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Effect of Phytase and/or Citric Acid on Some Blood Biochemical Parameters and Organs Morphology in Broiler Chicks (Part II.)

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Abstract

Part II is a continuation of the previous study (part I) using the same experimental groups. It was carried out to investigate the effect of phytase and citric acid (CA) either alone or in combination on some blood traits and organ morphology in male broiler chicks from 8-40 days of age. The results revealed that all supplemented groups did not show any significant changes in hematological parameters. CA did not result in any significant changes in all measured blood biochemical parameters. Phytase did not induce any significant changes in AST activity, total cholesterol, HDL, triglycerides, urea, creatinine and glucose concentrations, while it increased ALT activity both in the overall mean and during most of the sampling periods. Supplementation of broilers feeds with mixed additives produced a significant increase in AST activity at 40 days of age, while total cholesterol concentration was significantly decreased. Triglycerides level was significantly increased at 24 days of age. Total protein level was significantly increased than phytase and CA supplemented groups at 24 days of age and than control group at 32 days of age. Blood glucose level was significantly increased at 24 days of age vs all experimental groups and at 32 days of age vs control and phytase supplemented groups. Histopathological examination revealed that the liver of chicks supplemented with phytase showed mild vacuolization in the cytoplasm of hepatocytes. The spleen showed lymphoid hyperplasia in white pulb of all supplemented groups, and the pancreas showed activation and proliferation in islets of Langerhans.

Key words: broilers, CA, Phytase, blood parameters, histopathological changes.

Introduction

Chickens are lacking or limited in phytase, the enzyme that is necessary for breaking down of the phytate molecule and subsequent release of phytate bound phosphorus (P), Ca, protein and other nutrients (O'Dell et al., 1972). Phosphorus (P) is a micro mineral in broiler nutrition. In growing broilers, besides its requirement for proper bone development, it is also involved in almost all metabolic processes. Poor P availability results in decreased productivity and poor health status. P availability from plant derived feeds is affected by an anti-nutritional factor "phytate", which forms a variety of insoluble salts with most of the minerals including P, calcium, magnesium, zinc and copper. So phytate is responsible for considerable nutrient losses (Khalid et al. 2013). Phytase has the ability to break down undigested phytic acid in grains and oilseeds releasing digestible calcium and phosphorus in monogastric animals (Ravindran et al., 1999; Applegate and Angel, 2004). Microbial phytase was found to positively influence nutrients such as amino acids and phosphorus to be advantageous (Yi et al., 1996; Namkung and Leeson, 1999). It may also reduce the degree of foam formation, thus alter the utilization of energy of fats in the intestines (Ravindran, 2001) Some studies however, showed that phytase does not degrade dietary phytate efficiently and thus the negative influence of phytate on protein digestibility is not completely removed by phytase supplementation (Khalid et al., 2013). Addition of organic acid to phytase supplemented diets was found to improve the efficacy of phytase. Several studies support the statement that addition of citric acid (CA) to broilers ration improved weight gain (Afsharmanesh and Pourreza, 2005; Nezhad et al., 2007), increased feed consumption (Moghadam et al., 2006) and improved feed efficiency (Abdel-Fattah et al., 2008). Its addition to broilers feed was also found to increase retention of P (Liem et al., 2008; Brenes et al., 2013), tibial ash (Rafacz-Livingston et al., 2005; Martinez-Amezcua et al., 2006) and toe ash (Atapattu and and Nelligaswatta, 2005) in broiler chicks. It was also found to decrease pH of caecal digesta, crop and gizzard (Andrys et al., 2003) and intestine (Denli et al., 2003) and improve immune response in broiler chicks (Rahmani and and Speer, 2005; Abdel-Fattah et al., 2008). In continuation to our previous study on Phytase and CA both phytase and CA were found to improve growth performance of broilers and to liberate the phytate bound P, making P more

available to the bird. The present study was designed to determine the effect of phytase, CA and their combination on some blood traits and organs morphology in broiler chicks.

Materias And Methods

2.1. Birds

Two hundreds, one-day-old chick (Cobb strain) were used in this study. Chicks were kept on lighting regimen of 23 hrs light daily (Mousa, 2008) and temperature were kept at 31°C using air warmers during the first 2 weeks then decreased gradually till 29°C according to recommendations obtained from the providing company. The chicks were handled carefully to avoid any pain or harm. Chicks were fed on starter ration during the first two weeks, grower ration during the next two weeks and finisher ration during the rest of the experiment (table 1). At 8 days of age, it could be able to distinguish between male and female chicks. Females were excluded and the experiment was continued on males only.

2.2. Feed Additives

2.2.1 Phytase enzyme (Microtech 5000): A concentrated phytase enzyme (5000 U/gm). It was a gift from Dr.Hamed El-Banna, Delta Vet.Cennter, Cairo, Egypt.

