <sup>b</sup>
Garlic (0.2%)	9	0.99 <sup>ab</sup>	0.99	0.33	3.02 <sup>a</sup>
Garlic (0.6%)	9	1.16 <sup>a</sup>	1.02	0.36	3.73 <sup>a</sup>
Garlic (1%)	9	0.96 <sup>ab</sup>	1.03	0.40	3.11 <sup>a</sup>
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 <sup>b</sup>	0.89 <sup>b</sup>	0.34	3.01 <sup>b</sup>
Garlic (0.2%)	9	1.15 <sup>ab</sup>	1.18 <sup>a</sup>	0.35	4.17 <sup>b</sup>
Garlic (0.6%)	9	1.39 <sup>a</sup>	1.41 <sup>a</sup>	0.56	6.10 <sup>a</sup>
Garlic (1%)	9	1.42 <sup>a</sup>	1.32 <sup>a</sup>	0.50	5.86 <sup>a</sup>
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 ( $\pi$ )  $\times$  (height)  $\times$  (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 <sup>2</sup> )
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 <sup>b</sup>	0.57 <sup>b</sup>	0.47	1.16 <sup>b</sup>
Garlic (0.2%)	9	0.75 <sup>ab</sup>	0.67 <sup>ab</sup>	0.46	1.62 <sup>ab</sup>
Garlic (0.6%)	9	0.84 <sup>a</sup>	0.73 <sup>a</sup>	0.54	1.97 <sup>a</sup>
Garlic (1%)	9	0.78 <sup>ab</sup>	0.78 <sup>a</sup>	0.56	1.89 <sup>ab</sup>
SEM	-	0.04	0.05	0.08	0.51
P-value	-	0.02	0.01	0.07	0.02

<sup>a,b</sup> 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  $(\pi) \times (\text{height}) \times (\text{width})$ ; 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  $\beta$ -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<sup>2+</sup> 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 <sup>2</sup> )
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 <sup>b</sup>
Garlic (0.2%)	9	1.08	1.13	0.54	3.89 <sup>ab</sup>
Garlic (0.6%)	9	1.32	1.24	0.43	5.06 <sup>a</sup>
Garlic (1%)	9	1.36	1.11	0.38	4.72 <sup>a</sup>
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 <sup>b</sup>	0.97 <sup>b</sup>	0.40	2.88 <sup>b</sup>
Garlic (0.2%)	9	1.28 <sup>ab</sup>	1.19 <sup>ab</sup>	0.54	4.81 <sup>a</sup>
Garlic (0.6%)	9	1.47 <sup>a</sup>	1.33 <sup>a</sup>	0.49	6.35 <sup>a</sup>
Garlic (1%)	9	1.40 <sup>a</sup>	1.30 <sup>a</sup>	0.52	5.71 <sup>a</sup>
SEM	-	0.12	0.31	0.05	0.81
P-value	-	0.01	0.03	0.33	0.01

<sup>a,b</sup> 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  $(\pi) \times (\text{height}) \times (\text{width})$ ; 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 <sup>2</sup> )
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 <sup>b</sup>	0.78	0.44	1.97 <sup>b</sup>
Garlic (0.2%)	9	0.99 <sup>ab</sup>	0.99	0.33	3.02 <sup>a</sup>
Garlic (0.6%)	9	1.16 <sup>a</sup>	1.02	0.36	3.73 <sup>a</sup>
Garlic (1%)	9	0.96 <sup>ab</sup>	1.03	0.40	3.11 <sup>a</sup>
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 <sup>b</sup>	0.89 <sup>b</sup>	0.34	3.01 <sup>b</sup>
Garlic (0.2%)	9	1.15 <sup>ab</sup>	1.18 <sup>a</sup>	0.35	4.17 <sup>b</sup>
Garlic (0.6%)	9	1.39 <sup>a</sup>	1.41 <sup>a</sup>	0.56	6.10 <sup>a</sup>
Garlic (1%)	9	1.42 <sup>a</sup>	1.32 <sup>a</sup>	0.50	5.86 <sup>a</sup>
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  $(\pi) \times (\text{height}) \times (\text{width})$ ; 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 <sup>2</sup> )
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 <sup>b</sup>	0.57 <sup>b</sup>	0.47	1.16 <sup>b</sup>
Garlic (0.2%)	9	0.75 <sup>ab</sup>	0.67 <sup>ab</sup>	0.46	1.62 <sup>ab</sup>
Garlic (0.6%)	9	0.84 <sup>a</sup>	0.73 <sup>a</sup>	0.54	1.97 <sup>a</sup>
Garlic (1%)	9	0.78 <sup>ab</sup>	0.78 <sup>a</sup>	0.56	1.89 <sup>ab</sup>
SEM	-	0.04	0.05	0.08	0.51
P-value	-	0.02	0.01	0.07	0.02

<sup>a,b</sup> 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  $(\pi) \times (\text{height}) \times (\text{width})$ ; 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  $\beta$ -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<sup>2+</sup> 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.

