

## SOME PHYSIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF PHENYLACTIC ACID IN BROILER CHICKS

A.A. Askar

Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

### SUMMARY

Some blood haemato-biochemical and immunological traits of broiler chicks have been investigated under the effect of phenylactic acid (PA) treatment. One hundred and fifty unsexed one-day-old Hubbard chicks (body weight of  $47.9 \pm 0.8$  g) were randomly allotted into five treatment groups. Birds of first group served as control and fed control diet (basal diet), while other four groups were received basal diet plus L-3-phenylactic acid in different levels, 0.075% (PA1); 0.150% (PA2); 0.225% (PA3) or 0.300% (PA4). Blood serum contents of total protein, Albumin and Globulin were differed significantly ( $p < 0.05$  or  $0.01$ ) due to PA treatment. Birds group that received 0.150 % of PA was accompanied by the highest value of blood proteins. No differences either among the other groups of PA or with the control group were noticed in blood proteins content. Highly significantly differences were observed in blood contents of total lipids and cholesterol in response to phenylactic acid treatment. In general, birds treated with 0.75, 0.150 or 0.225% of PA had lower levels of cholesterol than those of other experimental groups including the control one. All PA treated groups had lower levels of total lipids than those of control one. Birds group that received 0.150% of PA recorded the lowest value of total lipids comparing with other experimental groups including the control one. Blood glucose levels were affected significantly ( $P < 0.001$ ) by PA treatment, since the significantly lowest value was recorded when birds supplemented by 0.75 % PA, while the highest one was observed in birds of control group. Effects of PA treatment on transaminase enzymes of liver (AST and ALT) were significant ( $P < 0.01$ ) only for ALT. Birds treated with 0.075, 0.225 or 0.300 % of PA showed low levels of ALT enzyme versus those recorded by birds received 0.150 % of PA or by those of control group. Red blood cells (RBCs) count, hemoglobin (Hb) and packed cell volume (PCV %) percentage of blood were affected significantly ( $P < 0.01$ ) due to PA treatment. Birds received 0.300% PA showed significantly higher values of these traits than those of control group birds that had the lowest ones. Number of WBC's, lymphocyte number percentage and relative weights of bursa of Fabricius and spleen were increased significantly in response to PA ( $P < 0.05$  or  $0.001$ ). The later traits of birds received 0.150, 0.225 or 0.300% of PA had nearly similar values and higher than those of birds received 0.075 of PA or those of control group. Most of the present results indicated that the treatment by PA showed a valuable improvement in some blood haemato-biochemical and immunological traits of broiler chicks, especially in birds received 0.150 % of PA.

**Key words:** Phenylactic acid, blood picture, blood components, immunological traits, broiler chicks.

### INTRODUCTION

A potential health hazard for humans may occurs due to using antibiotics in birds to enhance their biological performance through the development of antibiotic-resistant pathogens that can be transferred to humans (Kelley *et al.*, 1998) and consuming antibiotics residues that may be present in bird products (Dipeolu *et al.*, 2005). The inclusion of antibiotics in the diets of livestock has been prohibited in the European Union since January 2006. Researchers attempted other alternatives claiming to enhance the biological performance of broiler chicken. Organic acids and their salts are generally regarded as safe and have been approved by most member states of EU to be used as stimulators for the biological performance of animals (Sheikh Adil *et al.*, 2010).

Recently, Acidifiers as potential alternatives to antibiotics were used in the poultry industry to enhance the biological performance and reducing

disease. Organic acids can play this role due to their antimicrobial activity, which reduces microbial competition with the host for nutrients and endogenous nitrogen losses, and by lowering the incidence of subclinical infections and the secretion of immune mediators (Dibner and Buttin, 2002, La Ragione and Woodward, 2003 and Denli *et al.*, 2003). Organic acids in nondissociated (nonionised, more lipophilic) form can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of microorganisms (Dhawale, 2005). Also, they reduce the pH of digesta, increase the pancreatic secretion and have trophic effects on mucosa layer of gastro-intestinal tract (Dibner and Buttin, 2002). Acidification with various organic acids has been reported to reduce the toxic components production by pathogens population, thus preventing the damage in epithelial cells of intestinal wall (Langhout, 2000), also improve the digestibility of proteins, calcium, phosphorus, magnesium, and zinc, and serve as substrates in

the intermediary metabolism (Kirchgessner and Roth, 1988). These beneficial effects in turn improve the immune status and the physiological conditions of animal and poultry.