2.2.2 Citric acid: Citric acid used in this study was obtained from El Gomhoria Company, Egypt.

2.3. Experimental Design

On the 8th day of age, 120 male chicks having approximately the same weight (500 - 633g average body weights) were chosen and randomly divided into 4 equal groups of 30 birds each with 3 replicate cages having 10 birds in each. They were treated as follows: The first group was kept as a control group and fed on a basal ration without any additives. The second group was fed on a basal ration to which phytse powder was added at a dose equal to 0.1g / kg as recommended by Delta Vet Company. The third group was fed on a basal ration to which 1%citric acid (10g/kg) was added . The fourth group was fed on a basal ration to which phytase powder (0.1g / kg) and citric acid (10g / kg) were added.

Table 1.Composition of percentage and calculated nutrients profile of the basal diets.

Ingredients %	Starter (1-15 day)	Grower (16-30 day)	Finisher (31-40 day)
Yellow corn	51.70	56.15	61.15
Corn gluten meal	5.00	5.00	5.00
Soybean meal (44% CP)	37.30	31.50	25.90
Soy oil	2.20	3.50	4.00
Dicalcium phosphate	1.60	1.60	1.70
Limestone	1.40	1.45	1.44
Common salt	0.40	0.40	0.40
DL-Methionine	0.05	0.05	0.06
L-Lysin	0.05	0.05	0.05
Vitamin&mineral premix*	0.30	0.30	0.30
Calculated analysis:			
ME (Kcal/kg)	2951.80	3049.55	3124.07
Crude protein%	23.20	21.29	19.00
Crude fat%	6.00	6.92	8.00
Crude fiber%	4.50	4.80	5.20
Calcium%	1.00	1.00	1.00
Non-phytate phosphorus%	0.45	0.45	0.45

*Per kg premix: 1 200 000 IU-vit. A, 350 000 IU - vit.D_3, 4 000 mg - vit. E, 250 mg - vit.B_1, 800 mg - vit.B_2, 600 mg - vit. B_6, 3.2 mg-vit. B_{12}, 450 mg - vit. K_3, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, 40 mg Co.

2.4. Sampling

2.4.1 Blood Samples: Blood samples for biochemical parameters were collected from 10 chicks in each group at 16, 24 and 40 days of age by slaughtering. Sera were obtained and stored at -20°C till assays were carried out. Except for glucose which was measured immediately after sample collection. Samples for hematological parameters were obtained on

EDTA (0.1 mg/5 ml blood) at 24 and 40 days of age.

2.4.2 Tissue Samples: At the end of the experiment 5 birds from each group were slaughtered. Tissue samples from liver, spleen and pancreas were obtained for histopathological examination.

1.5. Measured parameters

2.5.1 Hematological parameters: Red blood cells (RBCs) were counted under the microscope by using improved double Neubaur haemocytometer (Natt and Herrick, 1952), the microhematocrite method (Schalm et al., 1975) was used in determination of haematocrit value (HCT), haemoglobin was measured by the cyanomethemoglobin method (Pilaski, 1972) using reagent obtained from Diamond Diagnostics, El Gomhoria Company, Egypt, Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration(MCHC) were calculated.

2.5.2 Biochemical parameter: Alkaline phosphatase activity (ALP) was estimated by the kinetic UV method of (Wenger et al., 1984). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Creatinine were estimated by the colorimetric-kinetic methods of (Murray et al., 1984). Cholesterol was estimated by the colorimetric method of (Meiattini et al., 1978). Triglycerides were estimated by the colorimetric method of (Fossati and Prencipe, 1982). Glucose was estimated by the colorimetric method of (Burtis and Ashwood, 1999). All parameters were measured in the serum using kits obtained from Spinreact Diagnostic Company and purchased from New Star Company in Kasr El-Ainy Street.

2.5.3 Histopathological examination: At the end of the experiment 5 birds from each group were slaughtered and Autopsy samples were taken from the liver, spleen and pancreas, fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns slidge microtome.The obtained tissue section were collected on glass slides, deparaffinised and stained by hematoxylin and eosin stains for histopathological examination through the electric light microscope (Banchroft et al., 1996).

2.6 Statistical analysis

All Data were presented as Mean ± Standard error. They were subjected to one way analysis of variance test (ANOVA), followed by the Tukey-Kramer multiple comparison test using statistical analysis system program (Instat-3).

Results

3.1 Effect on haematological parameters

Table 2) represents the effect of phytase and/or citric acid on red blood cells count (RBCs), haemoglobin concentration (Hb), haematocrit value (HCT), Mean corpuscular Hb (MCH), mean corpuscular volume (MCV) and mean corpuscular Hb concentration at 24 and 40 days of age. No significant changes were recorded in all measured haematological parameters between the four experimental groups.

