



## Effects of Dietary Inclusion of Turmeric (*Curcuma longa*) and Cinnamon (*Cinnamomum verum*) Powders on Performance, Organs Relative Weight and Some Immune System Parameters in Broiler Chickens

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### Abstract

A total of 240 Ross 308 day-old male broiler chicks within a completely randomized design were used in this experiment to evaluate the effects of dietary inclusion of turmeric powder and cinnamon powder on performance and some immune responses of broiler chickens. Dietary treatments were as follow: a corn-soybean meal basal diet (control); basal diet + 10 ppm avilamycin; basal diet + 2.5 g/Kg turmeric powder; basal diet + 7.5 g/Kg turmeric powder; basal diet + 2.5 g/Kg cinnamon powder; and basal diet + 7.5 g/Kg cinnamon powder. During the experiment, feed intake, body weight gain, and feed conversion ratio were measured in the beginning and the end of starter (0-21 d.) and grower (22-42 d.) periods. On 21 d. of age, two chicks from each replicate were randomly selected and blood samples were taken. Differential enumeration of heterophils, lymphocytes, and monocytes were done. Also, antibody titers against IBV, IBD, and NDV vaccines were measured. Addition of 2.5 g/Kg turmeric powder increased body weight gain in the starter period and improved feed conversion ratio in the starter and entire periods of the experiment, when compared to the control ( $P<0.05$ ). Also, inclusion of 10 ppm avilamycin into diet improved feed conversion ratio in the grower and entire periods of the experiment, comparing to the control ( $P<0.05$ ). Inclusion of turmeric powder at 2.5 g/Kg or 7.5 g/Kg and cinnamon powder at 7.5 g/Kg declined the heterophil to lymphocyte ratio ( $P<0.05$ ). The results of this study showed that turmeric powder at the level of 2.5 g/Kg would be a potential alternative for antibiotic growth promoters. Also, reducing heterophil to lymphocyte ratio by turmeric and cinnamon powders, introduce them as potential stress alleviators in broiler chickens.

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## Introduction

Phytogenic products have been used as food spices and traditional remedies for many centuries, but their application in feed industry is almost new. Phytogenic products have received more interest to be used as feed additives after the ban on antibiotic growth promoters in animal feed industry by European Union in 1999 (Hertrampf, 2001). On the other hand, increasing concerns of consumers regarding the safety of the foods have resulted to generation of "organic" livestock production concept which in turn, more accelerates the search for natural alternatives to antibiotic growth promoters. Phytogenic feed additives are natural products originated from different parts of the plants, mostly in the form of powder or extracts. Conducted researches with phytogenics have revealed a number of various characteristics such as antioxidative and antimicrobial effects, enhancing palatability of the diet, regulation of the gut function, and growth promoting effects for this products; and most of these effects has been attributed to the plants intermediary metabolites such as alkaloids, phenolic and polyphenolic compounds, terpenoids, saponins, and flavonoids (Windisch *et al.*, 2008).

Turmeric and cinnamon are plant-derived products with a long history in human nutrition as spices in different parts of the world. Turmeric is a yellow powder driven from the rhizome of "*Curcuma longa*" with extensive use as spices particularly in south and south-east Asia and Middle East countries. Curcumin, the yellow pigment of turmeric, has been reported as the main active component in turmeric (Jagetia and Aggarwal, 2007). Antioxidative properties have been reported for turmeric and curcumin in several studies (Reddy and Lokesh, 1994; Wei and Shibamoto, 2007). Also, it has been shown that turmeric and curcumin possess hypolipidemic effects (Dixit *et al.*, 1988; Babu and Srinivasan, 1997). Anti-inflammatory and immune system modulating effects of turmeric have been investigated (Joe and Lokesh, 1997; Joe *et al.*, 1997; South *et al.*, 1997).

Cinnamon (driven from *Cinnamomum verum*) is another spice with a good history in human nutrition which potentially can be used as feed additive. Cinnamaldehyde is the major component of cinnamon, creating about 65 percent of the extracted essential oil (Mountzouris *et al.*, 2009). Immune system stimulating effects has been reported for cinnamaldehyde (Nofrarias *et al.*, 2006). Also, considerable antibacterial (Tabak *et al.*, 1999; Chang *et al.*, 2001) and antifungal (Montes-Belmont and Carvajal, 1998; Soliman and Badea, 2002) properties have been found for cinnamon essential oil. This study was conducted to further investigate the efficacy of turmeric and cinnamon powders as phytogenic feed additives in broilers nutrition.

