Effects of phenyllactic acid on production performance, egg quality parameters, and blood characteristics in laying hens

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Primary Audience: Nutritionists, Researchers, Veterinarians

SUMMARY

Organic acids have been widely used as feed additives to replace antibiotics in livestock feeds. Data on the use of phenyllactic acid (PLA) are lacking. The effects of PLA on production performance, egg quality parameters, and blood characteristics in laying hens were studied in a 35-d experiment. A total of 240 ISA Brown 36-wk-old layers were divided into the following 4 treatments: 1) control (basal diet), 2) control + 0.1% PLA, 3) control + 0.2% PLA, and 4) control + 0.3% PLA. Although supplementing the diet with PLA did not affect ADFI and FE, it linearly improved egg production rate, eggshell breaking strength, and Haugh units. Egg weight, eggshell thickness, and egg yolk color were not significantly altered by supplementing the diet with PLA. White blood cell, red blood cell, total protein, and albumin concentrations were higher in the control + 0.1% PLA and control treatment groups ($P < 0.05$), whereas the groups fed the control + 0.2% PLA and control + 0.3% PLA diets had greater ($P < 0.05$) lymphocyte concentrations. In conclusion, PLA may exert some positive effects to the immune system and egg production over the short term. It may be beneficial to supplement the diets of laying hens with PLA in the absence of antibiotics. This experiment involved a small experimental sample and a short time, so the beneficial effects of PLA should be studied further in commercial farms over the long term.

Key words: blood characteristic, egg quality, laying hen, phenyllactic acid, production performance

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DESCRIPTION OF PROBLEM

Historically, antibiotics have been used in the poultry industry and by poultry veterinarians to enhance growth and FE while reducing disease. Unfortunately, the use of antibiotics in bird diets to promote growth may lead to the development of antibiotic-resistant pathogens that can be transferred to humans [1, 2]. Additionally, it is possible for antibiotic residues to be present in bird products, especially eggs. These residues may pose a potential health hazard to humans [3, 4]. The inclusion of antibiotics in the diets of livestock has been prohibited in the European Union since January 2006 (Regulation 1831/2003/EC). Current candidate replacements include organic

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acids, probiotics, prebiotics, plant extracts, and enzymes. Organic acid treatments composed of individual acids and blends of several acids have been found to exert antimicrobial activities similar to those of antibiotics. Organic acids can improve bird performance because of their antimicrobial activity, which improves protein and energy digestibility by reducing 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 [5].

Phenyllactic acid (**PLA**) is an organic acid that is produced as a by-product of phenylalanine metabolism [6]. Phenyllactic acid has recently been identified in cultures of *Lactobacillus plantarum*, and it is known to inhibit the growth of a wide variety of fungal strains [7, 8] and pathogenic bacteria, such as several strains of *Staphylococcus aureus* and *Escherichia coli* [9]. In addition, PLA had less odor than other organic acids, such as acetic acid. Phenyllactic acid may also have potential as an alternative to antibiotics when applied to the diets of animals. However, few studies have been conducted to evaluate the application of PLA or its salts to the diets of poultry.

This study was conducted to evaluate the potential of adding PLA to the diets of laying hens to function as an alternative to antibiotic growth promoters. Specifically, we evaluated the effects of PLA on egg production performance, egg quality parameters, and blood characteristics.