Table (2): Effect of phytase and/or citric acid on blood parameters

Paramerters	RBCs		Hb		HCT		MCH		MCV		MCHC	
Age/day / Group	24	40	24	40	24	40	24	40	24	40	24	40
Control	3.94 ± 0.17	3.91 ± 0.17	10.14 ± 0.43	10.06 ±0.43	30.49 ± 1.33	30.20 ± 1.30	25.40 ± 0.21	25.52 ±0.10	78.42 ± 0.89	76.92 ± 0.19	32.27 ±0.34	31.57 ± 0.37
Phytase	4.04 ± 0.16	4.06 ± 0.14	10.34 ± 0.40	10.40 ±0.36	31.02 ±1.22	31.21 ±1.08	25.54 ±0.16	25.56 ±0.10	78.64 ±0.82	76.96 ±0.15	32.18 ±0.23	31.78 ± 0.29
Citric acid	3.89 ± 0.16	3.89 ± 0.16	9.9 ± 0.41	9.95 ±0.41	29.84 ±1.24	29.84 ±1.24	25.63 ±0.15	25.31 ±0.08	79.06 ±0.67	76.58 ±0.16	32.20 ±0.26	31.13 ± 0.33
Mixed additives	3.98 ± 0.17	3.97 ± 0.17	10.17 ± 0.44	10.17± 0.43	30.52 ±1.33	30.51 ± 1.28	25.12 ±0.26	25.46 ±0.10	77.51 ±1.47	76.72 ±0.17	32.21 ±0.34	31.31 ± 0.35

• Each value is expressed as Mean ± Standard Error

3.2 Effect on blood chemistry

3.2.1 Aspartate aminotransferase (AST): Table (3) shows that there were no significant differences between control and all supplemented groups in the activities of AST at 16, 24 and 32 days of age. However, at 40 days of age AST activity was significantly higher in group supplemented with mixed additives than control and other supplemented groups. There were no significant differences in the overall mean of AST activity between all experimental groups.

Table (3): Effect of phytase and/or citric acid on AST activity (U/L) in the serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	132±4.9	122.0±5.9	135.0 ± 2.4	134.4±5.4
24	126.6±6.7	160.4±8.9	148.0±19.9	185.6±21.0
32	182.2±15.3	157.8±1.4	130.6±17.5	133.0±16.0
40	115.8±21.2^{a}	141.5±13.5	121.4±3.8^{b}	188.0±4.5^{ab}
Overall mean	139.6±9.8	146.5±7.2	133.8±6.6	159.5±9.7

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

3.2.2. Alanine aminotrasferase (ALT): Data presented in table (4) indicate that ALT activity was significantly higher in phytase supplemented group than control and other supplemented groups during most of the sampling periods. No significant differences were recorded in ALT activity between control group and groups supplemented with citric acid and mixed additives all over the sampling periods. The overall mean of ALT activity was also significantly higher in group supplemented with phytase vs control and other supplemented groups.

Table (4): Effect of phytase and/or citric acid on ALT activity (U/L) in serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	4.0±0.3^{a}	11.0±0.6^{abc}	7.2±1.3^{b}	6.2±0.9^{c}
24	7.8±1.0	7.0±0.5^{a}	7.0±0.6^{b}	10.5±0.9^{ab}
32	7.0±0.4^{a}	10.4±0.9^{abc}	6.2±0.8^{b}	4.8±0.5^{c}
40	7.9±1.4^{a}	13.5±1.8^{abc}	6.2±0.9^{b}	7.0±1.0^{c}
Overall mean	6.7±0.5^{a}	10.5±0.7^{abc}	6.6±0.4^{b}	7.1±0.6^{c}

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

3.2.3. Total cholesterol: It is known from table (5) that there were no significant differences in the overall means of cholesterol levels between control and all supplemented groups. No significant differences in total cholesterol level were also recorded between control and all supplemented groups during most of the sampling periods.

Table (5): Effect of phytase and/or citric acid on total cholesterol level (U/L) in the serum of chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	129.4±2.9	122.2±6.8	109.6±3.	122.4±5.7125.0
24	131.4±5.2	150.8±5.0^{a}	114.0±6.6^{ab}	151.0±3.7^{b}
32	156.8±6.7	170.8±10.1	163.6±1.8	152.8±12.1
40	175.6±14.2^{a}	198.8±4.2^{bc}	153.3±2.2^{b}	131.3±3.2^{ac}
Overall mean	146.7±6.0	160.6±7.2^{a}	135.1±5.7^{a}	139.4±4.4

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different.

3.2.4. High density lipoprotein (HDL): Data presented in table (6) revealed that there were no significant differences in HDL concentration between experimental groups during all sampling periods.

Table (6): Effect of phytase and/or citric acid on HDL level (mg/dl) in the serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	8.9±1.1	8.8±1.0	10.5±0.7	8.2±1.1
24	4.9±1.0	4.0±0.5	3.3±0.2	3.2±0.2
32	7.8±1.0	8.2±1.7	13.3±0.5	11.2±1.9
40	4.0±0.4	6.0±1.3	3.6±0.9	4.8±1.0
Overall mean	6.4±0.6	6.8±0.7	7.7±1.0	6.8±0.9

- Each value is expressed as Mean ± Standard Error

3.2.5 Ttriglycerides: As shown in table (7) that there were no significant differences in the overall means of triglycerides levels between control and all supplemented groups. No significant differences in the triglycerides level were also recorded between control and all supplemented groups during most of the sampling periods. Group supplemented with mixed additives exhibited the highest triglyceride level during most of the sampling periods.

