Physiological, Biochemical and Histopathological Changes of Ethylenediaminetetraacetic acid (EDTA) and Vitamin C Supplementation in Broiler Chicks Diets.

Abdelrafea A. El-Shafei.,* Arafa, M. M.,** Abdelrahman, A. S.,** Gihan, H. Elsakkar***

* Animal Production Dept. Faculty of Agric. Al-Azhar University, Nasr City, Cairo, Egypt. **Bioch. Dept. Anim. Health Res. Instit. Agric. Res. Center, Ministry of Agric., Dokki, Giza, Egypt. ***Pathol. Dept, (Zagazig Branch), Anim. Health Res. Instit. Agric. Res. Center, Ministry of Agric., Dokki, Giza, Egypt.
dr_abdel-elshafei@hotmail.com

Abstract: A total number of 540 Ross 308 chicks one week old were used in five weeks study to detect the effects of gradual levels of dietary EDTA disodium and Vitamin C alone or in combination on performance, physiological, biochemical, histopatholgical, heavy metals and trace elements changes of broiler chicks. The basal broiler diet was supplemented with EDTA and Vitamin C to compose 12 experimental diets, namely as follows: T1 (control), T2 (0.5g EDTA/kg feed), T3 (1.0g EDTA/kg feed), T4 (2.0g EDTA/kg feed), T5 (0.5gVC/kg feed), T6 (1g VC/kg feed), T7 (0.5g EDTA+0.5g VC/kg feed), T8 (0.5g EDTA+1g VC/kg feed), T9 (1g EDTA+0.5g VC/kg feed), T10 (1g EDTA+1g VC/kg feed), T11 (2g EDTA+ 0.5g VC/kg feed), T12 (2g EDTA+ 1g VC/kg feed). In conclusion, the present study showed that addition of EDTA and VC to diets of Ross 308 chicks revealed no significantly effect on body weight and carcass characteristics. The obtained results revealed that dietary treatments of group fed 0.5g EDTA (T2), group fed 0.5g EDTA plus 1g VC (T8), group fed 1g EDTA plus 0.5 g VC (T9) and group fed 2g EDTA (T4) were significantly ($P \leq 0.05$) improved feed conversion ratio compared with the control group. There are no clear effects of EDTA and VC on thermoregulation parameters. Muscles crude protein % significantly ($P \leq 0.05$) increased for all treated groups compared to the control group while either extract % was significantly ($P \leq 0.05$) decreased for all treated groups compared to the control group. Serum ALT and AST activities increased significantly ($P \leq 0.05$) as EDTA levels increased alone or by combined with VC in the diets. While, the addition of VC alone decreased ALT and AST activities to be less than the control group. Also treated groups with high level of EDTA alone (T3 and T4) or high level of EDTA combined with VC (T11 and T12) recorded significantly ($P \leq 0.05$) increase in serum AP compared to the control group. On the other hand, serum Chol and TG levels were significantly ($P \leq 0.05$) decreased with addition of EDTA and VC either individual or in a combination in the broiler diets. Serum TP and Glob were significantly ($P \leq 0.05$) increased by using EDTA and VC in broiler diets compared to the control group. Broiler chicks fed 1g VC/kg diet (T6) was significantly ($P \leq 0.05$) higher in serum and breast muscles of calcium, phosphorous, sodium and potassium compared to the control and other groups. This study showed that addition of EDTA and VC individual or in a combination to diets of Ross 308 chicks, reduced significantly ($P \leq 0.05$) the lead and cadmium levels in both of breast muscles and serum, helped to eliminate heavy metals from the bird bodies as compared to non treated birds. Results of Macroscopically, the examined organs appear normal in treated chicks with 1g EDTA in addition to 0.5g EDTA either alone or with 0.5 and 1gVC. But the treated chicks with 2g EDTA either alone or with 0.5 or 1g VC showed variable degree of lesion including slightly focal swollen and congestion in liver, spleen, kidney and heart. Macroscopically, liver shows necrosis of epithelial lining bile duct and fibrosia in portal triads (T4) and also, liver showing cytoplasmic vacuolization of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration, (T4). The kidney showing congestion of interlobular blood vessels (T4). The spleen showing atrophied lymphoid follicle (T4) and heterophilic cells infiltration (T11). Moreover, the brain showing necrosis of necrons (T4 and T11).Meanwhile, the examined heart showing myolysis of focal mycotes (T4) in addition to intermuscular edema (T4, T11, T12). Finally thymus gland showing focal hemorrhage (T4).

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1. Introduction

Antioxidants are the vitamins, minerals, enzymes, or other chemical compounds such as ethylene diamine tetra-acetic acid (EDTA) that give up an electron to stop free radicals from causing oxidation. They play a key role in neutralizing the estimated thousands of oxidative hits in each cell suffers a day. In other words, antioxidants are able to destroy free radicals in cells before they can attack DNA or cause lipids to oxidize. EDTA is a synthetic amino acid used orally and intravenously to cleanse, detoxify and remove heavy metals from the body.

EDTA chelating therapy was approved for use by the Food and Drug Administration (FDA) in 1950. Physicians and alternative medicine practitioners also use EDTA to treat cardiovascular disease to improve circulation, remove plaque and improve oxygen flow to the brain since it was approved by the FDA in 1950. **(World Resources Institute, 2002; Vega-Lopez *et al.*, 2004, Pennathur and Heinecke, 2004; Krishnaiah *et al.*, 2007; Flora *et al.*, 2008; Pham-Huy *et al.*, 2008; Flora, 2009; Roussel *et al.*, 2009).**

Nezhad *et al*., (2010) stated that, EDTA is an organic acid which has similar potential with citric acid and it increases availability of some minerals. EDTA is a strong chelated and it improves the absorption rate of minerals in diets of poultry. Supplementation of ascorbic acid at 200mg/ kg in broiler diet reduced cadmium in blood serum, liver, kidney, muscle and reduces the oxidative stress generated by cadmium and protective against many of the adverse effects of cadmium (**Erdogan *et al.*, 2005).** Also, added vitamin C (VC) at level of 100mg/kg in broiler diet reduced copper concentration in liver and blood and reduced lipid peroxidation **(Ajuwon and Idowu, 2010).** VC is a water-soluble vitamin required by the body to maintain normal metabolic activities, and is synthesized in the body to meet all physiological and biological requirements in poultry **(Bardakioúlu *et al.*, 2005).** Poultry have the ability to synthesize ascorbic acid, or VC in their body **(McDowell, 2000)** hence, no recommended requirement is established by the **NRC, (1994**). However, environmental and pathological stressors are known to alter VC use or synthesis or both in the fowl, thus increase VC in the diet is useful to reduce environment stressors.

