Comparative studies on the panzyme and citric acid on the immunomodulatory, some selective biochemical and growth promoting parameters in broiler chicks

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Abstract: One hundred and fifty, one day old, chicks were divided into 5 groups and reared for 6 weeks. Group I: control group fed on balanced commercial ration. Groups II, III, IV and V: treated groups fed on balanced commercial ration supplied with 0.5%,1% citric acid and 0.05%, 0.1% panzyme for 6 weeks respectively. Immunological, some biochemical and growth performance parameters were investigated at 3rd and 6th week. Also, parts from the liver, kidney, intestine, spleen, thymus and bursa were obtained for histopathological examination. Our results revealed significant lymphocytic leukocytosis in the group fed 0.05% panzyme all over the experimental period. There was significant decrease in the level of IL10 in the 0.05% panzyme fed group, and on the contrary, there were significant increases in the 0.5, 1% citric acid groups in comparison with the control group. While, IL6 and TNF-α were significantly increased in panzyme groups at 3 weeks, while, at 6 weeks there were significant decrease in citric acid groups. The bacteriological analysis of the caecal content revealed significant increase in the total bacterial and *coliform* count in the citric acid fed groups with significant decrease in the count of *lactobacillus spp*. All the experimental groups showed no effect on serum albumin and uric acid levels. Whereas, significant decrease in AST and creatinine was recorded in the 0.05% panzyme group. Furthermore, 0.05% panzyme group showed significant increase in TP, globulin and glucose along with hypocholesterolemia in the 0.05% panzyme fed group when compared with the other groups of the experiment. The addition of 0.05% of panzyme to the diet of broilers results in improved growth with increased intestinal villus height. We could conclude that panzyme at the level 0.05% in the diet has a prospective effect on the growth performance, nonspecific and specific immune response in broilers.

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1. Introduction:

The poultry industry is one of the most important sources of protein all over the world today. However, commercial poultry production is challenged by microbial infections and unavailability of good quality feeds on a sustainable basis at stable prices **(Ohimain and Ofongo, 2012).** The biggest single expense in any system of poultry production is feed accounting for up to 70% of total production cost per bird. Poultry naturally produces enzymes to aid the digestion of feed nutrients. However, they do not have enzyme to break down fiber completely and need exogenous enzymes in feed to aid digestion. The benefits of using enzymes in poultry diets include not only enhanced bird performance and feed conversion but also less environmental problems due to reduced output of excreta **(Khattak *et al.*, 2006).** Feeding enzymes to poultry is one of the major nutritional advances in the last fifty years. Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, often because the animal cannot produce the necessary enzyme to degrade them. Nutritionists can help the animal by identifying these indigestible compounds and feeding a suitable enzyme **(Wallis, 1996).**

Furthermore, organic acids have a long history of being utilized as food additives to prevent food deterioration and extend the shelf life of perishable food ingredients **(Ricke, 2003)**. Their supplementation in broiler feeds, however, has shown conflicting results, in part because of the different organic acids, doses, microbial challenges, or evaluated responses that have been used in the published experiments **(Patten and Waldroup, 1988; Leeson *et al.*, 2005** and **Vieira *et al.*, 2008)**.

This study aims to compare the effect of dietary supplementation of citric acid and an enzyme preparation (panzyme)on the growth performance,

immunomodulatory effect beside the changes of serum biochemistry of broilers.

2. Material And Methods

1- Experimental chickens:

One hundred and fifty, one day old, apparent healthy chicks, Cobb breed were obtained from Ismailia- Masr Poultry Company Serapum City, Egypt. Chickens were reared in litter under standard environmental and hygienic conditions. Chickens were fed on a balanced ration full-fill the requirements according to **NRC (1994)**, free from antibacterial agents and water ad libitum. All chickens vaccinated at 5th day and 18th of age, with Hitchner and Lasota respectively, whereas at 14th and 24th day of age, vaccinated with Gumboro.

2-Dietary supplements:

1- Citric Acid Monohydrate (Monohydrate), C6H8.H2O, LOBA Chemie Pvt. Ltd. 107,Wodehouse Road, Mumbai 400005, India.

2- Enzyme preparation (Pan Zyme), Baytara for Pharmaceuticals Technology, under license of VTR Company, Sadat Industrial City.

*Pan Zyme is an enzyme preparation containing multiple enzymes: Xylanase 15.000.000 IU/kg; Acidic Protease 540.000 IU/kg; Neutral Proteinase 450.000 IU/kg and Cellulase 600.000 IU/kg.