Table (7): Effect of phytase and/or citric acid on triglycerides level (mg/dl) in the serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	193.9±7.1	176.0±14.7	188.2±12.6	214.4±23.0
24	205.2±3.4^{a}	180.0±2.8^{b}	201.0±8.7^{c}	281.8±9.4^{abc}
32	196.0±6.8	169±11.9^{ab}	216.8±4.1^{a}	205.2±5.8^{b}
40	201.0±6.4	216.0±5.4^{ab}	173.2±12.0^{a}	181.0±3.5b
Overall mean	199.3±2.9	181.0±7.1^{a}	195.2±5.9	220.9±10.4^{a}

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different

3.2.6 Urea and creatinine: As shown from tables (8, 9), there were no significant differences in the overall mean of serum urea and creatinine between all experimental groups. No significant differences were also detected between all experimental groups during most of the sampling periods.Table (8): Effect of phytase and/or citric acid on urea level (mg/dl) in the serum of chicks.

Table (8): Effect of phytase and/or citric acid on urea level (mg/dl) in the serum of chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	9.6±0.2	8.5±0.2	7.5±0.7	8.6±0.7
24	12.0±1.1	11.6±1.2	10.4±1.4	11.2±0.4
32	11.9±1.5	13.0±1.4	10.4±1.6	9.3±1.4
40	10.4±1.6	17.2±2.1	14.2±0.7	15.7±2.7
Overall mean	11.0±0.6	12.6±1.0	10.6±0.8	11.2±1.0

- Each value is expressed as Mean ± Standard Error.

Table (9): Effect of phytase and/or citric acid on creatinine level (mg/dl) in the serum of chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	0.32±0.04	0.56±0.04	0.46±0.02	0.50±0.03
24	0.38±0.04	0.48±0.02^{a}	0.30±0.03^{a}	0.36±0.04
32	0.43±0.02	0.48±0.04	0.32±0.06	0.40±0.03
40	0.36±0.04	0.30±0.03	0.3±0.03	0.40±0.03
Overall mean	0.43±0.03	0.45±0.03^{a}	0.36±0.02^{a}	0.42±0.02

- Each value is expressed as Mean ± Standard Error

3.2.7. Total protein: Total protein concentration was significantly higher in group supplemented with mixed additives than phytase and citric acid supplemented groups at 24 days of age and than control group at 32 days of age (Table 10).

Table (10): Effect of phytase and/or citric acid on total protein level (g/dl) in the serum of chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	6.5±0.4	7.2±0.3	6.1±0.5	6.2±0.3
24	8.3±0.2	7.4±0.1[a]	7.5±0.2[b]	8.6±0.4[ab]
32	7.2±0.5[a]	7.8±0.4	8.7±0.5	9.4±0.3[a]
40	7.7±0.3	7.6±0.4	7.0±0.3	7.2±0.3
Overall mean	7.5±0.3	7.3±0.2	7.3±0.3	7.9±0.3

- Each value is expressed as Mean ± Standard Error

3.2.8. Glucose: Table (11) demonstrates that the highest blood glucose level was recorded in the group supplimented with mixed additives. It was significantly higher than control and other experimental groups at 24 days of age and significantly higher than phytase supplemented group at 32 days of age. There was no significant difference in the overall mean of blood glucose level between all experimental groups except for group supplemented with mixed additives which exhibited a significantly higher blood glucose level than phytase supplemented group.

Table (11): Effect of phytase and/or citric acid on glucose level (g/dl) in the serum of broiler chicks

Age/days	Control	Phytase	Citric acid	Mixed additives
16	204.1±14.8	181.0±7.3	182.4±16.1	206.7±9.7
24	160.3±6.2[a]	145.1±6.2[b]	148.1±9.4[c]	211.9±15.6[abc]
32	130.7±5.0[a]	136.3±11.7[b]	174.7±12.5	212.6±14.2[ab]
40	216.6±10.4	237.6±10.1	246.5±6.7	228.9±5.2
Overall mean	204.2±6.8	181.1±8.2[a]	192.6±8.6	215.5±5.4[a]

- Each value is expressed as Mean ± Standard Error
- Means having the same letter in the same raw are significantly different

3.3 Histopathological findings

Figures 1-12 show the results of histopathological examination of the liver, spleen and pancreas of all experimental groups at the end of the experiment.