Several researchers observed significant improvement in growth of chicks by the addition of VC to poultry diets under high temperature. Broilers fed diets containing VC were less stressed due to having reduced body temperature and respiratory rates (**Thaxton and Pardue, 1984; Pardue and Thaxton, 1986; Kassim and Norziha, 1995)** and showed higher feed intake **(Kutlu and Forbes, 1993; Mckee and Harrison, 1995)** than those of control birds. Substantial reports are available that show under field conditions feeding VC enhanced productivity, immune response, disease resistance and survivability under stressful conditions **(Zulkifli, *et al*., 1995; Lohakare, *et al*., 2005; Ajakaiye *et al.*, 2010).**

The present study was carried out for a period of five weeks to investigate the effect of EDTA disodium and VC supplementation levels alone and their combinations in the diets on performance, physiological, biochemical profile, eliminate of some heavy metals and histopathological changes in broiler chicks.

2. Materials and Methods

Location, Experimental Birds and Management of the Flock:

An experiment was performed at Poultry Experimental Station, Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Most of experimental analyzes of this study were done in the Laboratories of Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, El-Dokki, Giza, Egypt. A total number of 540 Ross 308 chicks one week old were used in the present study. The experiment was lasted for 5 weeks during summer and birds were housed on floor and had free access to food and water *(ad libitum*). All experimental diets were isocaloric, isonitrogenous and were formulated to meet the requirements of the strain. Diet specifications and composition analysis are given in Table (1). All birds were reared under similar managerial and hygienic conditions. Temperature degree and humidity percentage were recorded daily through the experiment and were ranged between 33.6^{o}C to 37 ^{o}C and 75 to 80 % respectively as average.

Experimental Design and Procedures:

At the beginning of the experiment, the birds were randomly distributed into 12 experimental groups and each group was divided into three replicates 15 birds for each. Averages of body weight for the 12 experimental groups were apparently uniform. The basal broiler diet was supplemented with EDTA disodium and VC (L-ascorbic acid) to compose 12 experimental diet groups, namely as follows:

T1 (control).
T2 (0.5g EDTA/kg feed).
T3 (1.0g EDTA/kg feed).
T4 (2.0g EDTA/kg feed).
T5 (0.5gVC/kg feed).
T6 (1g VC/kg feed).
T7 (0.5g EDTA+ 0.5g VC/kg feed).
T8 (0.5g EDTA+ 1g VC/kg feed).
T9 (1g EDTA+ 0.5g VC/kg feed).
T10 (1g EDTA+ 1g VC/kg feed).
T11 (2g EDTA+ 0.5g VC/kg feed).
T12 (2g EDTA+ 1g VC/kg feed).

VC and EDTA were mixed with feed every other day to avoid decomposition. Weekly body weights of birds were recorded to the nearest (0.1g).

Carcass and immune organs test.

At the end of experiment, 72 birds were slaughtered 6 from each group for carcass and immune organs tests.

Blood Sampling:

At the end of the experiment, 6 samples from each group were taken randomly to blood sample. Birds were fasted overnight before bleeding via jugular vein in

unheparinzed tubes to determine the blood profiles and serum was separated and stored frozen at –20^{O} C until analyzed.

Main Data Collection

1- *Productive data*

a- Body weight. **b-** Body weight gain. **C-** Feed intake. **d-** Feed conversion ratio. **e-** Carcass yield data.

Body weight, body weight gain, feed consumption, feed conversion ratio were calculated weekly during the experimental time.

2- Physiological and biochemical data

a- Thermoregulation parameters: respiration rate, skin, cloaca and feather temperatures were measured 2 times weekly till the end of experiment.

b- Chemical examinations of breast muscles:

Samples of breast muscles at the end of the experiment were analyzed for moisture, protein, fat and ash contents. Moisture, Protein and fat content samples were measured according to **AOAC (1995).** Ash was determined according to the person's chemical analysis of food ***(*Quasem *et al.*, 2009*)*.**

c- Serum biochemical parameters:

Alanine and aspartate amino transaminase (ALT and AST) activities were determined colorimeterically according to the method of **Retiman and Francle (1957).** A serum alkaline phosphatase was determined according to the modified methods of **Kind and King, (1954).** Serum creatinine was estimated according to **Husdan and Rapoport, (1968).** Serum uric acid was measured according to **Arliss and Entvistle, (1981).** Serum total protein was determined according to **Weichselbaum, (1946).** Albumin was measured according to **Doumas, (1971).** Serum globulin and albumin to globulin ratio were calculated mathematically. Serum cholesterol and triglycerides were measured using commercially available kits from Sigma Diagnostics Company, (Taufkirchen, Germany) on an auto analyzer apparatus.

Serum calcium was measured according to **Gindler and King, (1972)** and serum phosphorus was measured according to **Goldenberg, (1966).** Serum potassium and sodium was measured using flame photometer according to **Oser, (1979).** The concentration of lead, cadmium, zinc and copper in the serum samples were taken directly from digital scale reading of atomic absorption spectrophotometer according to method of **Amodio- Cocchieri and Fiore (1987).**

d- Estimation of heavy metals in breast muscle:

At the end of the experimental period broiler chicks breast muscle of 6 birds from each group were prepared and digested according to the technique recommended by **Khan *et al*., (1995).** The filtrated extract was collected in tubes and kept at room temperature until analyzed by using atomic absorption spectrophotometer. Calcium, phosphorus, sodium, potassium, lead, cadmium, zinc and copper were measured by using Air/acetylene flame atomic absorption spectrophotometer (UNICAM 969 AA spectrometer).

3- Pathological examination:

At the end of experiment target organs were collected from 6 birds of each group for histopathological examination. Specimens were collected from the liver, kidney, spleen, brain, heart and thymus gland of the sacrificed birds and directly fixed in 10% neutral buffered formalin. Five nicron paraffin sections were prepared, stained with hematoxylin and eosin according to **Bancroft and Gamble, (2008)** and examined microscopically.

Statistical analysis:

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package **(SPSS, 2001, version 11.0).** All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple range test **(Duncan, 1955)** at 5% level of significant. Data were analyzed by one way method using the following model. $Y_{ij} = u + N_i + e_{ij}$ Where Y_{ij} = the observed value, u = population means, N_i = the effect of treatment, e_{ij} = the standard error.