3- Experimental design:

One hundred and fifty, one day old, apparent healthy chickens were classified into 5 groups, each of 30 chickens. **Group I:** control group fed on basal ration free from antimicrobial agents. **GroupII:** fed on basal ration supplied with citric acid at a level of 0.5% (5 gm citric acid / kg ration). **GroupIII:** fed on basal ration supplied with citric acid at a level of 1% (10 gm citric acid / kg ration). **GroupIV:** fed on basal ration supplied with panzyme at a level of 0.05% (0.5 gm citric acid / kg ration). **GroupV:** fed on basal ration supplied with panzyme at a level of 0.1% (1gm citric acid / kg ration).Six random samples of serum were taken from all experimental groups at 3rd and 6th weeks of the experiment for investigation.

I- Immunological Parameters:

1-Leukogram assay:

Parameter of the leukogram was determined according to standard techniques described by **Jain, (1986)** and **Terry, (1988)** which includes total leukocytes count (TLC) and differential leukocytes count (DLC). Blood films were stained by Giemsa stain for differential leukocytic count. The percentage and absolute value for each type of white cells were calculated according to **Feldman *et al.* (2000).**

2-Cytokines (Interleukins):

- **Chicken Interleukin 10** (IL-10) ELISA kit (My BioSource Co, San Diego, California, USA).
- **Chicken Interleukin 6** (IL-6) ELISA kit (MyBioSource Co, San Diego, California, USA).
- **Chicken Tumor Necrosis Factor α** (TNF-α) ELISA kit (MyBioSource Co, San Diego, California, USA).

3-Bacteriological analysis of caecal content:

At 21st and 42nd days, 3 birds from each group were picked up randomly and sacrificed. The fresh excreta of one caecum per bird were gently squeezed and aseptically collected in a sterile test tube.One gram of caecal content was suspended in a tube containing 9 ml sterile buffered peptone water; the tube was shaken thoroughly by an electric touch mixer, the samples were further diluted by serial tenfold serial dilutions (from 10^1 to 10^{10}) and one tenth milliliter (0.1ml) from each dilution was withdrawn and spread on nutrient agar, for total bacterial count of caecal content; De Man-Rogosa-Sharpe (MRS) agar**,** for *Lactobacilli* count incubated at 37 ˚C for 48 hours **(De Man *et al.*, 1960)** and macConkey agar, for pathogenic *Coliform* count of caecal content **(Roberts *et al.*, 1996** and **Guban *et al.*, 2006).** The counts were reported as CFU/gm of digesta.

II- Biochemical Parameters:

The biochemical tests were performed using commercial test kits to determine ALT and AST (Randox Co. UK), total proteins and albumin(STANBIO kits, Texas), cholesterol, glucose and uric acid (SPINREACT, Spain), creatinine "kinetic" (Human, Germany). A/G ratio was calculated according to **Kaneko *et al.* (1997).**

III- Growth Performance Parameters:

a) Body weight:

Each chick was weighed at the beginning of the experiment (one day old) and at the end of 3rd and 6th week of the experiment. Individual live body weight was summed and divided by the number of chickens of each group to obtain the average live body weight/ week **(Brady, 1968).**

b) Body weight gain, Feed consumption (FC) and Feed conversion ratio (FCR):

The gain in body weight per week, FC and FCR were calculated according to **Brady (1968).**

IV- Histopathological studies:

Specimens of liver, kidney, intestine, spleen, thymus and bursa of scarified birds from all groups were fixed in 10 % neutral formalin, embedded in paraffin, sectioned at 5-micron thickness and stained with Haematoxylin and Eosin for histopathological examination **(Bancroft *et al.*, 1990)**.

V-Histomorphometrical analysis of the intestine:

Two cm tissue samples from the middle length of duodenum were transected; ingesta washed away using normal saline and fixed in 10% buffered formalin. Tissues were dehydrated by transferring through a series of alcohols with increasing concentrations,

placed into xylol and embedded in paraffin. A microtome was used to make 5 cuts that were 5 µm and they were stained with hematoxylin-eosin. Using digital photography and light microscopy the photos were taken and morphometric analyses were performed by means of an image analysis program (Image J software). In each of the five sections taken from the tissues, the villus height and crypts depth were determined by examining randomly 6 villi and 6 crypts. Later, the average of 30 values obtained for each chick was taken **(Rezaian *et al.*, 2007)**.

VI-Statistical analysis:

Data collected from the leukogram, serum biochemical, immunological, bacteriological and histomorphometrical analysis of treated groups of chicks were statistically analyzed in compare to control group for the mean and standard error using statistical software program (SPSS for Windows, version 15, USA). Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance (P<0.05).