## Materials and Methods

### Bird management, diets, growth performance

Two hundred and forty day-old male broiler chicks (Ross 308) were weighed and randomly assigned to 24 deep litter floor pens (0.80 × 1.20 m). Four pens were

then randomly assigned to each dietary treatment. Dietary treatments were as follow: 1) corn-soybean meal basal diet (control); 2) the basal diet supplemented with 10 ppm avilamycin; 3) the basal diet supplemented with 2.5 g/Kg turmeric powder; 4) the basal diet supplemented with 7.5 g/Kg turmeric powder; 5) the basal diet supplemented with 2.5 g/Kg cinnamon powder; and 6) the basal diet supplemented with 7.5 g/Kg cinnamon powder. The corn-soybean meal basal diet for the starter (from 0 to 21 d) and grower (from 22 to 42 d) periods were formulated to meet the NRC (1994) minimum requirements (Table 1). Grit was used as an inert material in the basal diet and was substituted for the plant additives to make dietary treatments. Turmeric and cinnamon powders were purchased from a local retailer.

**Table 1. Ingredients and chemical composition of the experimental diets**

Ingredients (g/Kg)	Control and avilamycin diets		Diets with 2.5 g/Kg additive		Diets with 7.5 g/Kg additive	
	Age (day)		Age (day)		Age (day)	
	0-21	22-42	0-21	22-42	0-21	22-42
Corn	585.5	646.2	585.5	646.2	585.5	646.2
Soybean meal (44%CP)	356.5	296.7	356.5	296.7	356.5	296.7
Sunflower oil	13.5	17.4	13.5	17.4	13.5	17.4
Limestone	13.3	14.1	13.3	14.1	13.3	14.1
Dicalcium phosphate	13.5	9.8	13.5	9.8	13.5	9.8
Salt	3.9	2.8	3.9	2.8	3.9	2.8
DL-Methionine	1.3	0.5	1.3	0.5	1.3	0.5
Vitamin premix <sup>1</sup>	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix <sup>2</sup>	2.5	2.5	2.5	2.5	2.5	2.5
Grit	7.5	7.5	5	5	---	---
Additive	---	---	2.5	2.5	7.5	7.5
<i>Calculated composition</i>						
ME (Kcal/Kg)	2900	3000	2900	3000	2900	3000
CP (%)	20.84	18.74	20.84	18.74	20.84	18.74
Lys (%)	1.18	1.024	1.18	1.024	1.18	1.024
Met + Cys (%)	0.81	0.68	0.81	0.68	0.81	0.68
Ca (%)	0.91	0.84	0.91	0.84	0.91	0.84
P <sub>a</sub> (%)	0.41	0.33	0.41	0.33	0.41	0.33

<sup>1</sup>Provides per Kg of diet: all-trans-Retinyol acetate, 2.72 mg; Cholecalciferol, 0.05 mg; all-rac- $\alpha$ -Tocopherol acetate, 4 mg; Menadione (menadione sodium bisulphate), 2 mg; Thiamine (thiamine mononitrate), 1.8 mg; Riboflavin, 6.6 mg; Niacin, 9.8 mg; Ca-pantothenate, 29.7 mg; Pyridoxine, 1.18 mg; Folic acid, 1 mg; Cobalamin, 0.015 mg; Biotin, 0.1 mg; Choline chloride, 500 mg.

<sup>2</sup>Provides per Kg of diet: 76 mg Mn (as MnO<sub>2</sub>); 66 mg Zn (as ZnSO<sub>4</sub>); 40 mg Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 4 mg Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O); 0.64 mg I (as NaI); 0.2 mg Se (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O).

Birds were reared in an environmentally controlled room and had free access to feed and water. Temperature was maintained at 32°C for the first two days and then was gradually reduced to 22°C (at the rate of 2.5°C per week), which was kept

at this temperature to the end of the experiment. Light was provided continuously. The research station had an altitude of 2049 m.