3. Results and Disscusion

Productive results:

Results of body weight, body weight gain, feed intake and feed conversion ratio of broiler chicks fed diets supplemented with different levels of dietary EDTA and VC are presented in Table (2). Results showed that the final body weight and daily gain were insignificant between treated groups, which mean that the dietary treatments had no effect on the body weight or daily gain. However, treatment fed 2g EDTA plus 0.5g VC (T9) was recorded a higher numeric value of body weight and daily gain followed by group fed 1g VC (T6), group fed 2g EDTA (T4) and group fed 0.5g VC /kg feed (T5). These results may attribute to that EDTA is (an organic acid) a strong chelator which appeared to improve the absorption rate and availability of minerals in poultry diets **(Vohra and Kratzer, 1965; Liem *et al*., 2008 and Nezhad *et al*., 2010).** The affinity to chelate with metal is quantitatively described as stability coefficient. EDTA has higher stability coefficient with all of the minerals and this is the reason for metal sweeper of EDTA and when EDTA is available in

system it could bind all cations **(Kratzer *et al.*, 1959 and Nezhad *et al.*, 2010).** Feeding ascorbic acid enhanced productivity, immune function, disease resistance, and survivability under stressful conditions **(Zulkifli, *et al.*, 1995; Lohakare, *et al.*, 2005).** Furthermore, the reactive oxygen generated from metabolism of extraneous chemicals in the body can be removed by the antioxidant defense system which the EDTA and ascorbic acid part of it **(Droge, 2002 and Liu, *et al.*, 2006).** VC plays a major role in the biosynthesis of corticosterone, a hormone that enhances energy supply during stress. The improved performance resulting from the use of ascorbic acid is associated with the suppressed stress responses indicated by reduction in plasma corticosterone level **(Kutlu and Forbes, 1993; Mckee and Harrison, 1995; Mahmoud *et al.*, 2004)** and adrenocorticoropic hormone **(Sahin *et al.*, 2003[a]).** Under heat-stress condition, ascorbic acid supplementation in poultry feeds has been reported to have positive effect such as increased weight gain and improved immune response (**Puthpongsiriporn *et al.*, 2001; Lin *et al.*, 2003: Lin *et al.*, 2006).**

Total feed intake and daily feed intake were significantly ($P \leq 0.05$) lower for group fed 1g EDTA and 1g VC (T10) compared to control and other groups. At the same time, total feed intake and daily feed intake were significantly ($P \leq 0.05$) higher for group fed 2g EDTA and 0.5g VC (T11). The obtained results revealed that dietary treatments of group fed 0.5g EDTA (T2), group fed 0.5g EDTA plus 1g VC (T8), group fed 1g EDTA plus 0.5g VC (T9) and group fed 2g EDTA (T4) were significantly ($P \leq 0.05$) improved feed conversion ratio compared with the control group. While, group fed 2g EDTA and 0.5g VC (T11) recorded worst feed conversion ration compared with the control and other groups. The pervious results may be due to that EDTA and VC improving growth performance by alleviates the negative effects of stress and have positive effects such as increased weight gain and improved feed conversion **(Sahin, *et al.*, (2003[b]).** These results suggested that VC may tend to encourage effective conversion of feeds to weight. The obtained findings are in agreement with those reported by **(Sahin *et al.*, 2003[b]; Bolu and Olatunde, 2003).**

The effect of experimental treatments on the composition of the bird carcasses (in gram) and relative to live body weight (%) are given in Table (3). The results of carcass variables showing no significant effect of dietary EDTA and VC treatments on carcass yield characteristics among all groups. Although, the live body weight and dressing weight improved for all treated groups compared to the control group but not significantly. The mode of action of VC in this specific application could be explained via the modulation of the release of corticosteroid hormones and alleviation of the disturbance in the electrolyte balance, thus reducing catabolism of body reserves and preventing a strong dehydration in the bird **(Cafantaris 1995; Lohakare *et al.*, 2005).** Abdominal fat pad % was recorded a lower % but not significant for group fed 2g EDTA (T4) and group fed 0.5g EDTA plus 1g VC (T8) compared to all other experimental groups. In general, there were no significant differences among the groups on carcass variable yields in broiler chicks submitted to this study. These results are in general disagreements with those obtained by **Lohakare *et al.*, 2005,** that found adding gradual levels of ascorbic acid from 10 to 200 ppm/Kg diet enhanced the carcass yield. However, the abdominal fat % was in agreement with the result of **Lohakare *et al.*, 2005.**

Physiological results:

The obtained results of immunocompetent organ weights indicate that there were no significant differences in the weights of lymphoid organs between all experimental groups Table (4). There was no structural changes were observed in shape of bursa, thymus or the spleen of any group. However, group fed 0.5g EDTA plus 0.5g VC (T7) recorded a higher value but not significant in spleen weight while group fed 0.5g EDTA plus 1g VC (T8) registered a higher numerical value in bursa weight compared to the other groups. At the same time, group fed 2g EDTA (T4) scored higher value in thymus gland weight but not significant compared with the other groups. These results are agreement with results of **(Elagib and Omer, 2012)** have been showed that supplementation of ascorbic acid with level of 150, 350 and 550 mg/Kg diet during heat stress had no beneficial effects on ratio of weight for bursa, thymus and spleen to body weight of heat-stressed birds.

Effect of EDTA and VC supplementation on (thermoregulation parameters) cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiratory rate (Rr) of broiler chicks are shown in Table (5). The addition of EDTA and VC to broiler diets of group fed 1g VC /kg feed (T6) registered a significantly ($P \leq 0.05$) higher value in Tc, Ts, Tf and Rr of birds compared to the other groups. While group fed 2g EDTA plus 1g VC (T12) was recorded a significantly ($P \leq 0.05$) lower value in Tc and Tf and was insignificantly lower in Rr compared with the other groups. Group fed 1g EDTA plus 0.5g VC (T9) was significantly ($P \leq 0.05$) lower in Ts compared to the control group. There was no significant effect of dietary EDTA and VC on Rr among all experimental groups. In general, the obtained results of above traits were recorded no significant effect of dietary EDTA and VC on those traits among all groups of experiment. These results are agree with the report of

Miraei-Ashtiani *et al.*, (2004) and Abioja *et al.*, (2012) found that the inclusion of VC in broiler diet did not result any difference in cloacal temperature compared to chickens receiving diet without VC supplementation. The results are in disagreement with findings of **Kutlu and Forbes (1993)** have been noted that ascorbic acid either in feed or water reduced skin and rectal temperature of broiler chickens. In that context, **Kutlu and Forbes (1993)** established that panting rate was reduced in heat-stressed chickens given ascorbic acid. **Shane, (1988)** and **Chaiyabutr, (2004)** declared that one of the physiological responses to heat-stress in birds is increase in respiratory frequency and that panting occurs when the deep body temperature of poultry reaches 41-43^{o}C because birds have no sweat glands like ruminants. Use of VC a reduction in panting rate occurs with supplemental adrenal ascorbic acid from water ingested **(Grieve, 2003**).