### References

- Al-Qattan KK, Thomson M, Al-Mutawa'a S, Al-Hajeri D, Drobiova H & Ali M. 2006. Nitric oxide mediates the blood-pressure lowering effect of garlic in the rat two-kidney, one-clip model of hypertension. *The Journal of Nutrition*, 136: 774S-776S. [\[Link\]](#)
- Ali M, Al-Qattan KK, Al-Enezi F, Khanafer RMA & Mustafa T. 2000. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. *Prostaglandins, leukotrienes and essential fatty acids*, 62: 253-259. [\[Link\]](#)
- Amagase H. 2006. Clarifying the real bioactive constituents of garlic. *The Journal of Nutrition*, 136: 716S-725S. [\[Link\]](#)
- Amagase H, Petesch BL, Matsuura H, Kasuga S & Itakura Y. 2001. Intake of garlic and its bioactive components. *The Journal of Nutrition*, 131: 955S-962S. [\[Link\]](#)
- Aqel MB, Gharaibah MN & Salhab AS. 1991. Direct relaxant effects of garlic juice on smooth and cardiac muscles. *Journal of Ethnopharmacology*, 33: 13-19. [\[Link\]](#)
- Baghbanzadeh A & Decuyper E. 2008. Ascites syndrome in broilers: physiological and nutritional perspectives. *Avian Pathology*, 37: 117-126. [\[Link\]](#)
- Balog JM. 2003. Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? *Avian and Poultry Biology Reviews*, 14: 99-126. [\[Link\]](#)
- Borek C. 2001. Antioxidant health effects of aged garlic extract. *The Journal of Nutrition*, 131: 1010S-1015S. [\[Link\]](#)
- Choi IH, Park WY & Kim YJ. 2010. Effects of dietary garlic powder and  $\alpha$ -tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. *Poultry Science*, 89: 1724-1731. [\[Link\]](#)
- Chowdhury SD & Smith TK. 2002. Effects of dietary garlic on cholesterol metabolism in laying hens. *Poultry Science*, 81: 1856-1862. [\[Link\]](#)
- Decuyper E, Vega C, Bartha T, Buyse J, Zoons J & Albers GAA. 1994. Increased sensitivity to