Phenylactic acid (PA) is an organic acid that is produced as a by-product of phenylalanine metabolism (Thierry and Maillard, 2002). PA has recently been identified in cultures of *Lactobacillus plantarum*, and it is known to inhibit the growth of a wide variety of fungal strains (Ström *et al.*, 2002 and Lavermicocca *et al.*, 2003) and pathogenic bacteria (Dieuleveux *et al.*, 1998). In addition, PA had less odor comparing with other organic acids, such as acetic acid. However, few studies have been conducted to evaluate the physiological and immunity effects of PA in broiler chicks. Therefore, this study was conducted to evaluate the physiological and immunity effects of using PA to enhance the biological performance of broiler chicks.

## MATERIALS AND METHODS

One hundred and fifty unsexed one-day-old Hubbard chicks (body weight of  $47.9 \pm 0.8$  g) were randomly allotted into five treatment groups of 30 chicks each (three replicates/group of 10 chicks). Birds were treated by L-3-phenylactic acid (PA) in powder form, which imported from Oxford Co., India by Al-Rahma office, Zagzig, Egypt. The PA was prepared by Qualikems Fine Chemicals Pvt. Ltd. Company (New Delhi, India). Birds of first group served as control and fed control diet (basal diet), while other four groups were received basal diet plus PA in different levels, 0.075% (PA1); 0.150% (PA2); 0.225% (PA3) or 0.300% (PA4). Two basal diets (starter and finisher) were formulated to cover the nutrients requirements of broiler chicks reported by NRC (1994) during the experimental period in two phases (starter and finisher), starter one from 0-21 days and finisher one from 22-49 days. The ingredients and chemical composition of the two basal diets are shown in Table 1. All birds of this trial were kept under similar conditions of management during whole period of the experiment (49 days). Chicks were provided with 8% sugar solution for the first 12 hours. Vitamins and electrolytes were added to the drinking water for the first three days to avoid stress of transportation. Temperature inside the house of chicks was controlled and gradually reduced from 32°C to 20°C on day 49. The chicks were maintained on a 24-hour consistent lighting schedule. A fresh feed and water were provided daily ad-libitum.

At the end of the experimental period (7-weeks of age), five birds from each treatment group were randomly taken for slaughtering and collecting blood samples. Blood received from each bird was collected in two tubes (heparinized or non-heparinized tubes). The heparinized

samples were used in determination the hematological parameters: red blood cells (RBC's) and white blood cells (WBC's) counts; hemoglobin content (Hb) and packed cell volume percentage (PCV%) according to Winterobe (1967). Two drops of blood from each sample were smeared on two glass slides and stained using May-Grunwald, Giemsa stains. One hundred of leukocytes cell including lymphocytes were counted on each slide to calculate lymphocyte percentage by dividing the number of lymphocytes by that of the whole leukocytes. The non-heparinized ones were centrifuged at 4000 rpm for 15 minutes. Serum was separated and stored in deep freezer at -20°C for chemical analysis. Total protein; albumin; alanine transaminase, ALT and aspartate transaminase, AST activities; total lipids; cholesterol; and glucose were estimated using commercial kits purchased from Diamond Diagnostics Company, Egypt.

Data collected from this study were statistically analyzed using one way ANOVA through the GLM procedures of SPSS program (1993) as the following model:  $Y_{ij} = \mu + \pi_i + e_{ij}$  Where,

$Y_{ij}$  = the observation on the  $ij$ th blood sample;

$\mu$  = general mean;

$\pi_i$  = fixed effect of  $i$ th phenylactic acid levels = 1;.....; 5 (0.000; 0.075; 0.150; 0.225; 0.300 %) and

$e_{ij}$  = error of the model.

Multiple range tests were used to determine significance of differences in all studied traits according to Duncan (1955).