3. Results and Discussion:

The present leukogram results, showed significant lymphocytic leukocytosis in the group fed 0.05% panzyme all over the experimental period **(Table 1)**.These results were partially agreed with **Mohammadbeygi *et al.* (2012)** who found that enzyme supplementation at the level of (0.5% purified β-glucanase) in common carp (*Cyprinuscarpio*) fed on barley based diet for 8 weeks result in higher white blood cells than other treatments. **Khaksar *et al.*(2012)** found that addition of an enzyme preparation (Endofeed W) at 0.05% to the wheat-based diet improved immunity in terms of heterophil to lymphocyte ratio. The other groups fed citric acid and 0.1% panzyme were insignificantly changed in T.L.C and D.L.C all over the experiment. These results were similar to **Tollba (2010)** who stated that citric acid did not significantly affect the leukocytic count and their differential countsin broilers till 40 days. Also, results obtained by **Ebru *et al.* (2011)** and **Khajepour *et al.* (2011)** agreed with our results. Meanwhile, disagreed with **Debi *et al.* (2010)** who observed the highest lymphocyte counted was in the dietary treatment having 2.5% citric acid in New Zealand White rabbits.

Interleukins are biologically active glycoproteins derived primarily from activated lymphocytes and macrophages **(Elmslie *et al.*, 1991)**. Interleukin 10 is a protein that inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3 and TNF produced by activated macrophages and by helper T cells. Our results showed significant decrease in the level of IL10 in the 0.05% panzyme fed group, and on the contrary, showed significant increase in the 0.5% and 1% citric acid groups in comparison with the control group.

Interleukin6, also called B-cell stimulating factor 2, T-cell activation factor, beta, interferon, and hepatocyte stimulating factor, is a glycoprotein secreted by blood monocytes, activated T lymphocytes and tissue macrophages **(Bauer *et al.*, 1988).** Meanwhile, Tumor Necrosis Factor, also known as (TNF alpha),is a cytokine that has a wide variety of functions. It can cause cytolysis of certain tumor cell lines; it is a potent pyrogen, causing fever by direct action or by stimulation of interleukin-1 secretion; it can stimulate cell proliferation and induce cell differentiation under certain conditions**(**Vilcekand Lee, 1991).The present results of levels of both IL6 and TNF-α revealed significant increase in panzyme groups at 3 weeks, while, at 6 weeks there were significant decrease in 0.5% and 1% citric acid groups in comparison with the control**(Table 2)**. **Feng *et al.* (2004)** reported increases in immunity-related responses such as natural killer cell activity and serum antibody titers when supplementing diets of poultry with exogenous enzymes. Moreover, **Soltan (2009)** found that enzyme supplementation to broiler chicks for 6 weeks improved phagocytic activity, phagocytic index and immune organs (spleen, bursa and thymus gland) relative weights.Also, **Khaksar *et al.* (2012)** found that addition of Endofeed W (an enzyme preparation) at 0.05% for 42 days to the wheat-based diet improved immunity in terms of hypersensitivity response and heterophil to lymphocyte ratio as compared to the control group, also induced competitive exclusion efficiently, in terms of increase in *Lactobacillus* and *Bifidobacterium* and reduction in *E. coli* counts. But, these results disagree with **Cardinali *et al.* (2008)** who recorded the better immune response in rabbits of FormaXol diet (mixture of micro incapsulated formic and citric acids and essential oils) could be lied to higher values of serum bactericidal activity and lower values of lysozyme. Also disagree with **Haque *et al.*(2010)** stated that the lymphocyte cells associated with immunity in the lymphoid organs (caecal tonsil, bursa fabricius and ileum) of broilers were more densely populated, suggesting an increased level of innate immunity in the 0.5% citric acid group. Our results came in harmony with the histopathological results, where there was focal hyperplasia of lymphoid organs in the panzyme groups, but there was lymphoid depletion in the citric acid groups**(Figures 4,5, 6 and 7)**.

Whereas, the obtained data from the bacteriological analysis of the caecal content as shown in **table (3),** revealed significant increase in the total bacterial and *coliform* count found in 0.5% and 1% citric acid groups at 6 weeks with significant decrease in the count of *lactobacillus spp*. **Aydin *et al.* (2010)**

who found that 3% citric acid supplementation of broiler diets didn't reduce the numbers of pathogenic bacteria in the ileum. **Açıkgöz *et al.* (2011)** showed that drinking water acidification with formic acid (pH 4.5) did not provide beneficial effects on intestinal microflora and carcass contamination in male broilers at 42 day of age. However, our results disagreed with **Tollba (2010)** found decrease in the counts of pathogenic intestinal bacteria (i.e., total aerobic bacteria, *E. coli*, salmonella and staphylococci) in ileum, caecum or fecal matter in chicks treated with citric acid (2g/kg feed) till 40 days of age. Moreover, **Ghazalah *et al.* (2011)** found that feeding broilers 2% citric acid in the diet increased Lactobacillus count and Coliforms in caeca content. Meanwhile, the group supplemented with 0.05% panzyme expressed significant increase in the *lactobacillus spp.* count along the experiment. This was in accordance with **Ohimain and Ofongo (2013).**