Feed intake and body weight gain were recorded weekly and feed conversion ratio was determined. Health status and mortalities were recorded daily during the experimental period. The animal experimentation was approved by Shahrekord University, Shahrekord, Iran.

### **Sample collection**

At the end of the experimental period (42 d of age) 2 birds from each replicate (8 birds per treatment) were randomly selected, weighed, and slaughtered by cervical dislocation (Khajali *et al.* 2011). Abdominal cavity was opened and spleen and bursa of Fabricius were dissected and weighed. In order to have a comparison for pulmonary hypertension status among treatments, heart, total ventricles, and right ventricle of each bird were weighed and the right ventricle weight to total ventricle weight (RV/TV) ratio was calculated as described by Khajali *et al.* (2011).

### **Blood cell counts**

At 21 day of age, two chicks from each replicate were bled via brachial vein and blood samples were collected in heparinized tubes. Blood films were prepared, dried at room temperature, and stained with Giemsa-Wright. Differential leukocytes count was done according to the standard avian guidelines of Ritchie *et al.* (1994). Microhematocrit tubes were filled with blood and centrifuged at  $8000 \times g$  for five minutes. Packed cell volume (PCV) was measured using a PCV auto-reader.

### **Humoral immunity**

In order to evaluate the effects of the dietary additives on humoral immune response, all chicks were vaccinated against infectious bronchitis virus (IBV) (H120, 4 d post hatch), Newcastle disease virus (NDV) (B1, 7 d posthatch), and infectious bursal disease (IBD) (D78, 11 d posthatch). At 21 d of age, two chicks from each replicate (8 birds per treatment) were randomly selected and blood samples were collected from brachial vein into non heparinized tubes. Blood samples were incubated at  $37^{\circ}\text{C}$  for 3 h, subsequently centrifuged at  $1500 \times g$  for 12 mins. Serum samples were isolated, transferred to 1.5 mL Eppendorf tubes and stored at  $-20^{\circ}\text{C}$  until analysis. Antibody titers to IBV, NDV, and IBD vaccines were determined in serum samples by using commercial ELISA kits (ProFLOK<sup>®</sup> IBV, AUCIBV900; ProFLOK<sup>®</sup> NDV *Plus*, AUCNDV+450; ProFLOK<sup>®</sup> IBD, AUCIBD900; SYMBIOTICS Corporation, Kansas City, MO 64163) according to the manufacturer's instructions.

### Statistical analysis

Data were analyzed according to a completely randomized design using the GLM (Generalized Linear Models) procedure of SAS (SAS, 2008). Whenever ANOVA results were significant ( $P < 0.05$ ), treatment means differences were determined by the new Duncan multiple-range test at  $\alpha$  value of 0.05 (Duncan, 1955).

## Results

### Growth performance

The results for feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) for different periods of the experiment are shown in Table 2. Feed intake was not affected by any of the dietary additives (avilamycin, turmeric powder, or cinnamon powder) during different periods of the experiment. Addition of 2.5 g/kg turmeric powder significantly ( $P < 0.05$ ) increased BWG during starter (0 to 21 d) period compared with control group. Dietary inclusion of turmeric powder, cinnamon powder, or avilamycin at the level used in this experiment, had no significant effect on body weight gain during grower (22 to 42 d) or entire (0 to 42 d) periods of the experiment. Addition of 2.5 g/Kg turmeric powder significantly ( $P < 0.05$ ) improved FCR compared to the control group during the starter as well as entire period of the experiment; and these effects were comparable with the effects of avilamycin addition (10 ppm) during these periods. Avilamycin addition (10 ppm), also improved FCR during the grower period ( $P < 0.05$ ). Addition of turmeric powder at the level of 7.5 g/Kg, and both levels of cinnamon powder (2.5 g/Kg and 7.5 g/Kg), had no significant effect on BWG and FCR during the experiment, comparing to the control group.