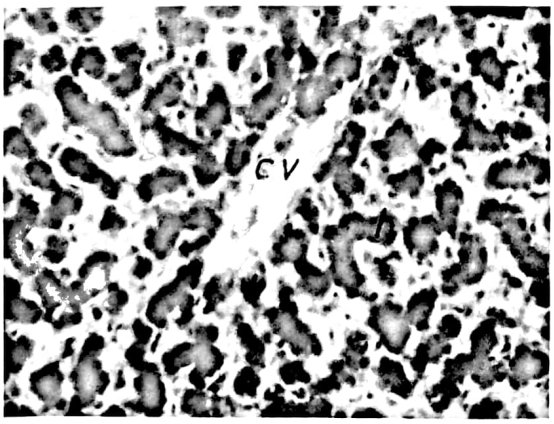

Figure 1.Liver of control chicks (H&E, X 80). Showing normal histopathological structure of the central vein (CV) and surrounding hepatocytes (h).

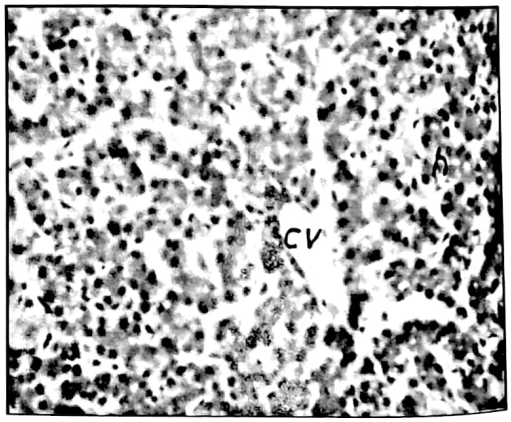

Figure 2. Liver of phytase supplemented group (H&E, X 80).Showing mild vacuolization in the cytoplasm of the hepatocytes (h)

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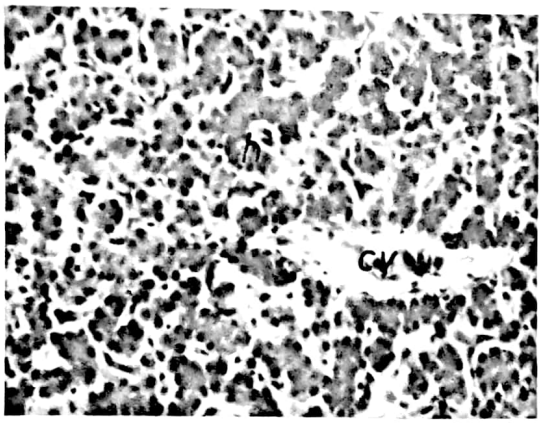
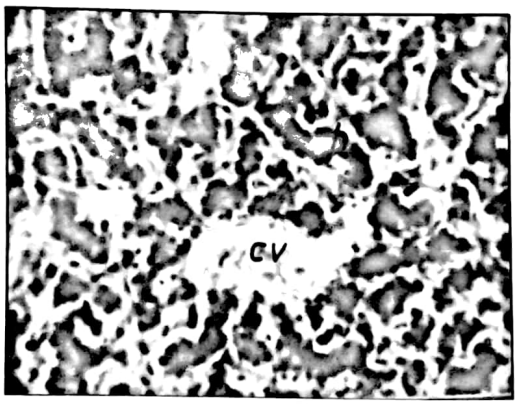


Figure 4.Figure 3. Liver of group supplemented with mixed additives (H&E, X 80). Showing intact normal histological structure of the central vein (CV) and surrounding hepatocytes (h) .

Figure 4.Figure 3. Liver of group supplemented with mixed additives (H&E, X 80). Showing intact normal histological structure of the central vein (CV) and surrounding hepatocytes (h) .



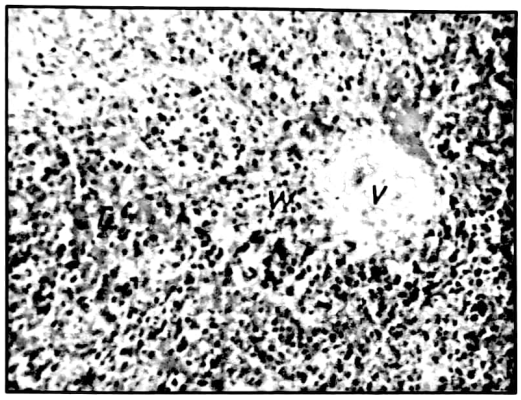


Figure 5.Spleen of control chicks (H&E,X 80). Showing normal histological structure of the follicular blood vessels (v), white pulp (w) and red

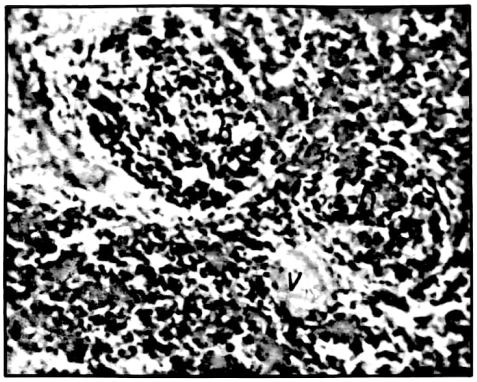


Figure 6. Spleen of phytase supplemented group (H&E, X 80). Showing lymphoid hyperplasia (h) in the white pulp (h)

Figure 7. Spleen of citric acid supplemented group. (H&E,X 80). Showing lymphoid hyoerplasia in white pulp (h).