- triiodothyronine ( $T_3$ ) of broiler lines with a high susceptibility for ascites. *British Poultry Science*, 35: 287-297. [\[Link\]](#)
- Elagib HAA, El-Amin WIA, Elamin KM & Malik HEE. 2013. Effect of dietary garlic (*Allium sativum*) supplementation as feed additive on broiler performance and blood profile. *Journal of Animal Science Advances*, 3: 58-64. [\[Link\]](#)
- Fleischauer AT & Arab L. 2001. Garlic and cancer: a critical review of the epidemiologic literature. *The Journal of Nutrition*, 131: 1032S-1040S. [\[Link\]](#)
- Hassanpour H, Momtaz H, Shahgholian L, Bagheri R, Sarfaraz S & Heydaripour B. 2011. Gene expression of endothelin-1 and its receptors in the heart of broiler chickens with  $T_3$ -induced pulmonary hypertension. *Research in Veterinary Science*, 91: 370-375. [\[Link\]](#)
- Hassanpour H, Afzali A & Bahadoran SH. 2013a. Diminished gene expression of cardiac heat shock proteins in pulmonary hypertensive chickens. *British Poultry Science*, 54: 581-586. [\[Link\]](#)
- Hassanpour H, Zamani Moghaddam AK, Khosravi M & Mayahi M. 2013b. Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. *Livestock Science*, 153: 116-122. [\[Link\]](#)
- Horton GMJ, Fennell MJ & Prasad BM. 1991. Effect of dietary garlic (*Allium sativum*) on performance, carcass composition and blood chemistry changes in broiler chickens. *Canadian Journal of Animal Science*, 71: 939-942. [\[Link\]](#)
- Khajali F & Fahimi S. 2010. Influence of dietary fat source and supplementary  $\alpha$ -tocopheryl acetate on pulmonary hypertension and lipid peroxidation in broilers. *Journal of Animal Physiology and Animal Nutrition*, 94: 767-772. [\[Link\]](#)
- Khajali F, Tahmasebi M, Hassanpour H, Akbari MR, Qujeq D & Wideman RF. 2011. Effects of supplementation of canola meal-based diets with arginine on performance, plasma nitric oxide, and carcass characteristics of broiler chickens grown at high altitude. *Poultry Science*, 90: 2287-2294. [\[Link\]](#)
- Kim-Park S & Ku DD. 2000. Garlic elicits a nitric oxide-dependent relaxation and inhibits hypoxic pulmonary vasoconstriction in rats. *Clinical and Experimental Pharmacology and Physiology*, 27: 780-786. [\[Link\]](#)
- Kim YJ, Jin SK & Yang HS. 2009. Effect of dietary garlic bulb and husk on the physicochemical properties of chicken meat. *Poultry Science*, 88: 398-405. [\[Link\]](#)
- Londhe VP. 2011. Role of garlic (*Allium sativum*) in various diseases-an overview. *Journal of Pharmaceutical Research & Opinion*, 1: 129 - 134. [\[Link\]](#)
- Mansoub HN & Nezhady MAM. 2011. The effects of using Thyme, Garlic and Nettle on performance, carcass quality and blood parameters. *Annals of Biological Research*, 2: 315-320.
- Martin N, Bardisa L, Pantoja C, Roman R & Vargas M. 1992. Experimental cardiovascular depressant effects of garlic (*Allium sativum*) dialysate. *Journal of Ethnopharmacology*, 37: 145-149. [\[Link\]](#)
- Miller M, Angeles F, Reuter B, Bobrowski P & Sandoval M. 2001. Dietary antioxidants protect gut epithelial cells from oxidant-induced apoptosis. *BMC Complementary and Alternative Medicine*, 1: 11. [\[Link\]](#)
- NRC. 1994. Nutrient requirements of poultry. National Academies Press.
- Onibi GE, Adebisi OE, Fajemisin AN & Adetunji AV. 2009. Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. *African Journal of Agricultural Research*, 4: 511-517. [\[Link\]](#)
- Pantoja CV, Martin NT, Norris BC & Contreras C. 2000. Purification and bioassays of a diuretic and natriuretic fraction from garlic (*Allium sativum*). *Journal of Ethnopharmacology*, 70: 35-40. [\[Link\]](#)
- Prasad R, Rose MK, Virmani M, Garg SL & Puri JP. 2009. Lipid profile of chicken (*Gallus domesticus*) in response to dietary supplementation of garlic (*Allium sativum*). *International Journal of Poultry Science*, 8: 270-276. [\[Link\]](#)
- Rahman K & Lowe GM. 2006. Garlic and cardiovascular disease: a critical review. *The Journal of Nutrition*, 136: 736S-740S. [\[Link\]](#)
- Siegel G, Walter A, Schnalke F, Schmidt A, Buddecke E, Loirand G & Stock G. 1991. Potassium channel activation, hyperpolarization, and vascular relaxation. *Zeitschrift fur Kardiologie*, 80: 9-24. [\[Link\]](#)
- Sobenin IA, Andrianova IV, Fomchenkov IV, Gorchakova TV & Orekhov AN. 2009. Time-released garlic powder tablets lower systolic and diastolic blood pressure in men with mild and moderate arterial hypertension. *Hypertension Research*, 32: 433-437. [\[Link\]](#)