Biochemical analysis of breast muscles:

Table (6) shows the results of biochemical analysis in breast muscle of broiler chicks fed different levels of EDTA and VC. Moisture content was recorded from 70.25±0.44 to 73.5±0.47 % and ash content from 1.38±0.04 to 1.5±0.37% without any significant difference among all groups. Crude protein % was significantly (P≤ 0.05) increased by increasing the levels of EDTA, VC and their combination in broiler diets compared with the control group. While ether extract was significantly (P≤ 0.05) decreased by increasing the levels of EDTA, VC and their combination in broiler diets compared with the control group. Group fed 2g EDTA (T4) and group fed 2g EDTA plus 1g VC (T12) had significantly (P≤ 0.05) lowest value in ether extract% compared to all other groups. These results suggest that the increase of crude protein in breast muscles of broiler fed diets contained levels of EDTA or VC alone or combined together may be due to the increase N retention and decreased N excretion and respect to the effect of VC on nutrient digestibility as reported by **Lohakare *et al.*, (2005).** In that concern **Bolu and Olatunde, (2003)** observed that broiler fed supplemental VC had better nutrient retentions than birds on non supplemented diet. This observation may suggest that VC enhances nutrient retention. **Gopal *et al.*, (2009)** showed that the reduction in the protein content after exposure to nickel chloride may be due to its effect on protein synthesis, which considered as the primary biochemical indicator for stress. This synthesizing is influenced by a large number of exogenous substances that may be due to proteolysis, lack of protein biosynthesis or inhibition of translation.

Serum and breast muscle biochemical analysis:

Results of ALT, AST, alkaline phosphatase, creatinine and uric acid are given in Table (7). As seen in Table (7), the serum ALT and AST activities increased significantly (P≤ 0.05) as EDTA levels increased alone or by EDTA combined with VC in the diets. While, the addition of VC individual decreased ALT and AST activities to be less than the control group. The significant changes in activities of these enzymes in blood serum indicate that tissue impairment caused by stress **(James *et al.*, 1991).** Also**,** alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm **(Khan and Sardar, 2005).** The increase of serum ALT and AST may be attributed to hepatocellular damage or cellular degradation and focal coagulative necrosis (cytoplasmic vacuolization of hepatocytes and pericholongiolar fibrosis) were noticed in T4,T11 and T12 (which have 2g EDTA), Figure (2-9) in liver and perhaps in heart and muscle as also shown by (**Yamawaki *et al.*, 1986**). High level of EDTA in broiler diet increased the ALT and AST enzymes activity which mean that this level is not optimal for broiler chicks that may be led to adverse effect by increasing lipid peroxidation caused by oxygen free radicals produce damage to liver tissue which is revealed by increase in serum ALT and AST concentration, which indicate that inability of liver to metabolize the ALT and AST **(Bharavi *et al.*, 2010)** and attribute to the outflow of these enzymes from the liver cytosol to the blood stream **(Cinar *et al.*, 2011).** These findings are supported by the hereafter results of liver histopathological examination presented in Fig. 1 to Fig. 10. The reduction occurred in serum ALT and AST of broiler groups fed levels of VC may be due to the anti-oxidants activities of VC as reported by **Ajakaiye *et al.*, (2010).**

The alkaline phosphatase (AP) activity showed significant (P≤ 0.05) a higher variation with the use of high level of EDTA alone or with high level of EDTA combined with VC (T4, T11 and T12) compared to the control and other groups. While, increase VC level in broiler diet, AP was significantly (P≤ 0.05) decreased. Alkaline phosphatase is one of the most frequently used biochemical markers of osteoblast activity and differentiation **(Magnusson *et al.*, 1999).** The higher AP activity is necessary for initiating mineralization, because AP can decompose phosphoric acid of organic matter, increase inorganic phosphoric acid concentration and thereby enhance mineralization. Osteoblasts secrete AP and calcium salt crystals into the extracellular matrix and up-regulate the concentration of phosphoric acid and then support mineralization **(Guo *et al.*, 2011).** Moreover, elevated AP activity may attributed to that AP is a hydrolase enzyme for removing many types of molecules, including nucleotides, proteins and alkaloids, and therefore its activity is high in tissue

involved in high level of metabolism. Increased AP activity is not limited to liver damage only but it is also associated with pathological changes in the bone, kidneys and biles. Therefore, the high level of ALT activity may be due to damage or disturbance in any of these organs. Increased AP activity has been associated with lead and other heavy metal poisonings **(Gursu *et al.*, 2004; Ambali *et al*., 2011).**

Creatinine and uric acid (UA) levels were significantly (P≤ 0.05) decreased for group fed 1g VC/kg feed (T6) compared with the control group (T1). While, group fed 2g EDTA/kg diet (T4) recorded a higher value in creatinine concentration which means that this level of EDTA (2g/kg diet) had deleterious effect on kidney function causing kidney interlobular congestion as shown in (Fig. 11 and 12). Both of creatinine and UA indicate better condition for kidney functions especially with VC alone or EDTA combined with VC supplementation. **Erdogan *et al.*, (2005)** found that ascorbic acid could be effective in the protection of cadmium- induced nephrotoxicity in broiler chicken. The reduction in concentration of serum UA may attribute to that VC might be beneficial in the prevention or management of gout **(Cinar *et al.*, 2010).** The effect of VC consumption on serum UA level has not been well documented. Previous study by **Huang *et al., (*2005)** suggested that VC had a uric acid modulation role that may be beneficial. In current study VC individual or in combination with EDTA decreased plasma UA levels. The mechanism by which VC reduces serum uric acid might include glomerular filtration and competition for renal reabsorption, i.e., VC and uric acid are both reabsorbed via anion exchange transport at proximal tubules **(Huang *et al.*, 2005; Saki *et al.*, 2010).**