**Table 2. Effect of turmeric or cinnamon powders supplementation on production performance of broiler chickens**

	Control	Avilamycin (10 ppm)	Turmeric powder		Cinnamon powder		SEM <sup>1</sup>
			2.5 g/Kg	7.5 g/Kg	2.5 g/Kg	7.5 g/Kg	
Feed intake (g)							
0-21	1018	1008	1005	1021	1011	1024	8.9
22-42	3252	2148	3237	3289	3225	3270	54.13
0-42	4270	4156	4242	4310	4237	4294	56.45
Weight gain (g)							
0-21	614 <sup>b</sup>	650 <sup>ab</sup>	666 <sup>a</sup>	637 <sup>ab</sup>	638 <sup>ab</sup>	630 <sup>ab</sup>	14.21
22-42	1596	1623	1628	1566	1588	1582	35.07
0-42	2211	2273	2294	2204	2227	2217	36.87
Feed conversion (Kg feed/Kg weight gain)							
0-21	1.67 <sup>a</sup>	1.55 <sup>ab</sup>	1.51 <sup>b</sup>	1.61 <sup>ab</sup>	1.58 <sup>ab</sup>	1.63 <sup>ab</sup>	0.044
22-42	2.04 <sup>ab</sup>	1.94 <sup>c</sup>	1.99 <sup>cb</sup>	2.10 <sup>a</sup>	2.03 <sup>ab</sup>	2.07 <sup>a</sup>	0.025
0-42	1.93 <sup>a</sup>	1.83 <sup>c</sup>	1.85 <sup>cb</sup>	1.96 <sup>a</sup>	1.90 <sup>ab</sup>	1.93 <sup>a</sup>	0.022

<sup>1</sup>Standard error of means.

<sup>a-c</sup>Means in the same row with different superscript differ significantly ( $P < 0.05$ ).

### Organs weight

Although heart weight was not affected by any of dietary additives, effects of the additives on the weight of RV and RV/TV ratio were significant (Table 3). Addition of cinnamon powder at the level of 7.5 g/Kg of the diet, caused a significant increase ( $P<0.05$ ) in the weight of RV and the RV/TV ratio compared to control (for RV weight), and control and avilamycin (for RV/TV ratio) groups. Although not significant, but avilamycin (10 ppm) tended to increase the weight of RV compared to control group.

Relative weight of bursa of Fabricius was not significantly affected by any of the additives (Table 3). However, the weight of bursa of Fabricius in either turmeric or cinnamon supplemented groups was numerically higher than that of control and avilamycin supplemented groups. Supplementation of the diet with cinnamon powder at the level of 2.5 g/Kg, caused a significant reduction in spleen weight compared to the control group ( $P<0.05$ , Table 3).

**Table 3. Effect of turmeric or cinnamon powders supplementation on organs relative weight (g/Kg of body weight) in broiler chickens (measured on 21 day of age)**

	Control	Avilamycin (10 ppm)	Turmeric powder		Cinnamon powder		SEM <sup>1</sup>
			2.5 g/Kg	7.5 g/Kg	2.5 g/Kg	7.5 g/Kg	
Heart	6.28	6.18	5.24	5.79	5.41	5.35	0.368
RV <sup>2</sup>	2.11 <sup>b</sup>	2.25 <sup>ab</sup>	2.06 <sup>b</sup>	2.26 <sup>ab</sup>	2.10 <sup>b</sup>	2.46 <sup>a</sup>	0.098
RV/TV <sup>3</sup>	0.21 <sup>b</sup>	0.21 <sup>b</sup>	0.21 <sup>b</sup>	0.22 <sup>b</sup>	0.23 <sup>ab</sup>	0.25 <sup>a</sup>	0.007
Spleen	1.50 <sup>a</sup>	1.22 <sup>ab</sup>	1.25 <sup>ab</sup>	1.13 <sup>ab</sup>	1.05 <sup>b</sup>	1.09 <sup>ab</sup>	0.125
Bursa	1.79	1.75	2.01	2.37	2.09	2.47	0.221

<sup>1</sup>Standard error of means.

<sup>2</sup>Right ventricle; <sup>3</sup>The ratio of right ventricle to total ventricle.

<sup>a,b</sup>Means in the same row with different superscript differ significantly ( $P<0.05$ ).