## RESULTS AND DISCUSSION

As shown in Table 2, blood serum contents of total protein, Albumin and Globulin were differed significantly ( $p < 0.05$  or  $0.01$ ) due to PA treatment, whereas birds group that received 0.150% of PA was accompanied by the highest value of blood proteins. No differences either among the other groups of PA or with the control group were noticed in blood proteins content. The present results are in partially agreement with those obtained in laying hens by Wang *et al.* (2009a). They found that total protein, and albumin concentrations were higher in birds treated with 0.1% PA and those of control treatment group ( $P < 0.05$ ) than those received 0.3% PA. However, PA treatment in pigs had no effect on IgG, and total protein concentration (Wang *et al.*, 2009b).

Highly significantly differences were observed in blood contents of total lipids and cholesterol in response to phenylactic acid treatment (Table 2). In general, birds treated with 0.75, 0.150 or 0.225% of PA had lower levels of cholesterol than those of other experimental groups including the control one. All PA treated groups had lower levels of total lipids than those

of control one. Birds group that received 0.150% of PA recorded the lowest value of total lipids comparing with other experimental groups including the control one. However, no comparisons with other studies could be made because there have been no other studies conducted to evaluate the effects of PA on the blood glucose, Sheikh Adil *et al.* (2010) observed that blood contents of cholesterol didn't differ significantly due to the treatment by some organic acids in broiler chicks.

Levels of blood glucose were affected significantly ( $P < 0.001$ ) by PA treatment, since the highly ones were observed for birds of control groups and those treated with 0.225% of PA (157.8 and 157.0 mg/dl, respectively) meanwhile, birds received 0.075 % of PA had the significantly lowest level of blood glucose (125.2 mg/dl) as shown in Table 2. However, no comparisons with other studies could be made because there have been no other studies conducted to evaluate the effects of PA on the blood glucose, Sheikh Adil *et al.* (2010) observed that blood glucose didn't differ significantly due to the treatment by some organic acids in broiler chicks.

The effects of PA treatment on transaminase enzymes of liver (AST and ALT) were significant ( $P < 0.01$ ) only for ALT (Table 2). Birds treated with 0.075, 0.225 or 0.300 % of PA showed low levels of ALT enzyme (45.2, 45.00 and 45.20 u/l, respectively) versus those recorded by birds received 0.150 % of PA or by those of control group (50.00 and 52.00 u/l). Similar observations were reported also by Sheikh Adil *et al.* (2010) in broiler chicks, they found that AST and ALT of blood serum didn't differ significantly due to the treatment by some organic acids

Blood content of red blood cells (RBCs), hemoglobin (Hb) and packed cell volume (PCV%) percentage of blood were affected significantly ( $P < 0.01$ ) due to PA treatment. Birds received 0.300% PA showed significantly higher values of these traits (2.66,  $\times 10^6/\text{mm}^3$ , 7.22, g/dl and 31.00 %, respectively) than those of control group birds that had the lowest ones. The available studies on PA effect on RBCs showed controversial results, Wang *et al.* (2009a) found in laying hens that red blood cell concentration was higher in birds dietary treated with 0.1% PA or those of control group ( $P < 0.05$ ) than in birds treated by 0.2 or 0.3% of PA, while Wang *et al.* (2009b) found that dietary treatment of PA had no effect on RBC in pigs.

The immunological status of broiler chicks was investigated under the effect of PA by determining WBC's number per  $\text{mm}^3$ , the percentage of lymphocyte number of total number of WBC's and the relative weights of bursa of Fabricius and spleen presented in Table 4. The later four measurements were increased