MATERIALS AND METHODS

Experimental Design, Birds, and Diets

All birds used in this trial were handled in accordance with guidelines set forth by the Dankook University Committee on Laboratory Animal Care. A total of 240 ISA Brown laying hens [10] were randomly assigned to 1 of 4 treatments in an experiment that was conducted with hens from 36 to 41 wk of age. Hens were weighed individually at the onset of the experiment and then assigned to treatments in a randomized block design based on house location and BW. There were 10 replicates for each treatment, with 3 adjacent cages (2 hens/cage, 38.1-cm width × 50-cm length × 40-cm height) representing a replicate. The hens were housed in a windowless and environmentally controlled room that was maintained at 26°C with a daily lighting schedule of 16L:8D. There was 1 empty cage between every 3 cages. Hens were provided with free access to water and feed through the nipple of an automatic drinker and a common trough feeder, respectively. The composition of the corn and soybean meal basal diet is shown in Table 1. Dietary treatments consisted of the corn and soybean meal basal diet supplemented with 0 (control), 0.1 (**PLA1**), 0.2 (**PLA2**), or 0.3% PLA (**PLA3**) [11]. The experimental diets were formulated in accordance with recommendations in the breeder's manual for ISA Brown hens and to meet NRC [12] recommendations, and were provided in mash form. Before the beginning of the experiment, 24-wk-old hens were purchased and provided with a basal diet for a 7-wk adjustment period, and birds not laying well were replaced.

Sampling and Measurements

Ten birds were selected at random from each treatment (1 hen per replication) on the initial and the final day of the experiment. Blood samples were collected from the wing vein of the same laying hens using a sterilized syringe and both nonheparinized tubes and K_3EDTA vacuum tubes [13] to obtain serum and whole blood, respectively. The blood samples were then centrifuged at 2,000 × *g* at 4°C for 20 min within 1 h of collection to separate the serum. The total protein and albumin in the serum were analyzed using an automatic biochemistry blood analyzer [14]. The IgG was analyzed using nephelometry [15]. The concentrations of white blood cells (**WBC**), red blood cells, and lymphocytes in the whole blood samples were determined using an automatic blood analyzer [16].

Daily records of egg production and weekly records of feed consumption were maintained. Egg production was expressed as average hen-day production. A total of 30 salable eggs (no shell defects, cracks, or double yolks) were randomly collected at 1700 h from each treatment (3 per replicate, n = 30) on a weekly basis and used to determine the egg quality at 2000 h the same day. Eggshell breaking strength was evaluated using an eggshell force gauge, model

Table 1. Basal diet composition (as-fed basis)

Item	Diet
Ingredient, %	
Corn	50.4
Soybean meal (CP 46%)	18.7
Wheat grain	10.0
Corn gluten meal	2.00
Wheat bran	5.00
Animal fat	4.40
Limestone	7.50
Tricalcium phosphate (18% P)	1.40
Salt	0.30
DL-Met (50%)	0.10
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Calculated value, %	
AME, kcal/kg	2,904
CP	15.00
Lys	0.80
Met + Cys	0.92
Ca	3.25
P	0.61
Analyzed composition, % of DM	
CP	15.65
Lys	0.89
Met + Cys	1.02
Ca	3.43
P	0.68

[1]Provided per kilogram of diet: 125,000 IU of vitamin A; 2,500 IU of vitamin D_3; 10 mg of vitamin E; 2 mg of vitamin K_3; 1 mg of vitamin B_1; 5 mg of vitamin B_2; 1 mg of vitamin B_6; 15 mg of vitamin B_{12}; 500 mg of folic acid; 35,000 mg of niacin; 10,000 mg of Ca-pantothenate; and 50 mg of biotin.

[2]Provided per kilogram of diet: 8 mg of Mn (as MnO_2); 60 mg of Zn (as $ZnSO_4$); 5 mg of Cu (as $CuSO_4 \cdot 5H_2O$); 40 mg of Fe (as $FeSO_4 \cdot 7H_2O$); 0.3 mg of Co (as $CoSO_4 \cdot 5H_2O$); 1.5 mg of I (as KI); and 0.15 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

II [17]. Eggshell thickness was measured on the large end, equatorial region, and small end, respectively, using a dial pipe gauge [18]. Finally, egg weight, egg yolk color, and Haugh units (**HU**) were evaluated using an egg multitester [19].