### Blood cell counts

Effects of the dietary additives on blood cell profile are shown in Table 4. The percentage of packed cell volume (PCV) and monocytes in peripheral blood were not affected by dietary additives. However, the percentages of lymphocytes, heterophils, and the ratio of heterophils/lymphocytes were affected by the dietary additives. Turmeric powder at the levels of 2.5 g/Kg and 7.5 g/Kg of the diet, and cinnamon powder at the level of 7.5 g/Kg of the diet, significantly increased lymphocytes percentage compared with the control group ( $P<0.05$ ). Also, the percentage of heterophils significantly was reduced by turmeric powder at the level of 2.5 g/Kg of the diet ( $P<0.05$ ). In addition, supplementation of the diet with turmeric powder (2.5 g/Kg and 7.5 g/Kg), and cinnamon powder (7.5 g/Kg) significantly reduced the ratio of heterophils/lymphocytes compared with the control group ( $P<0.05$ ).

### Humoral immunity

Effects of dietary additives on antibody titers to IBD and NDV vaccines (Table 5) were not significant ( $P>0.05$ ). However, supplementation of the diet with turmeric powder at the level of 2.5 g/Kg of the diet, caused a significant increase in anti-IBV titer compared to the control group ( $P<0.05$ , Table 5).

**Table 4. Effect of turmeric or cinnamon powders supplementation on PCV values and leucocyte counts in broiler chickens (measured on 21 day of age)**

	Control	Avilamycin (10 ppm)	Turmeric powder		cinnamon powder		SEM <sup>1</sup>
			2.5 g/Kg	7.5 g/Kg	2.5 g/Kg	7.5 g/Kg	
PCV <sup>2</sup> (%)	36.39	35.12	35.87	35.17	36.37	36.5	0.89
M <sup>3</sup> (%)	6.75	5.87	6.25	6.12	7.12	6.62	0.64
L <sup>4</sup> (%)	50.00 <sup>b</sup>	56.50 <sup>ab</sup>	61.75 <sup>a</sup>	60.75 <sup>a</sup>	57.62 <sup>ab</sup>	60.62 <sup>a</sup>	2.55
H <sup>5</sup> (%)	39.25 <sup>a</sup>	35.00 <sup>ab</sup>	29.75 <sup>b</sup>	30.87 <sup>ab</sup>	33.62 <sup>ab</sup>	31.62 <sup>ab</sup>	2.59
H/L <sup>6</sup>	0.79 <sup>a</sup>	0.64 <sup>ab</sup>	0.48 <sup>b</sup>	0.51 <sup>b</sup>	0.60 <sup>ab</sup>	0.53 <sup>b</sup>	0.074

<sup>1</sup>Standard error of means; <sup>2</sup>Packed cell volume; <sup>3</sup>Monocytes; <sup>4</sup>Lymphocytes; <sup>5</sup>Heterophils; <sup>6</sup>Heterophils to lymphocytes ratio.

<sup>a,b</sup>Means in the same row with different superscript differ significantly ( $P<0.05$ ).

**Table 5. Effect of turmeric or cinnamon powders supplementation on humoral immunity in broiler chickens (measured on 21 day of age)**

	Control	Avilamycin (10 ppm)	Turmeric powder		Cinnamon powder		SEM <sup>1</sup>
			2.5 g/Kg	7.5 g/Kg	2.5 g/Kg	7.5 g/Kg	
IBV <sup>2</sup>	2151 <sup>ab</sup>	1403 <sup>b</sup>	3181 <sup>a</sup>	1637 <sup>b</sup>	1489 <sup>b</sup>	1629 <sup>b</sup>	452
IBD <sup>3</sup>	373	192	270	181	275	238	91
NDV <sup>4</sup>	2620	3620	2870	2860	4370	2000	1020

<sup>1</sup>Standard error of means; <sup>2</sup>infectious bronchitis virus; <sup>3</sup>infectious bursal disease; <sup>4</sup>Newcastle disease virus.

<sup>a,b</sup>Means in the same row with different superscript differ significantly ( $P<0.05$ ).