Regarding the results of biochemical investigation as shown in **table (4)**, there were significantly decreased activities of AST in the panzyme fed groups with non-significant changes in other groups, while ALT was non-significantly changed in all groups. The reduced activities of ALT and AST enzymes observed might be due to improvement in the physiological condition of the liver and increase in the hepatic metabolic reserve **(Dobicki *et al.*, 2007)**. **Paul *et al.* (2010)** observed that enzyme 1 ml/L of drinking water supplementation significantly decreased AST and ALT values of broilers. Our results came in agreement with **Abdel-Fattah *et al.* (2008)** and **Agustin *et al.* (2003)**where ALT and AST were insignificantly altered in citric acid groups. Also, **Adil *et al.* (2010)** found that the addition of organic acids had no effect on the concentration of ALT and AST activities. Our results differed from **Yousefian *et al.* (2013)** and **Shehab *et al.* (2012)** who concluded the tested dietary enzyme had any significant effect on AST. **Abdel-Azeem *et al.* (2000)** demonstrated that the activity of AST was reduced in growing rabbits fed supplemental citric acid, although ALT was not significantly affected. Our pathological results showed normal histological hepatic structure, which was proved by our biochemical results **(Figure 2).**

Serum total proteins and globulin were significantly increased in the 0.05% panzyme fed group with non-significant increase in albumin all over the experiment. The increase in total protein in this group may be related to increased production of other serum protein fractions as globulin. Globulin level has been used as indicator of immune responses and source of antibody production. Our results agreed with **Alaeldein (2012)** and **Yousefian *et al.* (2013)**,but disagree with **Mohammadbeygi *et al.* (2012)** and **Shehab *et al.* (2012).** On the other hand, at 6 weeks age, total proteins and globulin were significantly decreased in groups fed citric acid. These results differed from those reported by **Ghazalah *et al.* (2011); Khajepour *et al.* (2011)** and **Brzóska *et al.* (2013).**

The inclusion of non-starch polysaccharides in the basic diet of monogastric animals has been reported to delay the intestinal absorption of glucose **(Sinha *et al.*, 2011).** The present results of glucose level showed hyperglycemia in the panzyme fed groups at 6 weeks, were compatible with **Yuan *et al.* (2008)** results who reported that enzyme inclusion in levels of 180 and 360 mg/kg significantly increased the amount of blood sugar due to breaking the NSPs to small residues of glucose. Similar results were achieved by **Ao *et al.*(2010)** and **Mohammadbeygi *et al.* (2012)**, but differed from the results recorded by **Gao *et al.* (2007 & 2008); Dingyuan *et al.* (2009)** and **Shehab *et al.* (2012)** who didn't find any significant effect on the concentration of blood glucose in enzyme supplemented group. On the other hand, insignificant changes in the glucose level were recorded in organic acids fed groups, this result agree with **Mahdavi and Torki (2009); Adil *et al.* (2010); Khajepour *et al.* (2011)** and **Brzóska *et al.* (2013)**.

Concerning cholesterol level, the present results revealed hypercholesterolemia in the o.5% citric acid group at 3 weeks, while at 6 weeks, 0.5%,1% citric acid groups were non-significantly changed in the cholesterol level. Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids **(Engberg *et al*., 2000)**. Our findings were in accordance with **Mahdavi and Torki (2009); Adil *et al*. (2010)** and **Brzóska *et al*. (2013)**. These results differ from **Abdel-Fattah *et al*. (2008);Moghadam *et al*. (2009)** and **Mohamed (2012)** who reported hypocholesterolemia with organic acids supplementation in poultry. Meanwhile, the 0.05% panzyme fed group showed hypocholesterolemia, but 0.1% panzyme group was non-significantly changed along the experiment. These findings differed with **Hajati (2010)** and **Mohammadbeygi *et al.* (2012)** who reported significant increase in cholesterol with the supplementation of enzyme preparations. **Gilliland *et al*. (1985)** hypothesized that *Lactobacillus* are able to incorporate cholesterol into their cellular membrane, thus, cholesterol assimilated by *Lactobacillus* which in turn reduce cholesterol absorption in the system, therefore reduced in blood.