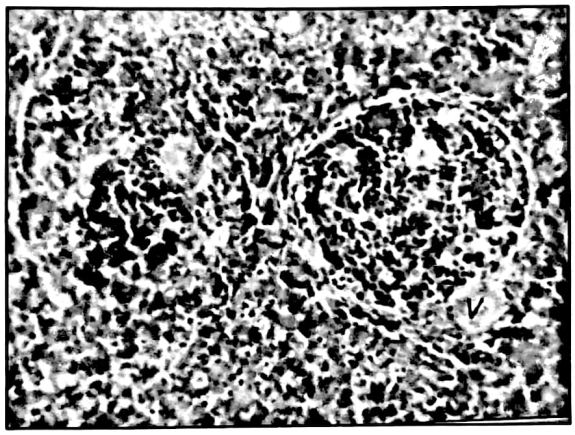


Figure 8. Spleen of group supplemented with mixed additives (H&E, X 80). Showing lymphoid hyperplasia in white pulp (h).

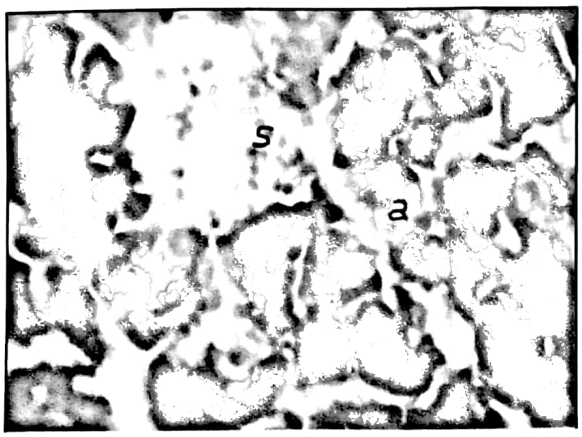


Figure 9. Pancreas of control chicks (H&E,X160) Showing normal histological structure of island of Langerhans cells (S) and surrounding exocrineacini (a)

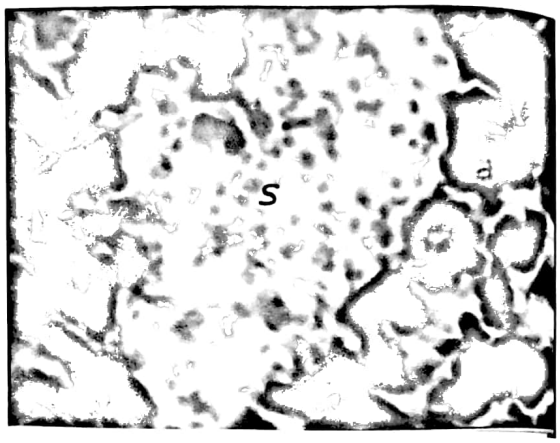


Figure 10. Pancreas of phytase supplemented group(H&E, X160). Showing activation and proliferation in island of Langerhans cells (S)

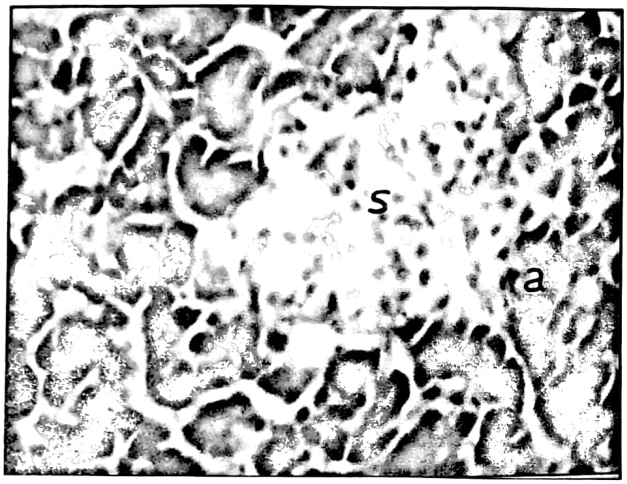


Figure 11. Pancreas of citric acid supplemented group (H&E, X160) Showing activation and proliferation in the islets of Langerhans cells (S)

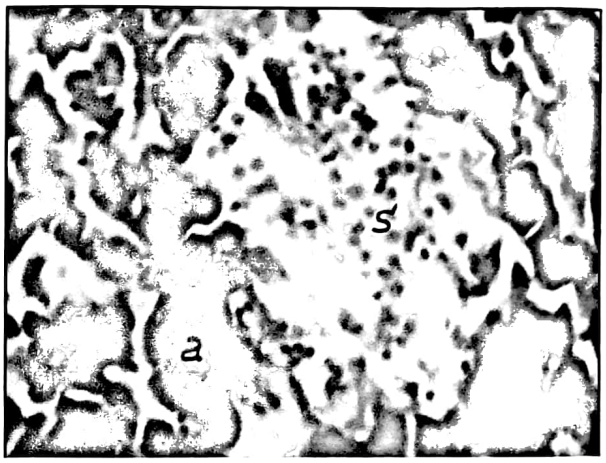


Figure 12. Pancreas of group supplemented with mixed additives (H&E, X160). Showing activation and proliferation in islets of Langerhans cells (S).