### Discussion

Consistent with our results, Kumari *et al.* (2007) reported a significant increase in BWG of broiler chickens with addition of 1 g/Kg turmeric powder into diet. Also, Al-Sultan and Gameel (2004) reported higher BWG in broilers fed diet supplemented with 2.5, 5, and 10 g/Kg of turmeric powder. Similar to our findings, Durrani *et al.* (2006) reported a significant improvement in BWG and FCR with addition of 5 g turmeric per Kg of the diet. A linear improvement in BWG and FCR of broiler chickens with addition of up to 5 g turmeric per Kg of the diet, has been reported (Al-Sultan, 2003). Furthermore, improvement in BWG and FCR of broiler chickens due to supplementation of the diet with curcumin at 200 mg/Kg has been reported (Rajput *et al.*, 2013). On the other hand, Sugiharto *et al.* (2011) reported no improvement in BWG and FCR of broiler chickens when fed diets supplemented with turmeric extract up to 800 mg/Kg live body weight. In contrast to our results, Emadi and Kermanshahi (2006) reported no improvement in FCR of broiler chickens fed turmeric powder supplemented diets.

Beneficial effects of turmeric have been reported to be associated with stimulation of bile production in the liver of broiler chickens (Al-Sultan and Gameel, 2004) and dogs (Eigner and Scholz, 1999). The increase in production and secretion of bile salts in human due to turmeric consumption has also been reported (Soni *et al.*, 1997). Also, Rajput *et al.* (2013) reported an improvement in the utilization of apparent metabolizable energy due to supplementation of broiler diets with curcumin. Furthermore, enhanced activities of trypsin and amylase in pancreas and small intestine of broiler chickens fed diets supplemented with essential oils, has been reported (Lee *et al.*, 2003; Jang *et al.*, 2004). Therefore, the improvement in BWG and FCR of the birds in our study can be partly attributed to the effects of turmeric on bile and digestive enzymes production and secretion and consequently better digestion and absorption of the dietary nutrients.

In contrast to our results, Emadi and Kermanshahi (2007) reported a significant reduction in heart weight with supplementation of the broiler diets with turmeric powder and indicated this as a good factor for prevention of incidence of ascites syndrome. A reliable indicator of subclinical ascites in broiler chickens is the ratio of RV/TV. The RV/TV ratios higher than 0.25 manifest subclinical ascites (Khajali *et al.*, 2011). The increase in the weight of RV and the RV/TV ratio due to supplementation of the diet with cinnamon powder (7.5 g/Kg) shows that this herbal preparation would be considered as a potential accelerator of ascites syndrome in broiler chickens.

Al-Sultan (2003) reported an increase in relative weights of bursa of Fabricius, thymus, and spleen when 5 g/Kg turmeric was added to broiler diets. In the current study, bursa of Fabricius relative weight was not significantly affected by any of the additives (Table 3); however the weight of bursa of Fabricius in either turmeric or cinnamon supplemented groups was numerically higher than that of the control and avilamycin supplemented groups.

Heterophils to lymphocytes ratio has been identified as a reliable indicator for evaluation of stress in poultry (Gross and Siegel, 1988). It has been indicated that various stressors such as fear, hunger, thirst, and crowd can considerably increase the ratio of heterophils to lymphocytes (Hocking *et al.*, 1993). Although we did not use any special stressors in this study, the effects of turmeric and cinnamon powders in reducing heterophils to lymphocytes ratio indicates that these herbal preparations would be considered as stress alleviators in poultry. In contrast to our results, Kumari *et al.* (2007) reported an increase in heterophils and a decrease in lymphocytes absolute counts, when 1 g/kg turmeric was added to broiler diets. A gross increase in erythrocyte and leucocyte counts has been reported when broiler diets were supplemented with 5 g/kg or 10 g/kg turmeric powder (Al-Sultan, 2003).

Immunostimulatory effects of turmeric have been demonstrated (Chandrasekaran *et al.*, 2013). Kumari *et al.* (2007) reported an increase in antibody titer to NDV and IBV vaccines in broilers due to administration of turmeric into the

diet. Supplementation of the broilers diets with turmeric increased serum Zn concentration which can be related to increased antioxidant defense (Kumari *et al.*, 2007). The increase in CD4 T-lymphocytes as well as B-lymphocytes in mice consuming 1 gram curcumin per kilogram of the diet, has been reported (Churchill *et al.*, 2000).

### Conclusion

The results of this study indicated that turmeric powder at the level of 2.5 g/Kg of the diet would be a potential alternative for antibiotic growth promoters. Besides, its potential to reduce the heterophils to lymphocytes ratio implies its stress reducing and sedative roles in broiler chickens.

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