On the other hand, Serum cholesterol (Chol) and triglycerides (TG) levels were significantly (P ≤ 0.05) decreased with addition of EDTA and VC either individual or in a combination in the broiler diets Table (8). Also, combination of EDTA with VC in the broiler chick diets has synergistic effects to reduce the serum Chol and TG levels significantly (P ≤ 0.05) at 6 weeks of age. In this connection, similar findings were agreed with report of VC supplementation by **Sahin *et al*., (2002and 2003)** have been suggested that free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane. VC itself plays important roles in cellular anti-oxidant defenses, not only by reacting with all oxygen species through formation of dehydroascorbyl, a particular inert radical, but also by transferring radical equivalents from lipid phases to aqueous compartment **(Bartov and Frigg, 1992; Zuprizal *et al*., 1993**). These results may be attributed to that VC helps in the metabolism of Chol, increasing its elimination and thereby assisting lower blood Chol **(Iqbal *et al.*, 2004).** Similarly, the decreases of TG concentrations observed during this experiment confirmed as well by previous studies (**Sahin *et al.*, 2003[a,b]; Kucuk *et al.*, 2003**). Several metabolic pathways would be involved in the reduction of lipid mobilisation and catabolism. Firstly, when birds were supplemented with ascorbate, the corticoid secretion was reduced and the lipoprotein and tissue lipases were consequently not stimulated. As a result, lipids and Chol were not mobilised from tissues. Secondly, ascorbate is necessary for the transformation of Chol to bile acids by controlling the microsomal 7α-hydroxylation. As this reaction is the rate-limiting step of the Chol catabolism in liver, ascorbic acid deficiency induces a marked slowing down of this reaction, leading to Chol accumulation in liver and in blood **(Seyrek *et al.*, 2004).** By contrast, ascorbate supplementation will accelerate the transformation of Chol into bile acids, decreasing Chol concentrations in liver and in serum. Because Chol is transported in blood by lipoprotein complexes (VLDL, LDL and HDL), Chol and lipoprotein concentrations were positively correlated **(Linne and Ringsrud, 1999; Guyton and Hall, 2006)**. So, in VC-deprived birds, high Chol concentrations were accompanied by high VLDL concentrations, whereas in-groups supplemented with ascorbate the VLDL concentrations were significantly reduced. Thirdly, as ascorbate is required for carnitine synthesis, the mitochondrial concentrations of this amino-alcohol is increased. By transporting long chain fatty acids from cytoplasma into mitochondrial matrix of muscle cells, carnitine improves beta-oxidation of lipids, leading to reduction of serum TG concentrations **(Ness *et al.*, 1999; Seyrek *et al.*, 2004; Guyton and Hall, 2006; Al-Daraji and Amen, 2011).**

There was significant (P ≤ 0.05) increase in serum total protein (TP), globulin (Glob) and (albumin but not significant) of groups treated with EDTA and VC alone or in a combination between them as presented in Table (8). Elevate the value of Glob in serum of treated groups than the control may reflect the enhanced effect of EDTA and VC on the immunity status of these groups especially group T12 (2g EDTA+1g VC) and T10 (1g EDTA+1g VC) which recorded a significantly (P ≤ 0.05) increased in Glob value than the control and other groups. These results are agreed with those obtained by **Ajakaiye *et al.*, (2010)** found that adding 200mg VC to layer chicken diet increased but not significantly TP in plasma. Regarding to the results of plasma protein concentration as compared to the control group may be attributed to the hormonal regulation of protein metabolism, for example growth hormone increased the synthesis of cellular protein, glucocosteroids

increased break down of most tissue proteins. The increasing of corticosterone hormone and glucocorticiods which are secreted by the adrenal cortex increased the quantity of protein in most tissues while decreased the amino acids concentration in the plasma, as well as decreased both liver protein and plasma proteins, or may be due to the decrease of thyroxin secretion, which thyroxin increases the rate of metabolism of all cells and, as a result indirectly affects protein metabolism **(Guyton and Hall, 2006; Al-Daraji and Amen, 2011).** Many researchers **(Donkoh, 1989; Kutlu and Forbes, 1993)** have reported that VC supplementation increases serum Alb concentrations. In the present study, there was also elevated in serum Alb concentrations but not significant in birds received VC and EDTA. It has been reported (**Kutlu and Forbes, 1993; Seyrek *et al.,* 2004)** that ascorbic acid supplementation reduces the synthesis of corticoid hormones in birds under heat stress. As corticoids induce gluconeogenesis from non carbohydrate precursors such as lactate, amino acids and glycerol **(Linne and Ringsrud, 1999),** decrease of glucocorticoids secretion could limit lipid and protein catabolism (**Kucuk *et al.,* 2003; Seyrek *et al.,* 2004).** The increases of serum Alb and Glob concentrations observed in experimental groups could be partially explained, by the reduction of synthesis and secretion of corticoids in birds received VC and EDTA. Regarding the A/G ratio, the obtained results indicated that there was significant ($P \leq 0.05$) decrease with groups combined EDTA with VC in the diets compared to the control.

Table (9) shows the trace elements in breast muscles and serum of broiler chicks fed diets supplemented with different levels of EDTA and VC. Calcium (Ca) levels in breast muscles and serum were significantly ($P \leq 0.05$) decreased with treatments T4, T3, T11 and T12. These results revealed that by increasing EDTA levels alone or integrated with VC in broiler diets led to significantly ($P \leq 0.05$) decreased Ca concentration in breast muscles and serum compared to the control and other groups. Similar findings were obtained by **Sifri *et al.* (1978)** recorded that, plasma Ca level was significantly decreased by added Na_2EDTA in adult quail. Meanwhile, broiler groups fed with VC alone appeared slightly increase in Ca concentrations but not significant in both of muscles and serum. The reduction in Ca concentration in breast muscles and serum may refer to that addition of dietary EDTA in rat at level of 300 and 800mg/kg diet had no influence on Ca absorption and retention but increased urinary Ca excretion (**Hurrell *et al.,* 1994).** In conclusion adding VC with EDTA improves the EDTA application by decreasing depletion of Ca from breast muscles and serum.

Also, the same trend of calcium found with results of phosphorus (P) in breast muscles and serum whereas increase EDTA level in the diets decreased P concentration. Groups T4 and T3 were significantly ($P \leq 0.05$) lower in P concentration compared to all other groups. These results may be due to the significant decrease in Ca concentration of the same treatments in both of muscles and serum. This finding may be referring to the properties of EDTA as a chelating agent to minerals. The results of Ca, P and Zn either in breast muscles or serum are in harmony with those established by **Kabuage, *et al.,* (2002)** which recorded that there were significantly decreased in these parameters when broiler fed diets contained 2% EDTA. Furthermore, **Liem *et al.,* (2008)** demonstrated that adding EDTA up to 3.65% in broiler diets decreased Ca and increased P concentrations significantly in plasma of broiler chicks.