Statistical Analysis

The average weekly data, except blood parameters (5 wk for each of the treatments), were statistically analyzed by ANOVA using the GLM procedure of SAS for a randomized complete block design [20]. The differences between the means of groups were separated by Duncan's multiple range test. The significance level was set at $P < 0.05$. In addition, orthogonal comparisons were conducted using polynomial regression to measure the linear and quadratic effects of increasing dietary concentrations of supplemental PLA. For the blood data, an analysis of covariance was carried out using the GLM procedure, with the initial data used as the covariate. Least squares means adjusted for the covariate were used for comparison.

RESULTS AND DISCUSSION

Production Performance

The ADFI and FE (kg of feed/kg of eggs) were not affected by the supplementation of PLA (Table 2), whereas egg production in the PLA treatments was improved by 1.55% (PLA1), 2.64% (PLA2), and 2.69% (PLA3) compared with hens in the control group (linear effect, $P < 0.001$), respectively. The difference in egg production from d 0 to 35 was linearly increased as the PLA levels increased ($P = 0.005$). The expected effect may have occurred because of the antimicrobial activity of the organic acid. If so, this may have improved the total nutrient digestibility, thereby improving the feed efficiency and the rate of egg laying. Previous studies have shown that organic acids exerted an antimicrobial effect in the gastrointestinal tract and improved the FCR of broilers [21–23]. Furthermore, an in vitro evaluation of PLA revealed that it had a wide bactericidal activity against 19 strains of bacteria at levels of less than 10 mg/mL. Dieuleveux et al. [9] found that in vitro D-3-PLA inhibited the growth of *Listeria monocytogenes* cultured in liquid medium or ultra-high-temperature whole milk and the growth of several strains of *Staph. aureus*, *E. coli*, and *Aeromonas hydrophila* on solid medium. These effects were not observed in the present study, which may be mainly attributed to its short duration. On the other hand, because this is the first experiment in which PLA was applied as an acidifier in poultry, the levels we adopted, without any reference to rely on, may not have been adequate to exert significant effects. Further investigations are needed to determine the proper level of PLA and the pH and microbial status of the gastrointestinal system.

Egg Quality Parameters

The egg quality parameters did not decrease in response to the addition of PLA to the diet

(Table 3). The eggshell strength was improved at 8.73 and 7.08% when 0.2% PLA was included (quadratic effect, $P < 0.05$) on d 21 and 35, respectively, whereas the difference between d 0 and 35 also increased with the highest value in the PLA2 treatment (quadratic effect, $P < 0.05$). Eggshells are composed of approximately 94 to 97% calcium carbonate, and calcium plays a pivotal role in the breaking strength and shell thickness of an egg [24]. Mroz et al. [25] suggested that organic acid had a beneficial effect on the apparent ileal and total tract digestibilities, as well as calcium digestibility in growing pigs. This may be because the addition of organic acids to the diet lowered diet acidity. Lowering the dietary pH may increase the solubility of minerals, thereby increasing the effectiveness of calcium. This has yet to be determined in hens fed PLA diets.

In this study, dietary supplementation with PLA significantly increased shell breaking strength, but not eggshell thickness. Um and Paik [26] found that an improvement in breaking strength was not necessarily reflected in the shell thickness. The results of other studies that reported similar findings have suggested that shell thickness is primarily dependent on calcium aggregation as calcium carbonate, whereas shell hardness primarily depends on the texture, which is a result of the composition of calcium carbonate, organic materials, and trace minerals in the shell [27, 28].

The HU, which describes the height of thick albumen relative to the weight of the egg, is generally used as an indicator of storage quality. Although few data are available regarding the effect of organic acid on the HU index, the results of the present study indicate that feeding hens diets containing 0.2% PLA can improve the HU score.