significantly in response to PA ( $P < 0.05$  or 0.001). In general, the immunological traits in birds received 0.150, 0.225 or 0.300% of PA had nearly similar values and higher than those of birds received 0.075 of PA or those of control group. These results are similar to some extent with those obtained by Wang *et al.* (2009a). They found that PA can partially improve the profile of blood characteristics, such as lymphocytes and WBC's in the short term, whereas WBC's count in laying hens was higher in birds dietary treated with 0.1% PA or those of control group ( $P < 0.05$ ) than in birds treated by 0.2 or 0.3% of PA. Also, Wang *et al.* (2010) found in broiler chicks that PA treatment (0.1, 0.2 or 0.3%) led to an increase ( $P < 0.05$ ) in the concentrations of WBC's and lymphocyte percentage, while the relative weights of bursa of Fabricius and spleen were unaffected by any of the dietary supplementations. Wang *et al.* (2009b) found in pigs that lymphocyte percentage on d 42 increased linearly as dietary PA increased ( $P < 0.05$ ). Additionally, WBC's counts on d 42 tended to increase as PA levels increased ( $P < 0.10$ ). Reviewed and present findings suggested that PA, like other organic acids, could stimulate the immune system. Although organic acids effect on the immunological status has not been well documented, it has been clearly established that several organic acids can affect microorganisms population of gut intestine that are necessary for gut immune system development (Wang *et al.*, 2009a&b and Czerwiński *et al.*, 2010). Also, the attained reduction in subclinical infections due to antimicrobial effects of organic acids can contribute in nutrient digestibility improvement beside the reduction in nutrients demand for immune tissues of gut. Reducing pH value of the diet due to organic acids may improve the utilization of minerals, such as Ca, which have positive effects on the immunity ( Mroz *et al.*, 2000), as well as, citric acid can help to release minerals bound to the phytate molecule, which in turn increase the utilization of Ca, P, and Zn (Kirchgessner and Roth, 1988; Boling *et al.*, 2000 and Liem *et al.*, 2008). Moreover, organic acids can improve the digestibility of proteins, Ca, P, Mg, and Zn, and serve as substrates in the intermediary metabolism (Sheikh Adil *et al.*, 2010). These additional minerals may provide another explanation for the improved immune status indicators.

In conclusion, PA treatment could be exerting some positive effects on blood haemato-biochemical traits and immune system. It may be beneficial to supplement the diets of broiler chicks with PA to improve and/or maintain it's physiological and immunity status. Most of blood profile traits, except immune status indicators, were not continually improved by PA levels. It is

likely that there was no level of PA responses on the improvement of blood parameters.

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**Table 1. Composition and Calculated chemical analysis of the basal diet**

Ingredient	Starter (%)	Finisher (%)
Ground yellow corn	54.50	60.90
Soybean meal 44%	32.00	26.00
Corn gluten 60%	6.56	5.00
Sun flower oil	3.50	4.70
Dicalcium phosphate	1.65	1.60
Limestone	1.00	1.00
Salt	0.42	0.40
Vit. Min. Premix*	0.30	0.30
DL-methionine	0.07	0.10
<b>Total</b>	<b>100.0</b>	<b>100.0</b>
Calculated analysis**		
Crude protein	22.94	19.90
ME Kcal/kg	3091	3219
Calcium %	0.85	0.82
Av. Phosphors %	0.46	0.44

\*Supplied per kg of the diet: Vit. A 2000 IU; Vit. D3 2200 IU; Vit. E 10 mg; Vit. K3 2 mg; Vit. B1 1 mg, Vit. B2 5 mg, Vit. B6 1.5 mg; Vit. B12 0.01 mg; Niacin 30 mg; Folic acid 1 mg; Biotin 0.05 mg; Choline chloride 300 mg; Copper 10 mg; Iron 30 mg; Manganese 60 mg; Zinc 50 mg; Iodine 1 mg; Selenium 0.1 mg; cobalt 0.1 mg.

\*\*Calculated according to NRC (1994).

**Table 2. Means ( $\bar{X}$ ) and standard errors (SE) of some blood serum components in broiler chicks as affected by phenylactic acid.**