Sodium (Na) concentration showed that group fed 2g EDTA (T4), group fed 2g EDTA plus 0.5g VC (T11) and group fed 2g EDTA combined with 1gVC (T12) were significantly ($P \leq 0.05$) higher in breast muscles Na compared to the control and other groups but were significantly ($P \leq 0.05$) lower in serum Na of the same groups which may be lead to muscular edema and degeneration as shown in histopathological pictures of heart and liver muscles in groupT4, T11 and T12 (Fig. 9, 21 and 22). Also, the reduction in plasma level of Na may be due to heat stress (33.6-37 C°) occurred during the experimental time and may also probably as a result of hemodilution following increased water consumption and high levels of Na may reduce the negative effect of heat stress **(Ribeiro, *et al.,* 2008).** Furthermore, the variation levels of serum Na and other minerals could refer to the disturbances in acid-base balance of birds due to serve heat stress during experimental period. The results are similar with results of **Khattak, *et al.,* (2012)** have been reported that birds under continuous panting result in respiratory alkalosis. Respiratory alkalosis is characterized by excessive removal of blood carbon dioxide. Carbone dioxide in the blood is a source of H_2CO_3. This is a source H ion in the blood a lack of H ion can lead to poor Na and K reassertion, causing more Na and K in the urine and leaving less Na and K in the blood (**Khattak, *et al.,* 2012).**

Potassium concentration (K) was significantly ($P \leq 0.05$) decreased in breast muscles as EDTA level increased in the diets of T2, T3 and T4. Also, the K concentration increased gradually with increasing VC level in the diet, whereas group fed 1g VC /kg diet was significantly ($P \leq 0.05$) higher in breast muscle K. While, serum K was insignificantly decreased as EDTA level increased in diets. These results revealed that there were no significant changes between all tested and the control groups in serum K.

In conclusion, adding VC with EDTA improves the EDTA application by decreasing depletion of trace elements from breast muscles and serum. Researchers have reported a reduction in plasma concentration of K and Na due to heat stress and probably as a result of hemodilution following increased water consumption of birds (**Belay and Teeter, 1993; Ribeiro, *et al.*, 2008).** The reason for the variation in serum minerals may be referred to the disturbances in acid-base balance of birds due to serve heat stress during experimental period **Khattak, *et al.*, (2012)**. The lower serum K concentration in treated groups compared to the control group could be due to the fact that K ions shift between muscle and extra cellular fluid during heat stress to maintain cellular atmospheric pressure **(Keskin and Durgun, 1997)** or it may be due to reason that K excretion from kidney was increased and also the uptake was increased by erythrocyte and skin **(Simth and Teeter, 1987).**

Serum and breast muscle heavy metals analysis:

Heavy metals in breast muscles and serum of the broiler fed diets supplemented with different levels of EDTA and VC are given in Table (10). Lead (Pb) and cadmium (Cd) in breast muscles and serum were significantly ($P \leq 0.05$) decreased with increasing the EDTA and VC levels in the diet compared with the control group. But the decreasing of Pb and Cd in breast muscle and serum was significantly ($P \leq 0.05$) lower and was more effective when EDTA was combined with VC (T7, T8, T9, T10, T11 and T12) compared with those of control group. Addition of EDTA and VC in this study may prevent the accumulation of these heavy metals in the analyzed tissue and serum. Also, VC and EDTA may decrease of the toxic effect of Pb and Cd by producing complexes with heavy metal cations which facilitates their elimination from the organism. Furthermore VC and EDTA probably reduce the absorption of Pb or Cd from the gastrointestinal tract **(Cinar *et al.*, 2011).** The reduction in Pb and Cd concentration in breast muscles and serum of treated groups may be due that VC and EDTA act as antioxidants that give up an electron to inhibit production of free radicals by blocking lipid peroxidation. Antioxidants play a very important biological role in the body by protecting against oxidative damage particularly oxidative damage to DNA **(Sahin *et al.*, 2002; Erdogan *et al.*, 2005; Ribeiro *et al.*, 2008; Seven *et al.*, 2009; Khattak *et al.*, 2012). Ajuwon and Idowu, (2010)** reported that VC supplementation for two weeks in broiler diet was effective in scavenging free radicals, including hydroxyl radicals, aqueous peroxyl radicals and superoxide anions. It acts as a two electron reducing agent and confers protection by contributing an electron to reduce free radicals, thus neutralizing these compounds in the extracellular aqueous environment prior to their reaction with biological molecules.

On the other hand, zinc (Zn) in breast muscles and serum had not been affected throughout the experimental period by the supplementation of EDTA, VC and their combination compared to the control group except T4 (2g EDTA/kg diet) was significantly ($P \leq 0.05$) lower in the serum Zn compared to all other groups. The results of Zn in this study show that slightly increment but not significant in muscle Zn. **Hurrell *et al.*, (1994)** found that addition $NaFe^{3}$+EDTA at level of 50 and 100mg/kg in rat diet might improve Zn and Cu absorption from low- Zn-bioavailability diet. While, **Hill *et al.*, (1987)[1] and Hill *et al.*, (1987)[2]** established that EDTA had no effect on zinc absorption in rats, swine or chickens whether in breast or serum. EDTA is a strong chelator which appeared to improve some of the minerals absorption in poultry diet. Previous investigation showed that addition of EDTA to broiler (**Kabuage *et al.*, 2002 and Nezhad *et al.*, 2010)** and layer chickens (**Nezhad *et al.*, (2008**) that contains plant proteins improved zinc absorption. The affinity to chelate with metal is quantitatively described as stability coefficient. EDTA has higher stability coefficient with all of the minerals and this is the reason for metal sweeper of EDTA and when EDTA is available in system it could bind all cations (**Nezhad *et al.*, 2010).** These results are in agreement with discovery of **Hill *et al.*, 1987[1,2] ; Hurrell *et al.*, 1994; Kabuage *et al.*, 2002; Nezhad *et al.*, 2008; 2010).**

While, copper (Cu) concentrations in breast muscles increased significantly ($P \leq 0.05$) in all groups compared with the control group. Similar finding was reported by **Nezhad *et al.*, (2010)** when studied interaction effect of EDTA+MP (microbial phytase) in broiler chick diets on concentration of Cu which was significantly ($P \leq 0.05$) increased. Also, found that addition of different levels of EDTA to diets not supplemented with MP and contained low level of available phosphorus increased concentration of serum Cu of broiler chicks. In addition, Cu concentrations in serum were significantly ($P \leq 0.05$) decreased with groups (T2, T3 and T4) which had EDTA alone while, group T6 (1g VC/kg diet) was significantly ($P \leq 0.05$) higher in serum Cu compared with the control and other groups. **Nezhad *et al.*, (2008)** reported that EDTA is strong chelatore and appeared that it improved some minerals absorption such as Cu and Zn in poultry. **Nezhad *et al.*, (2010)** documented that addition of different levels of EDTA to diets which contained low level of available phosphorus decreased concentration of Cu in serum. But, when VC added to EDTA, the Cu depletion prevented and sometimes Cu content increased. VC may be beneficial in preventing copper-induced

oxidative damage in poultry and shows potential for veterinary use. **Waters *et al.*, (2001)** expressed that there was significantly increased in urinary losses of lead, cadmium, zinc and calcium following EDTA chelating therapy.