Blood Characteristics

White blood cells and total protein concentrations were significantly increased in the PLA1 treatment group, whereas the red blood cell level was significantly higher ($P < 0.05$) in the PLA1 treatment than the PLA2 and PLA3 treatments (Table 4). The PLA1 treatment also had higher

Table 2. Effects of phenyllactic acid (PLA) supplementation on ADFI, FE, egg production, and egg weight (36 to 41 wk of age)[1]

						P-value	
Item	Control	PLA1	PLA2	PLA3	SEM	Linear	Quadratic
ADFI, g							
0 d	105	112	110	108	3.62	0.598	0.844
21 d	101	100	102	103	2.34	0.952	0.625
0–35 d	101	105	106	106	1.49	0.574	0.876
Difference[2]	−4	−7	−4	−2	2.35	0.325	0.508
FE, g of feed/g of egg							
0 d	2.13	2.18	2.03	2.10	0.19	0.777	0.694
21 d	1.88	1.81	1.84	1.87	0.24	0.954	0.218
0–35 d	1.95	1.98	1.93	1.99	0.17	0.433	0.937
Difference[2]	−0.18	−0.20	−0.10	−0.11	0.12	0.659	0.792
Egg production, %							
−7–0 d	87.87	88.87	88.09	87.90	1.25	0.357	0.771
21 d	87.19	89.38	90.14	89.89	0.94	0.428	0.293
0–35 d	88.42	89.79	90.59	90.80	1.48	<0.001	0.176
Difference[2]	0.55	0.92	2.50	2.90	1.02	0.005	0.387
Egg weight, g							
−7–0 d	58.41	58.96	59.41	58.56	1.69	0.664	0.491
21 d	59.26	58.95	60.14	59.69	0.78	0.467	0.920
0–35 d	59.95	60.93	60.20	60.63	1.24	0.162	0.920
Difference[2]	1.54	1.97	0.97	2.75	2.08	0.690	0.671

[1]Each mean represents 10 observations per treatment. Diets: control = basal diet; PLA1 = basal diet with PLA 0.1%; PLA2 = basal diet with PLA 0.2%; PLA3 = basal diet with PLA 0.3%.

[2]Difference between d 0 and 35.

($P < 0.05$) albumin levels than the PLA3 treatment. In addition, the lymphocyte level was significantly higher in layers fed the PLA3 diet than in those provided the other dietary treatments. Because of the large SEM, the differences between d 0 to 35 were not statistically significant in any of the parameters we measured.

The effect of organic acids on immune responses has not been well documented. However, it is well known that intestinal microorganisms are necessary for the development of the gut immune system. The results of the present study suggested that PLA, like other organic acids, could stimulate the immune system to some extent.

Total protein and albumin concentrations are generally responsible for the protein status of the blood. Laborde et al. [29] suggested that the albumin fraction of total protein is more reflective of the long-term protein status because of the additional nonalbumin fractions of serum total protein. The nonalbumin fraction of total protein consists of globulin and other fractions, such as fibrinogen, peptide hormones, enzymes, and amino acids [30], which have beneficial functions for the body. In addition, previous studies have suggested that the IgG concentration increases as the total protein concentration increases [31]. However, in this study the IgG concentrations were below the detection limit in all treatment groups. In addition, Lumeij et al. [32] demonstrated that a significant correlation existed between total calcium and total protein concentration in birds, which may serve as evidence that the total calcium concentration was improved by the addition of PLA. On the other hand, most of the blood profile traits, except lymphocytes, were not continually improved by the levels PLA. It is likely that there were no dose responses of PLA on blood parameters. Because only a limited number of immune-related parameters were measured, further research is necessary to evaluate additional components such as IgG, IgA, and IgM.

CONCLUSIONS AND APPLICATIONS

1. Linearly improved egg production was observed in the PLA-supplemented groups when compared with the control group.

Table 3. Effect of phenyllactic acid (PLA) supplementation on egg quality parameters (36 to 41 wk of age)[1]