The concentration of blood uric acid can accurately reflect the state of protein metabolism and balance of amino acids; the concentration is low when urea synthesis is reduced by improvement of dietary amino acid profile **(Borg *et al.*, 1987)**. Concerning the results of the present study, which revealed

insignificant change in the serum uric acid in all groups all over the experimental period, these results were compatible with those obtained by **Dingyuan *et al.* (2009); Mahdavi and Torki (2009); Shehab *et al.* (2012)** and **Yousefian *et al.* (2013)**,but incompatible with **Yin *et al.* (2001)** and **Hajati (2010)**. Meanwhile, groups fed panzyme revealed significant decrease in the level of creatinine at 6 weeks with non-significant changes in the organic acids fed groups. Creatinine is a chemical waste molecule that is generated from muscle metabolism, the kidneys maintain the blood creatinine in a normal range, the lower values derived that no muscular wastage which might have been possibly caused by inadequacy of protein in animals **(Polat *et al.*, 2011).** These results disagree with **Shehab et al., (2012)** who found no effect of enzyme supplementation on creatinine level in blood. **Abd-AlGadir *et al.* (2009)** observed significant gradual increase in the serum creatinine and urea nitrogen levels in the rats with increasing the dose of benzoic acid and benzoic with citric acid. The pathological results of the present work revealed normal histological architecture of both renal glomeruli and renal tubules**(Figure 3)**.

Table (1): Leukogram (Mean values ± S.E.) in chickens administrated citric acid and panzyme for 3 and 6 weeks.

Group	TLC 10^3 /µl	Heterophils 10^3/µl	Eosinophils10^3 /µl	Basophils 10^3 /µl	Lymphocytes 10^3/µl	Monocytes 10^3 /µl
At 3 weeks						
I	27.75^{b} ±1.25	5.82^{bc} ±0.53	1.58^{abc} ±0.43	0.08^{a} ±0.08	16.26^{bc} ±1.85	4.01^{a} ±0.66
II	29.50^{b} ±1.26	3.06^{c} ±0.82	1.11^{abc} ±0.30	0.00^{a} ±0.00	20.71^{ab} ±1.21	4.55^{a} ±0.62
III	29.23^{b} ±0.48	4.52^{c} ±0.81	0.29^{c} ±0.17	0.00^{a} ±0.00	18.93^{abc} ±0.70	5.51^{a} ±0.65
IV	35.75^{a} ±2.17	10.41^{a} ±0.38	1.92^{ab} ±0.51	0.00^{a} ±0.00	22.96^{a} ±1.17	4.72^{a} ±1.15
V	26.75^{b} ±1.93	8.7^{ab} ±1.28	0.72^{bc} ±0.04	0.00^{a} ±0.00	14.27^{c} ±1.60	3.05^{a} ±0.50
At 6 weeks						
I	32.00^{bc} ±3.08	8.37^{a} ±1.50	1.88^{a} ±0.34	0.00^{a} ±0.00	18.89^{bc} ±2.40	2.87^{ab} ±0.23
II	24.25^{c} ±2.53	6.92^{a} ±0.52	2.42^{a} ±0.80	0.00^{a} ±0.00	13.26^{c} ±1.73	1.65^{b} ±0.25
III	28.00^{bc} ±3.03	7.51^{a} ±1.46	2.02^{a} ±0.24	0.00^{a} ±0.00	16.30^{bc} ±1.40	2.18^{ab} ±0.72
IV	41.00^{a} ±1.08	9.10^{a} ±2.13	1.64^{a} ±0.29	0.00^{a} ±0.00	27.64^{a} ±2.30	2.63^{ab} ±0.97
V	33.25^{ab} ±1.55	9.68^{a} ±1.86	1.39^{a} ±0.43	0.00^{a} ±0.00	19.88^{b} ±1.17	2.30^{ab} 0.25

Means with the same letter in the same column are non significant at $P<0.05$

The present results of significantly increased body weight and weight gain; decreased total feed intake and feed conversion ratio in group fed 0.05% panzyme in comparison with control, as shown in **table (5)**, these results came in agreement with **Café *et al.* (2002); Abudabos (2010)** and **Ohimain and Ofongo (2013).** The improvement in feed conversion efficiency include alterations in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients **(Yeo and Kim, 1997).** Another study stated that, improvements in growth performance are frequently attributed to the composition and activity of the gut microflora which affects nutrient utilization**(Yang *et al.*, 2009),** this was proved by our results of bacteriological analysis**.** As well as, our results were proved by the histopathological findings, where panzyme showed higher intestinal villi with normal mucosa, also the intestinal glands showed normal histological morphology **(Figure 8)**. On the other hand, the 0.5%, 1% citric acid groups showed reduction in the growth, this may be due to the increased count of harmful bacteria and the reduction of the beneficial bacteria promoting the growth. These results were consistent with **Pinchasov and Elmalich (2000)** where depressed weight gain was observed with application of acetic acids in diets of broilers. Moreover, **Agustin *et al.* (2003); Abd El-Hakim *et al.* (2009)** and **Ao *et al.* (2009)** reported significant reduction in weight gain and feed consumption of broiler chicks with the diet acidification. Our results were proved by the histopathological studies, where mild focal degeneration of intestinal mucosa, hyperplasia of goblet cells, atrophy of intestinal glands, leukocytic infiltration along with shorter intestinal villi in the citric acid groups **(Figure 9)**. However, in some reports application of organic acids not only did not influence the performance of broilers but in some cases had

harmful effects **(Cave, 1984).** Our results differed from those obtained by **Afsharmanesh and Pourreza (2005); Abdel-Fattah *et al.*, (2008)** and **Islam *et al.*, (2008).** Pathogenic microbial flora in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat-soluble vitamins due to deconjugating effects of bile acids **(Engberg *et al.*, 2000).** This leads to depressed growth performance and to increased incidence of disease.