Items	Traits									
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (g/l)	Cholesterol (mg/dl)	Glucose (mg/dl)	AST (u/l)	ALT (u/l)		
	**	*	**	**	**	**	Ns	**		
Phenylactic acid (%)	0.000	X	4.90 <sup>b</sup>	1.80 <sup>b</sup>	3.10 <sup>b</sup>	6.03 <sup>a</sup>	143.0 <sup>a</sup>	157.8 <sup>a</sup>	32.50	50.00 <sup>a</sup>
		SE	0.11	0.07	0.06	0.038	1.10	1.24	0.38	1.10
	0.075	X	4.80 <sup>b</sup>	1.84 <sup>b</sup>	2.96 <sup>b</sup>	5.78 <sup>b</sup>	131.8 <sup>b</sup>	125.2 <sup>b</sup>	32.66	45.20 <sup>b</sup>
		SE	0.11	0.07	0.06	0.034	1.20	0.97	0.59	0.97
	0.150	X	5.92 <sup>a</sup>	2.10 <sup>a</sup>	3.82 <sup>a</sup>	4.56 <sup>c</sup>	120.2 <sup>b</sup>	155.8 <sup>a</sup>	32.68	52.00 <sup>a</sup>
		SE	0.19	0.07	0.15	0.031	1.50	1.02	0.39	1.00
	0.225	X	4.80 <sup>b</sup>	1.78 <sup>b</sup>	3.02 <sup>b</sup>	5.33 <sup>d</sup>	120.2 <sup>b</sup>	157.0 <sup>a</sup>	31.16	45.00 <sup>b</sup>
		SE	0.14	0.07	0.11	0.026	1.50	1.64	0.83	0.84
	0.300	X	5.00 <sup>b</sup>	1.84 <sup>b</sup>	3.16 <sup>b</sup>	5.58 <sup>c</sup>	143.3 <sup>a</sup>	155.8 <sup>a</sup>	31.82	45.20 <sup>b</sup>
		SE	0.12	0.06	0.07	0.032	2.62	1.02	0.42	0.80

\* = significant (P<0.05)\*\* = highly significant (P<0.01) and ns = not significant.

Means in the same column bearing the same superscripts did not differ from each other; otherwise they differ (P≤0.05 or 0.01).

**Table 3. Means ( $\bar{X}$ ) of blood picture traits in broiler chicks as affected by phenyllactic acid**

Items	Traits				
	RBC's ( $\times 10^6$ )/mm <sup>3</sup>	Hb (g/dl)	PCV (%)		
	**	**	**		
<i>Phenyllactic acid</i> (%)	0.000	X	2.20 <sup>b</sup>	6.10 <sup>b</sup>	26.00 <sup>c</sup>
		SE	0.08	0.16	1.14
	0.075	X	2.60 <sup>a</sup>	7.08 <sup>a</sup>	29.20 <sup>ab</sup>
		SE	0.12	0.12	0.73
	0.150	X	2.50 <sup>a</sup>	6.86 <sup>a</sup>	28.00 <sup>bc</sup>
		SE	0.06	0.08	0.45
	0.225	X	2.65 <sup>a</sup>	7.00 <sup>a</sup>	30.80 <sup>a</sup>
		SE	0.06	0.16	0.86
	0.300	X	2.66 <sup>a</sup>	7.22 <sup>a</sup>	31.00 <sup>a</sup>
		SE	0.04	0.10	0.89

\*\* = highly significant (P<0.01).

Means in the same column bearing the same superscripts did not differ from each other; otherwise they differ (P≤0.001).

**Table 4. Means ( $\bar{X}$ ) and standard errors (SE) of some immunological traits in broiler chicks as affected by phenyllactic acid.**

Items	Traits					
	WBC's ( $\times 10^3$ )/mm <sup>3</sup>	Lymphocyte <sup>1</sup> (%)	Bursa of Fabricius <sup>2</sup> (%)	Spleen <sup>2</sup> (%)		
	**	*	*	**		
<i>Phenyllactic acid</i> (%)	0.000	X	19.2 <sup>c</sup>	69.40 <sup>b</sup>	0.188 <sup>b</sup>	0.123 <sup>d</sup>
		SE	1.06	0.72	0.059	0.068
	0.075	X	35.2 <sup>b</sup>	70.54 <sup>ab</sup>	0.199 <sup>ab</sup>	0.135 <sup>cd</sup>
		SE	1.59	0.67	0.062	0.066
	0.150	X	43.8 <sup>a</sup>	71.48 <sup>ab</sup>	0.215	0.143 <sup>bc</sup>
		SE	1.02	0.60	0.086 <sup>a</sup>	0.059
	0.225	X	40.2 <sup>a</sup>	72.60 <sup>a</sup>	0.219	0.160 <sup>b</sup>
		SE	1.36	0.73	0.059 <sup>a</sup>	0.057
	0.300	X	42.0 <sup>a</sup>	71.28 <sup>ab</sup>	0.201 <sup>ab</sup>	0.178 <sup>a</sup>
		SE	1.41	0.77	0.078	0.055

\* = significant (P<0.05) and \*\* = highly significant (P<0.01).