Discussion

Supplementation of broilers ration with CA in the current study did not result in any significant changes in hematological parameters (RBCs, Hb, HCT, MCH, MCV and MCHC) and all measured blood biochemical parameters (AST and ALT activities as well as total cholesterol, HDL, triglycerides, urea, creatinine, total protein and glucose levels). Generally the pattern of the results obtained during the current study indicated that addition of CA to broilers ration at the used dose is safe and did not alter hepatic or renal functions of broiler chicks. These results confirm the earlier findings of (Hernandez et al., 2006) that organic acid supplementation had no effect on the blood metabolites in broiler chickens. Moreover, (Abdel-Fattah et al., 2008) recorded that dietary supplementation of organic acids could be done up to the level of 3% in the diet of broiler chickens without causing any adverse effect on kidney and liver. These results are also in accordance with those of (Adil et al., 2010) who reported that supplementation of organic acids showed no significant differences in the concentration of serum glucose and cholesterol as well as the activities of ALT and AST. (Kalafova et al., 2014) also reported that consumption of CA during 42 days did not produce any significant changes in cholesterol and glucose levels in broiler chicks. Furthermore, (El-Afifi et al., 2001) reported no significant effect on serum lipids (triglycerides and cholesterol) after CA treatment. In the same concern (Nourmohammadi et al., 2010) reported

that CA did not significantly affect urea, triglycerides and total protein concentration as well as alkaline phosphatase activities. On the contrary (Abdel-Fattah et al., 2008) observed a tendency to increase total protein concentration and lipid metabolite reduction (cholesterol and triglycerides) by adding different levels of citric acid (0, 1.5 and 3%) to broiler feeds. However, (Kaya and Tuncer, 2009) after organic acid addition to broiler diets found a decrease in total protein and triglycerides and an increase in cholesterol concentration. The results of the present study does not agree with these observation. These contradictory results may be attributed to the difference in the dose of CA used and/or the type of feed offered. (Nourmohammadi et al., 2010) reported that addition of 3% CA significantly improved weekly growth performance, but adding 6% citric acid had a negative effect on weekly performance factor of broiler chicks.

Addition of phytase to broiler diets in the current study did not produce any significant changes in all measured hematological parameters. This finding is in agreement with the results reported by (Amer, 2014) who found that diets supplemented with phytase showed insignificant influence on all hematological parameters investigated (RBCs and WBCs count, H/L ratio, Hb concentration and PCV). Moreover, the inclusion of 0.1g phtase/kg broiler rations in the present study did not induce any significant changes in AST activity, total cholesterol, HDL, triglycerides, urea, creatinine and glucose concentrations. These results confirm the previous findings reported by (Shakmak, 2003; Al Harthi and El Deek, 2009; Amer, 2014) who found that phytase supplementation had no adverse effect on biochemical constituents of broilers plasma. Several studies support the statement that the addition of phytase to broiler standard ration (El-Deek et al., 2009; Jalali and Babaei, 2012) and low phosphorus ration (Mondal et al., 2007; Nuhriawangsa et al., 2011) did not significantly affect total protein concentration of chicks serum. Moreover, it was also reported that addition of phytase had no effect on blood glucose level of broiler chicks (Jozefiak et al., 2010; Aureli et al., 2011; Nuhriawangsa et al., 2011; Safamehr and Attarhoseini, 2011) and laying hens (Safamehr and Attarhoseini, 2011). In the same concern (Cowieson et al., 2011) reported that phytase did not produce any significant changes in blood glucose level of broiler chicks fed standard ration, while it significantly increased glucose level in broilers fed low calcium and phosphorus diet. Many investigators have also reported that addition of phytase to broiler diets had no effect on total plasma cholesterol (Jozefiak et al., 2010), HDL cholesterol (Jozefiak et al., 2010; Cowieson et al., 2013) On the other hand, the present results are contradictory to those of(Cowieson et al., 2013) who reported that phytase significantly reduced triglyceride level in broiler chicks fed standard ration and low phosphorus and calcium ration. These contradictory results might be due to the difference in the amount of available phosphorus, the amount of phytase added in the diet (Bingol et al., 2009) and/or the source of phytase used in the study. Meanwhile, phytase supplementation in the present study significantly increased ALT activity than control group and groups supplemented with CA and mixed additives both in the overall mean and during most of the sampling periods. Contrary to these results (Viveros et al., 2002) reported that dietary phytase addition decreased serum ALT activity by 27.9% in broilers fed non phytate phosphor diet. These conflicting results might be due to the difference in the type of phosphorus and/or type and dose of phytase used in the broiler feeds. (Bogin and Israeli, 1976) in their earlier study stated that plasma ALT activity has been reported to be low in all tissues of chickens. In the same concern (Viveros et al., 2002) added that specific diagnostic value of ALT in birds is poor. On the other hand (Cheesborough, 1991; Johnston, 1999) stated that ALT is principally found in the liver and is regarding as being more specific than AST for detecting liver cell damage. Moreover, (Zantop, 1997) reported that ALT activity often increases due to damage in many tissues. These differences among studies indicated that further investigation is recommended to know the exact effect of ALT

and consequently the effect of phytase supplementation on broilers health.