Histopathological examination:

Macroscopically the examined organs appear normal in treated chicks with 1g EDTA in addition to 0.5g EDTA either alone or with 0.5 and 1g VC. But the treated chicks with 2g EDTA either alone or with 0.5 or 1g VC showed variable degree of lesion including slightly focal swollen and congestion in liver, spleen, kidney and heart. The hepatic damage may be attributed to increase of serum ALT and AST in T4, T11 and T12 (which have 2g EDTA/kg diet), figure (2-10) in liver or may be attributed to the outflow of these enzymes from the liver cytosol to the blood (**Yamawaki *et al*., 1986: Cinar *et al.*, 2011).** The histopathological changes of liver treated high level of EDTA (Fig. 2-9) might be due to the formation of highly reactive radicals and subsequent lipid peroxidation. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis of hepatocellular damage **(Renugadevi and Prabu, 2010).** Meanwhile, these changes are in nearly agreement with those recorded by **Reuber, (1969)** found these changes could be due to accumulation of metabolites and increased the cytoplasmic osmotic pressure and inhibition of water into the cell. The examined kidney of the control chicks appears normal (Fig.11). Meanwhile in T4 there was a congestion of interlobular blood vessels was noticed (Fig.12). this is in moderate agreement with that recorded with **Wynn *et al*., (1970)** who failed to show any renal damage, and found that EDTA was not well tolerated at dietary levels above 5% and in fairly agreement with that reported by **(Ahrens and Aronson, (1971).** The examined control spleen appears normal (Fig.13). While in T4, atrophied lymphoid follicle was noticed (Fig.14). Also, T4 and T11, the examined spleen showed heterophilic cells infiltration (Fig.15 and 16) which may be due to high EDTA concentrations in chick diets. Meanwhile, the examined control brain appears normal (Fig.17), but in T4 and T11 showed necrosis of neurons was detected (Fig.18). In addition to the examined heart showed myolysis of focal mycotes and another examined case showed intermuscular edema. These changes may be due to high level of EDTA in diet (Fig. 20 -22). The examined thymus gland of control group, chicks appear normal (Fig. 23). While, in T4 there was a focal hemorrhage detected (Fig. 24).

Table (1): Compositions of experimental diets of broiler Ross 308 chicks.

Ingredients	Diets of broiler chicks		
	Starter (1-10 day)	Grower (11- 24 day)	Finisher (25-42 day)
Ground yellow corn 7.7%	58.00	59.02	61.50
Corn glutten meal 61%	9.14	9.00	8.00
Fish meal herring 72%	3.50	3.20	3.00
Soybean meal 42%	23.65	22.69	21.00
Sunflower oil 8500	1.00	2.67	3.30
Dicalcium phosphate	2.00	1.50	1.40
Limestone	1.59	1.11	1.10
Premix[1]	0.30	0.30	0.30
Sodium Chloride	0.30	0.30	0.30
Lysine	0.39	0.21	0.10
Methionine	0.13	-	-
Total (Kg)	100	100	100
Calculated			
Crude protein%	22.99	22.14	20.72
ME.Kcal/Kg	3029.77	3157.60	3206.95
C/P ratio	131.79	142.62	154.78
Calcium%	1.20	0.90	0.87
Available Ph.%	0.55	0.45	0.43
Lysine%	1.46	1.24	1.06
Methionine %	0.63	0.46	0.42
Methionine + Cystine%	0.98	0.82	0.79

[1]**Composition of vitamins and minerals premix.** Each 3Kg of vitamin and minerals mixture contain: Vit. A 10000000 IU, Vit. D_3 2000000 IU, Vit. E 10000 mg, Vit. K_3 1000 mg Vit. B_1 1000 mg, Vit. B_2 5000 mg, Vit. B_6 1500 mg, Vit B_{12} 10 mg, Niacin 20000 mg, Pantothenic acid 10000 mg, Folic acid 1000 mg, Biotin 50 mg, Choline chloride 500000 mg, Copper 4000 mg, Iodine 300 mg, Iron 30000 mg, Manganese 60000 mg, Zinc 50000 mg, Cobalt 100 mg and Selenium 100 mg.

Table (2): Effect of different levels of EDTA, VC and their combination in diets on final body weight (g) at 6th weeks of age, daily gain, total feed intake, daily feed intake and feed conversion during the total period (2 – 6 weeks of age) of broiler chicks.

Treatment	Final body weight (g/bird)	Daily gain (g/bird. day)	Total feed intake[2]	Daily feed intake[3]	Feed conversion ratio[4]
T1	1962.86^{lab}±44.03	47.13^{ab}±1.11	4026.33^{d}±1.00	115.04^{d}±0.03	2.051^{f}±0.0006
T2	1996.07^{a}±61.16	47.97^{ab}±1.68	3842.83^{i}±0.83	109.80^{i}±0.02	1.925^{i}±0.0008
T3	1912.31^{ab}±44.51	45.81^{ab}±1.30	3893.33^{g}±0.67	111.24^{g}±0.02	2.035^{d}±0.0009
T4	2024.17^{a}±59.62	49.15^{a}±1.83	3912.50^{f}±1.17	111.79^{f}±0.03	1.932^{h}±0.0006
T5	2023.33^{a}±43.16	48.78^{a}±1.19	4141.67^{c}±1.00	118.33^{c}±0.03	2.046^{c}±0.0005
T6	2060.00^{a}±43.39	49.71^{a}±1.25	4249.00^{b}±1.00	121.40^{b}±0.03	2.062^{b}±0.0004
T7	1925.50^{ab}±44.91	45.80^{ab}±1.31	3875.67^{h}±1.00	110.73^{h}±0.03	2.012^{g}±0.0013
T8	1947.14^{ab}±42.87	47.20^{ab}±1.28	3754.17^{j}±0.83	107.26^{j}±0.02	1.928^{i}±0.0008
T9	2061.15^{a}±48.40	50.14^{a}±1.46	3978.00^{e}±0.50	113.66^{e}±0.01	1.929^{i}±0.0007
T10	1817.31^{b}±30.42	43.51^{b}±0.91	3748.00^{k}±1.00	107.09^{k}±0.03	2.062^{b}±0.0014
T11	1931.79^{ab}±42.46	46.48^{ab}±1.03	4291.68^{a}±1.11	122.62^{a}±0.03	2.221^{a}±0.0005
T12	1961.92^{ab}±65.99	47.08^{ab}±1.91	3979.77^{e}±0.90	113.71^{e}±0.04	2.028^{e}±0.0007

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f,g,h,i,j,k Means having different letter exponents among columns are significantly different (P≤0.05).