						P-value	
Item	Control	PLA1	PLA2	PLA3	SEM	Linear	Quadratic
Eggshell breaking strength, kg/cm^2							
0 d	4.09	4.13	4.15	4.16	0.17	0.312	0.214
21 d	4.01	4.12	4.36	4.25	0.36	0.767	0.455
35 d	4.38	4.48	4.69	4.50	0.16	0.450	0.045
Difference[2]	0.29	0.35	0.54	0.34	0.09	0.214	0.027
Eggshell thickness, 10^{-2} mm							
0 d	39.0	38.0	38.0	39.0	0.57	0.681	0.964
21 d	37.0	38.0	39.0	39.0	0.74	0.301	0.375
35 d	38.0	38.0	38.0	39.0	0.87	0.764	0.685
Difference[2]	−1	0	0	0	0.48	0.281	0.967
Yolk color							
0 d	8.33	8.33	8.60	8.33	0.15	0.797	0.282
21 d	8.40	8.42	8.60	8.53	0.20	0.185	0.757
35 d	8.67	8.87	8.94	8.73	0.26	0.820	0.785
Difference[2]	0.34	0.54	0.34	0.40	0.18	0.254	0.975
Haugh units							
0 d	84.6	86.2	84.5	86.4	1.52	0.381	0.204
21 d	84.4	86.6	87.4	88.1	2.08	0.591	0.794
35 d	84.6	86.8	88.2	89.9	2.13	0.421	0.054
Difference[2]	0.04	0.62	3.75	3.53	0.65	0.141	0.047

[1]Each mean represents 10 observations per treatment. Diets: Control = basal diet; PLA1 = basal diet with PLA 0.1%; PLA2 = basal diet with PLA 0.2%; PLA3 = basal diet with PLA 0.3%.

[2]Difference between d 0 and 35.

Table 4. Effect of phenyllactic acid (PLA) supplementation on blood characteristics (36 to 41 wk of age)[1]

Item	Control	PLA1	PLA2	PLA3	SEM	*P*-value of the model	*P*-value	
							Linear	Quadratic
White blood cells, 10^5, n/mm^3								
0 d	3.34	3.70	3.23	3.44	0.33	0.61	0.440	0.211
35 d	3.37^{b}	3.84^{a}	3.20^{b}	3.33^{b}	0.15	0.01	0.334	0.421
Difference	0.03	0.14	−0.03	−0.11	0.95	—	0.520	0.591
Red blood cells, 10^6, n/mm^3								
0 d	2.39	2.51	2.39	2.47	0.11	0.77	0.634	0.730
35 d	2.40^{ab}	2.49^{a}	2.31^{b}	2.33^{b}	0.05	0.01	0.097	0.474
Difference	0.01	−0.02	−0.08	−0.15	0.68	—	0.597	0.710
Lymphocytes,[2] %								
0 d	68.7	61.5	68.5	65.5	2.67	0.35	0.654	0.140
35 d	65.0^{b}	67.7^{b}	69.8^{b}	72.7^{a}	3.23	0.39	0.110	0.961
Difference	−3.71	6.19	1.29	7.20	12.36	—	0.610	0.771
Total protein, g/dL								
0 d	5.50	5.78	5.20	5.22	0.29	0.35	0.251	0.394
35 d	5.08^{b}	5.44^{a}	5.43^{ab}	5.18^{ab}	0.27	0.03	0.800	0.227
Difference	−0.43	−0.34	0.23	−0.04	0.48	—	0.594	0.441
Albumin, g/dL								
0 d	2.28	2.34	1.98	2.04	0.11	0.15	0.384	0.691
35 d	2.13^{ab}	2.34^{a}	2.20^{ab}	2.19^{b}	0.10	0.04	0.594	0.028
Difference	−0.14	0	0.22	0.15	0.51	—	0.347	0.156

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Each mean represents 10 observations per treatment. Diets: Control = basal diet; PLA1 = basal diet with PLA 0.1%; PLA2 = basal diet with PLA 0.2%; PLA3 = basal diet with PLA 0.3%.

[2]Values are presented as a percentage of total WBC count.

2. The egg breaking strength and HU values were significantly higher in the group that received feed amended with 0.3% PLA.
3. Phenyllactic acid can partially improve the profile of blood characteristics, such as lymphocytes and WBC in the short term.
4. It may be beneficial to supplement the diets of laying hens with PLA in the absence of antibiotics. However, more research regarding the effects of PLA on laying hens is still necessary.

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