No available literature could be found regarding the effect of adding a mixture of phytase and CA to broiler feeds on hematological parameters and blood biochemical parameters of broiler chicks. However, the present study revealed that supplementation of broiler feeds with mixed additives did not produce any significant changes in the all measured hematological parameters, ALT activity, HDL, urea and creatinine levels both in the overall means and during all sampling periods. On the other hand AST activity was significantly increased at 40 days of age vs control and CA supplemented group. These results indicated that supplying of broiler feeds with mixed additives should not exceed 24 days to avoid any hepatic damage. (Lewandowski and Harrison, 1986) reported that activity of AST is not liver specific in birds, whereas, (Viveros et al., 2002) stated that elevated AST activity usually indicates liver or muscle damage. Total cholesterol concentration was significantly decreased at 40 days of age vs control group. This decrease might be due to the stimulatory effect of mixed additives for conversion of cholesterol to bile acid in order to facilitate digestion and absorption of nutrients.(Russell, 2003) reported that in the liver the conversion of cholesterol to bile acid is the principal pathway of cholesterol catabolism providing sufficient amount of bile acids as detergent for the digestion and absorption of lipid nutrients and removing excess cholesterol from the body. Low cholesterol levels may also be due to the effect of mixed additives on the conversion of cholesterol to fatty acids and cholesterol in order to increase the metabolic energy (Robak et al., 2004). Moreover, (Sato et al., 2006) stated that generally total cholesterol are broken down into fatty acids and glycerol as a source of metabolic energy. Triglycerides level was significantly increased at 24 days of age vs control and other supplemented groups. (Woyengo et al., 2010) found that addition of CA to the phytase supplemented diet significantly increased ambient metabolic energy (AME) which may have been due to increased phytic acid hydrolysis.. Total protein level was significantly higher in group supplemented with mixed additives at 24 days of age vs phytase and CA supplemented group and at 32 days of age vs control group. It was also appeared from the present study that blood glucose level was significantly increased at 24 days of age vs all experimental groups and at 32 days of age vs control and phytase supplemented group. The increase in blood glucose level might be due to the improvement in glucose digestion induced by adding a mixture of phytase and CA to broilers feed. Several mechanisms by which phytic acid reduces the digestibility of energy yielding nutrients have been proposed. They include 1) binding to protein in the stomach and small intestine (Maenz, 2001; Selle et al., 2006), 2) binding to carbohydrate and lipids in small intestine (Selle and Ravindran, 2007) and 3) binding to endogenous enzymes and metal cofactor of enzymes involved in hydrolysis of energy yielding molecules (Thompson et al., 1987).

No available literature could be found regarding the effect of supplementation of broilers feed with phytase and CA on the histopathological changes in the liver, spleen and pancreas of supplemented birds. In the present study histopathological examination of the liver revealed that supplementation of broilers feeds with CA and mixed additives for 40 days did not result in any histopathological changes in the liver of supplemented birds, whereas the liver of chicks supplemented with phytase showed mild vacuolization in the cytoplasm of hepatocytes. This confirms the obtained results regarding the effect of phytase on ALT activity in the serum of broiler chicks and supports the openion that further investigation is required using different types and doses of phytase to determine the exact effect of phytase supplementation on broilers health. Meanwhile, histopathological examination of the spleen in the present study showed lymphoid hyperplasia in white pulb of all supplemented groups. This lymphoid hyperplasia might be due to the immune stimulant effect of he used feed additives. (Ghahri et al., 2012) reported that

addition of phytase in the diet increased antibody production against Newcastle disease virus in broilers from 14-42 days of age. In the same concern (Amer, 2014) added that supplementation of broilers diet with phytase resulted in a reduction in blood H/L ratio and attributed this to the enhancement of immune system of broilers. Moreover, some investigators reported that supplementation of CA in broilers diet has appositive effect on immune status of broilers (Rahmani and and Speer, 2005; Abdel-Fattah et al., 2008; Liem et al., 2008; Chowdhury et al., 2009).

Histopathological examination of the pancreas showed activation and proliferation in islets of Langerhans. This activation might be due to stimulatory effect of phytase alone and in combination on pancreatic hormones. Addition of phytase to broilers feed was reported to increase blood insulin concentration in broilers (Cowieson et al., 2013) and pigs (Johnston et al., 2004). No available literature could be found regarding the effect of phytase and CA on other pancreatic hormone and this point needs further study.

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