From the previous results, we could conclude that dietary supplementation of panzyme at a concentration 0.05%had a prospective effect on the immune response and growth performance in broilers compared with citric acid supplementation.

Table (2): Interleukins values (Mean values ± S.E) in chickens administrated citric acid and panzyme for 3 and 6 weeks.

Groups	IL10 Pg/ml	IL6 Pg/ml	TNF-α Pg/ml
At 3 weeks			
I	9.49^{a} ±0.42	39.75^{bc} ±2.91	17.10^{d} ±1.04
II	8.27^{ab} ±0.96	32.07^{c} ±4.88	15.80^{d} ±3.87
III	6.29^{b} ±0.62	48.20^{b} ±3.87	21.00^{cd} ±1.51
IV	5.37^{b} ±0.84	69.23^{a} ±1.32	39.40^{a} ±0.61
V	7.97^{ab} ±1.30	66.33^{a} ±4.58	29.27^{b} ±1.09
At 6 weeks			
I	8.23^{b} ±0.01	69.15^{a} ±1.29	45.00^{ab} ±1.62
II	11.30^{a} ±0.29	57.93^{bc} ±1.42	35.60^{c} ±1.60
III	12.07^{a} ±0.23	64.37^{ab} ±3.09	32.27^{cd} ±0.57
IV	7.11^{c} ±0.10	72.90^{a} ±0.80	48.83^{a} ±2.10
V	7.92^{bc} ±0.23	72.13^{a} ±5.92	42.20^{b} ±2.81

Means with the same letter in the same column are non significant at $P<0.05$

Table (3): Caecal bacteriological count (Mean values ± S.E) in chickens administrated citric acid and panzyme for 3 and 6 weeks.

Groups	T.B.C. 10^4 CFU/gm	Coliform 10^4 CFU/gm	Lactobacilli 10^5 CFU/gm
At 3 weeks			
I	4.3^{b} ±0.9	5.3^{b} ±0.9	27.7^{bc} ±2.2
II	8.0^{a} ±0.6	11.7^{a} ±2.7	26.0^{bc} ±2.6
III	5.0^{ab} ±1.2	4.3^{b} ±0.9	22.7^{bc} ±3.9
IV	2.7^{b} ±0.9	2.0^{b} ±1.0	46.7^{a} ±3.5
V	4.0^{b} ±0.6	5.7^{b} ±0.9	33.0^{b} ±2.6
At 6 weeks			
I	9.3^{b} ±0.9	6.0^{b} ±1.5	71.7^{b} ±1.5
II	26.0^{a} ±3.1	18.0^{a} ±3.2	34.3^{c} ±2.3
III	25.0^{a} ±2.9	17.7^{a} ±1.9	19.7^{d} ±1.5
IV	4.0^{b} ±1.2	2.7^{b} ±1.2	123.0^{a} ±1.7
V	8.3^{b} ±0.9	6.0^{b} ±1.2	74.0^{b} ±2.6

Means with the same letter in the same column are non significant at $P<0.05$

Table (4): Some serum biochemical parameters (Mean values ± S.E.) in chickens administrated citric acid and panzyme for 3 and 6 weeks.