Means in the same column bearing the same superscripts did not differ from each other; otherwise they differ (P≤0.05 or 0.001).

<sup>1</sup>Values presented as a percentage of total white blood cells count.

<sup>2</sup>Values presented as a percentage of body weight.

## بعض التأثيرات الفسيولوجية والمناعية لحمض الفينيل أسيتك أسيد في بداري اللحم

علي عبدالرازق سيد أحمد عسكر

قسم الدواجن، كلية الزراعة، جامعة الزقازيق، الزقازيق

تم دراسة بعض صفات الدم الخلوية والكيميائية والمناعية تحت تأثير حمض الفينيل أسيتك أسيد. تم تقسيم ١٥٠ ككتوت غير مجنس عمر يوم عشوائيا (وزن الجسم ٤٧.٩ جم) الى خمس مجموعات تجريبية. استخدمت طيور المجموعة الاولى كمجموعة مقارنة غذيت علي العليقة القاعدية في حين زودت عليقة الاربع مجموعات الاخرى بحامض الفينيل أسيتك أسيد بمستويات مختلفة ٠.٠٧٥، ٠.١٥٠، ٠.٢٢٥ و ٠.٣٠٠%. محتوى سيرم الدم من البروتين الكلي والالبيومين والجلوبيولين اختلف معنويا نتيجة المعاملة بالحامض حيث سجلت الطيور المعاملة ب ٠.١٥٠% من حامض الفينيل أسيتك أسيد اعلي قيمة لبروتينات الدم. لم يلاحظ اختلاف في المحتوي من بروتينات الدم بين المجموعات الاخرى او بينها وبين مجموعة المقارنة. اثرت المعاملة بالحامض بدرجة عالية علي محتوي الدم من الدهون الكلية والكوليسترول. سجلت الطيور المعاملة ب ٠.٠٧٥، ٠.١٥٠، ٠.٢٢٥% من الحامض مستويات منخفضة من الكوليسترول عن تلك الخاصة بالمجموعات الاخرى بما فيها مجموعة المقارنة وسجلت الطيور المعاملة ب ٠.١٥٠% من الحامض اقل مستوي من الدهون الكلية مقارنة بالمجموعات الاخرى بما فيها مجموعة الكنترول. تأثرت مستويات الجلوكوز معنويا بالمعاملة بالحامض حيث سجلت الطيور المعاملة ب ٠.٠٧٥% من الحامض اقل قيمة من جلوكوز الدم بينما وجدت اعلي التركيزات في طيور المقارنة. تأثير المعاملة بالحامض عل انزيمات الكبد كان معنويا فقط علي ALT. اظهرت الطيور المعاملة ب ٠.٠٧٥، ٠.٢٢٥ او ٠.٣٠٠% من الحامض مستويات منخفضة من هذا الانزيم مقارنة بتلك الخاصة بالطيور المعاملة ب ٠.١٥٠% من الحامض او تلك الخاصة بطيور المقارنة. تأثر عدد كرات الدم الحمراء ومحتوي الدم من الهيموجلوبين والحجم الخلوي للدم معنويا بالمعاملة بالحامض. اظهرت الطيور المعاملة ب ٠.٣٠٠% من الحامض اعلي القيم لهذه الصفات مقارنة بتلك الخاصة بمجموعة المقارنة والتي سجلت اقل القيم. أدت المعاملة بالحامض الي زيادة عدد كرات الدم البيضاء والنسبة المئوية لعدد الخلايا الليمفاوية والوزن النسبي لكيس فابريشيوس والطحال بدرجة معنوية. سجلت الطيور المعاملة ب ٠.١٥٠، ٠.٢٢٥ و ٠.٣٠٠% من الحامض قيم تقريبا متماثلة لهذه الصفات وأعلي من تلك الخاصة بالطيور المعاملة ب ٠.٠٧٥% من الحامض او الخاصة بطيور المقارنة. معظم النتائج في هذه الدراسة تشير الي ان المعاملة بالحامض قد اظهرت تحسن ملحوظ في بعض الصفات الخلوية والكيميائية والمناعية للدم في بداري اللحم وخاصة المعاملة منها ب ٠.١٥٠% من حامض الفينيل أسيتك أسيد.