- Solis de los Santos F, Farnell MB, Tellez G, Balog JM, Anthony NB, Torres-Rodriguez A, Higgins S, Hargis BM & Donoghue AM. 2005a. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. *Poultry Science*, 84: 1092-1100. [\[Link\]](#)
- Solis De Los Santos F, Farnell MB, Tellez G, Balog JM, Anthony NB, Torres-Rodriguez A, Higgins S, Hargis BM & Donoghue AM. 2005b. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. *Poultry Science*, 84: 1092-1100. [\[Link\]](#)
- Stanačev V, Glamočić D, Milošević N, Puvača N, Stanačev V & Plavša N. 2011. Effect of garlic (*Allium sativum*) in fattening chicks nutrition. *African Journal of Agricultural Research*, 6: 943-948. [\[Link\]](#)
- Sun X & Ku DD. 2006. Allicin in garlic protects against coronary endothelial dysfunction and right heart hypertrophy in pulmonary hypertensive rats. *American Journal of Physiology-Heart and Circulatory Physiology*, 291: H2431-H2438. [\[Link\]](#)
- Wideman RF. 2001. Pathophysiology of heart/lung disorders: Pulmonary hypertension syndrome in broiler chickens. *World's Poultry Science Journal* 57: 289-307. [\[Link\]](#)
- Wideman RF, Rhoads DD, Erf GF & Anthony NB. 2013. Pulmonary arterial hypertension (ascites syndrome) in broilers: A review. *Poultry Science*, 92: 64-83. [\[Link\]](#)
- Zamani Moghaddam AK, Hassanpour H & Mokhtari A. 2009. Oral supplementation with vitamin C improves intestinal mucosa morphology in the pulmonary hypertensive broiler chicken. *British Poultry Science*, 50: 175-180. [\[Link\]](#)



## مکمل سیر، مورفولوژی مخاط روده در جوجه‌های گوشتی با هیپرتانسیون ریوی در حال تکامل را اصلاح می‌کند

Bahadoran S<sup>1</sup>, Hasanpour H<sup>2</sup> & Shafigh Z<sup>1</sup>

<sup>1</sup>گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران  
<sup>2</sup>گروه علوم پایه، بخش فیزیولوژی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران

Poultry Science Journal 2015, 4(2): 117-125  
DOI: 10.22069/PSJ.2016.10564.1174

### چکیده

هدف از این مطالعه، ارزیابی اثرات سیر اضافه شده در جیره بر بازده رشد، مورفولوژی مخاط روده و پاسخ هیپرتانسیون ریوی در جوجه‌های گوشتی با هیپرتانسیون ریوی القاء شده با ۳،۵،۳- ال تری‌یدوتیرونین بوده است. جوجه‌ها به مدت ۴۲ روز پرورش داده شده و با تری‌یدوتیرونین (۱/۵ میلی‌گرم در کیلوگرم جیره) و صفر (شاهد)، ۰/۲، ۰/۶ یا ۱ درصد پودر سیر تیمار شدند. قسمت‌هایی از روده (دوازده، تهی روده و ایلئوم) جدا شده تا متغیرهای پرز در آنها بررسی شوند. نسبت جوجه‌های با  $RV/TV \leq 0.29$ ، در همه گروه‌های تیمار شده با سیر کمتر از گروه شاهد بود ( $P < 0.05$ ). وزن بدن جوجه‌های تغذیه شده با ۰/۲ و ۰/۶ نسبت به گروه شاهد افزایش یافت ( $P < 0.05$ ). طول، عرض (در ۴۲ روز) و ناحیه سطحی (در ۲۸ و ۴۲ روز) پرز دوازده و تهی روده به طور معنی‌داری در اکثر گروه‌های جوجه‌ی تغذیه شده با سیر در مقایسه با شاهد افزایش داشتند ( $P < 0.05$ ). طول، ناحیه سطحی (در گروه ۰/۶ درصد سیر) و عرض (در گروه‌های ۰/۶ و ۱ درصد سیر) پرز ایلئوم در جوجه‌های تغذیه شده با مکمل سیر نسبت به شاهد در ۴۲ روزگی نیز افزایش یافتند ( $P < 0.05$ ). نتیجه گیری می‌شود که مکمل سیر (بخصوص غلظت‌های ۰/۶ و ۱ درصد) در جوجه‌های گوشتی با هیپرتانسیون ریوی در حال تکامل می‌تواند پاسخ هیپرتانسیون ریوی را تعدیل کرده و مورفولوژی مخاط روده را اصلاح کند.

### کلمات کلیدی

پرز روده  
سیر  
روده  
آسیت

### نویسنده مسئول

Shahab Bahadoran  
Bahadoran4@yahoo.com

### تاریخچه مقاله

دریافت: ۳ فوریه ۲۰۱۶  
ویرایش: ۱۴ می ۲۰۱۶  
پذیرش: ۳ اکتبر ۲۰۱۶

Please cite this article as: Bahadoran S, Hasanpour H & Shafigh Z. 2016. Garlic Supplement Improves Intestinal Mucosa Morphology in Broiler Chickens with Developmental Pulmonary Hypertension. *Poult. Sci. J.* 4(2): 117-125.

© 2016 PSJ. All Rights Reserved