Group	ALT U/L	AST U/L	T.P gm/dl	Alb. gm/dl	Glob. gm/dl	A/G ratio	Gluc. mg/dl	Choles. mg/dl	U.A. mg/dl	Creat. mg/dl
At 3 weeks										
I	36.84^{a} ±4.48	73.67^{a} ±8.01	2.40^{b} ±0.31	1.02^{a} ±0.29	1.38^{b} ±0.02	0.74^{a} ±0.21	321.07^{a} ±8.90	109.48^{bc} ±0.27	5.63^{ab} ±0.39	1.65^{a} ±0.20
II	33.94^{a} ±4.36	77.67^{a} ±6.36	2.42^{b} ±0.30	1.06^{a} ±0.22	1.36^{b} ±0.16	0.80^{a} ±0.17	341.06^{a} ±12.54	154.06^{a} ±5.06	4.67^{b} ±1.07	1.46^{ab} ±0.18
III	33.89^{a} ±1.05	65.67^{a} ±6.06	2.60^{b} ±0.48	0.85^{a} ±0.19	1.75^{b} ±0.34	0.50^{a} ±0.11	342.10^{a} ±8.55	98.12^{c} ±5.57	7.38^{a} ±0.95	1.22^{ab} ±0.09
IV	30.66^{a} ±0.10	45.33^{b} ±3.28	3.92^{a} ±0.10	1.26^{a} ±0.24	2.67^{a} ±0.29	0.51^{a} ±0.16	346.21^{a} ±9.86	78.20^{d} ±0.82	7.74^{a} ±0.13	1.26^{ab} ±0.05
V	33.16^{a} ±0.98	41.33^{b} ±4.10	2.47^{b} ±0.12	0.92^{a} ±0.11	1.55^{b} ±0.22	0.64^{a} ±0.17	341.72^{a} ±9.37	99.62^{c} ±4.50	6.20^{ab} ±0.37	1.05^{b} ±0.10
At 6 weeks										
I	30.93^{ab} ±0.70	57.00^{a} ±5.77	2.86^{b} ±0.12	1.28^{ab} ±0.12	1.59^{b} ±0.10	0.82^{a} ±0.11	315.01^{b} ±14.99	287.62^{ab} ±14.97	6.77^{abc} ±0.78	1.31^{ab} ±0.07
II	30.00^{b} ±1.44	61.67^{a} ±9.33	1.93^{c} ±0.13	1.02^{ab} ±0.13	0.91^{c} ±0.11	1.15^{a} ±0.21	285.58^{b} ±9.21	258.80^{ab} ±14.32	4.71^{c} ±0.15	1.61^{a} ±0.21
III	31.76^{ab} ±2.78	58.33^{a} ±8.67	1.98^{c} ±0.21	1.08^{b} ±0.09	0.90^{c} ±0.14	1.24^{a} ±0.17	296.99^{b} ±10.17	277.01^{ab} ±13.54	7.14^{ab} ±0.72	0.96^{bc} ±0.09
IV	28.66^{b} ±1.58	36.67^{b} ±5.78	4.02^{a} ±0.15	1.64^{a} ±0.18	2.38^{a} ±0.04	0.69^{a} ±0.09	475.12^{a} ±12.43	159.83^{c} ±14.56	8.06^{a} ±0.47	0.44^{d} ±0.07
V	29.06^{b} ±0.43	31.00^{b} ±2.65	3.05^{b} ±0.12	1.24^{ab} ±0.15	1.81^{ab} ±0.24	0.73^{a} ±0.20	407.59^{a} ±12.56	247.03^{b} ±12.00	7.38^{ab} ±0.53	0.56^{cd} ±0.13

Means with the same letter in the same column are non significant at $P<0.05$

Table (5): Growth performance parameters (Mean values ± S.E.) in chickens administrated citric acid and panzyme for 3 and 6 weeks.

Groups	Body wt gm	Body wt gain gm	Feed consumption gm/bird	FCR
At 3 weeks				
I	552.17^{b} ±12.61	507.53^{b} ±13.11	1000.00^{a} ±11.55	1.97^{a} ±0.05
II	523.17^{b} ±10.39	478.77^{b} ±10.26	800.00^{d} ±17.32	1.67^{b} ±0.04
III	529.00^{b} ±11.50	484.93^{b} ±11.58	826.67^{cd} ±14.53	1.71^{b} ±0.04
IV	610.00^{a} ±13.59	567.93^{a} ±13.87	870.00^{b} ±11.55	1.54^{c} ±0.04
V	522.00^{b} ±18.16	476.69^{b} ±18.14	850.00^{bc} ±11.54	1.79^{b} ±0.06
At 6 weeks				
I	1900.60^{b} ±11.80	1348.43^{a} ±23.03	2716.67^{a} ±17.64	2.03^{a}b ±0.09
II	1413.60^{e} ±27.12	890.43^{d} ±24.98	1816.67^{e} ±14.53	2.06^{ab} ±0.08
III	1721.20^{c} ±9.30	1192.20^{b} ±14.04	2333.33^{c} ±17.64	1.96^{b} ±0.02
IV	1989.00^{a} ±21.00	1379.00^{a} ±29.21	2430.00^{b} ±17.32	1.77^{c} ±0.04
V	1868.20^{b} ±19.27	1346.20^{a} ±24.16	2110.00^{d} ±23.09	1.57^{d} ±0.03

Means with the same letter in the same column are non significant at $P<0.05$

Table (6): Histomorphometrical parameters of the caecum (Mean values ± S.E.) in chickens administrated citric acid and panzyme for 3 and 6weeks.

Groups	At 3 weeks		At 6 weeks	
	Crypt depth (µm)	Villus height (µm)	Crypt depth (µm)	Villus height (µm)
I	248.26^{ab} ±6.40	1340.37^{bc} ±38.19	313.52^{b} ±13.28	1515.19^{c} ±10.29
II	249.41^{ab} ±4.68	1243.33^{d} ±27.93	399.60^{a} ±7.70	1280.56^{d} ±10.75
III	255.74^{a} ±6.85	1387.51^{b} ±15.41	316.84^{b} ±10.86	1134.85^{e} ±13.76
IV	223.57^{c} ±5.05	1593.24^{a} ±23.88	168.51^{c} ±4.72	2100.68^{a} ±4.71
V	253.49^{a} ±3.88	1375.23^{bc} ±22.62	293.48^{b} ±4.01	1576.42^{b} ±13.55

Means with the same letter in the same column are non significant at $P<0.05$

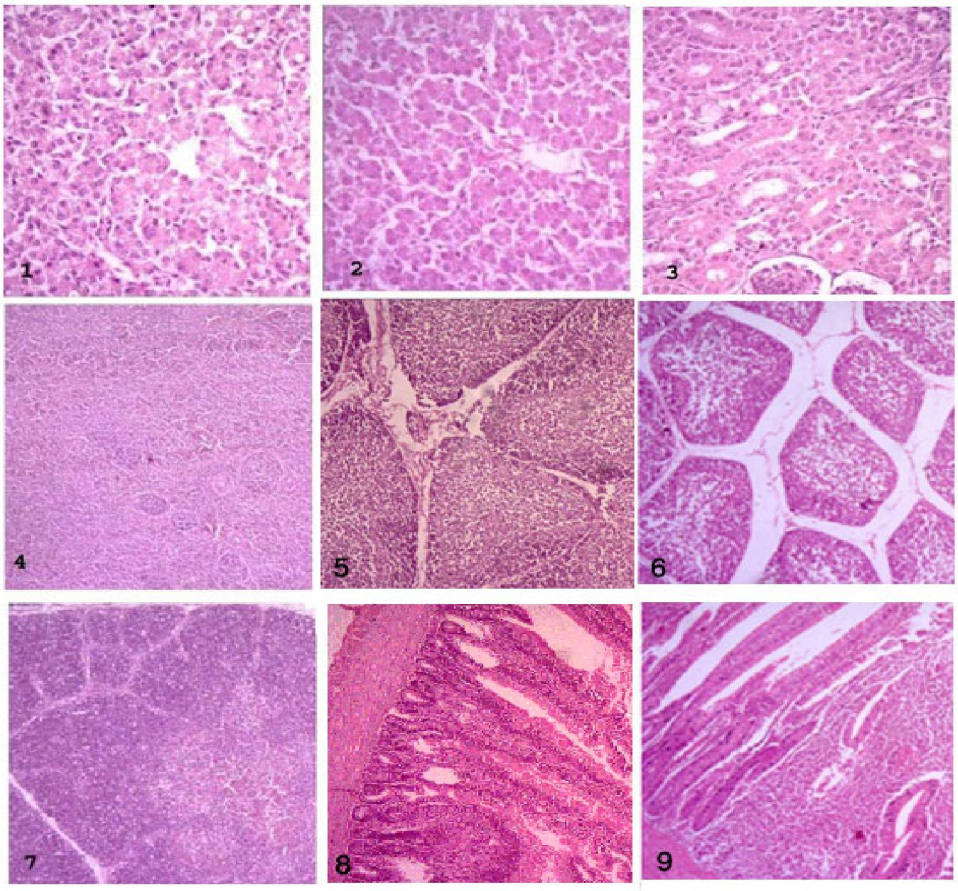

Figure (1): Liver of control group at 6 weeks, showing normal hepatocytes with normal arrangement of hepatic cords around the central veins. H&E. X 400.
Figure(2): Liver 0.05% panzyme group at 6 weeks, showing normal hepatocytes. H&E. X 400.
Figure(3): kidney of 0.05% panzyme group at 6 weeks, showing normal histological architecture of both renal glomeruli and renal tubules. H&E. X 400.
Figure(4): Spleen of 0.05% panzyme group at 3 weeks, showing multifocal to diffuse hyperplasia of lymphoid follicles. H&E. X 200.
Figure(5): Bursa of 0.05% panzyme group at 3weeks, showing normal histological architecture with normal follicular epithelium and hyperplasia of lymphoid follicle. H&E. X 200.
Figure(6): Bursa of 0.5% citric acid group at 6 weeks, showing mild depletion of lymphocytes in addition to edema around the lymphoid follicles. H&E. X 200.
Figure(7): Thymus of 0.05% panzyme group at 3 weeks, showing normal cortex and medulla long with pronounced hyperplasia of cortical follicles. H&E. X 200.
Figure(8): Intestine of 0.05% panzyme group at 3 week, showing normal intestinal villi and intestinal glands. H&E. X 200.
Figure(9): Intestine of 0.5% citric acid group at 6 weeks, showing mild focal destruction of intestinal villi and intestinal gland along with mononuclear cell infiltration. H&E. X 200.

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