[2] (g/bird. 5 weeks) from 2-6 weeks of age.

[3] (g/bird. day). [4] (g feed/1g weight gain. bird) from 2-6 weeks of age.

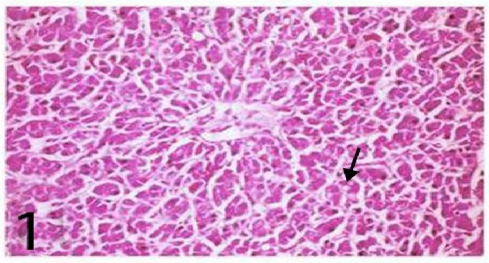

Fig.(1): Liver Control (Normal) H&E.X 400

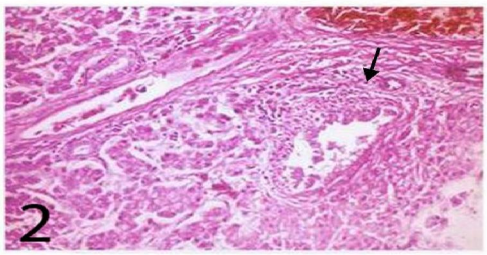

Fig.(2):Liver showing necrosis of epithelial lining bile duct and fibroplasia in portal triads H&E.X 400

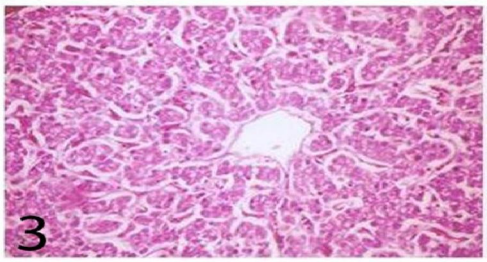

Fig. (3): Liver showing cytoplasmic vacuolization of hepatocytes (T4). H&E.X 400

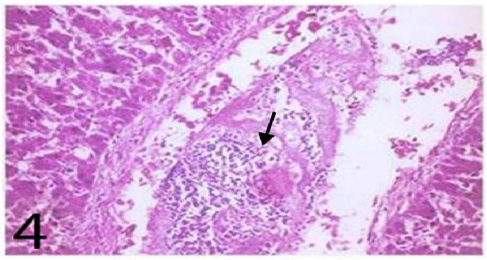

Fig. (4): Liver showing Thrombus formation in the central vein.(T11) H&E.X 400

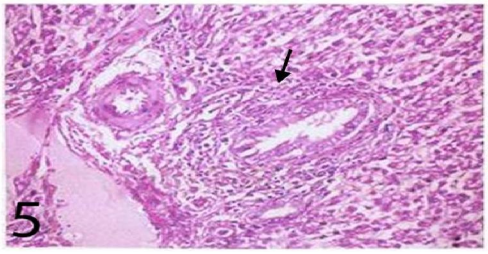

Fig. (5): Liver showing cytoplasmic vacuolization of hepatocytes and perichoangiolar (T12) H&E X400.

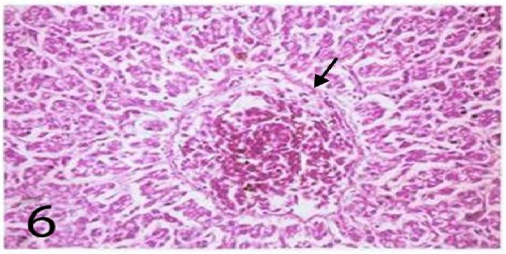

Fig. (6): Liver showing congestion of central vein and cytoplasmic vacuolization of hepatocytes (T3) H&E X400.

Table (3): Effect of different levels of EDTA, VC and their combination in diets on carcass characteristics broiler chicks at 6th weeks of age.

Treat.	Live body weight (g)	Dressing weight (g)	Dressing %	Giblets %	Gizzard %	Liver %	Heart %	Abdominal fat pad %
T1	2145.00[1]±172.90	1520.62±146.27	70.65±1.78	4.11±0.18	1.47±0.21	2.24±0.18	0.39±0.01	1.86[ab]±0.16
T2	2332.50±174.23	1633.74±142.07	69.87±1.53	4.20±0.17	1.45±0.12	2.32±0.13	0.43±0.04	1.58[ab]±0.27
T3	2315.00±150.40	1630.38±131.71	70.19±1.21	4.25±0.16	1.51±0.03	2.29±0.16	0.44±0.05	1.40[ab]±0.22
T4	2161.25±163.41	1471.41±128.62	67.91±0.81	3.93±0.19	1.46±0.23	2.04±0.19	0.43±0.03	1.16[b]±0.15
T5	2272.50±149.37	1617.64±114.96	71.11±1.12	4.52±0.60	1.37±0.11	2.74±0.55	0.41±0.04	1.74[ab]±0.24
T6	2266.25±182.94	1608.87±150.61	70.77±1.09	4.27±0.30	1.62±0.10	2.21±0.21	0.44±0.03	1.54[ab]±0.13
T7	2266.25±82.35	1592.05±62.61	70.23±1.10	4.55±0.08	1.56±0.13	2.57±0.17	0.43±0.04	1.51[ab]±0.17
T8	2288.75±148.76	1627.86±167.25	70.64±2.77	4.21±0.22	1.54±0.19	2.23±0.12	0.44±0.01	1.21[b]±0.15
T9	2396.25±156.65	1691.73±138.92	70.34±1.33	4.07±0.17	1.54±0.06	2.15±0.17	0.38±0.01	1.28[ab]±0.27
T10	2240.00±80.73	1561.35±51.20	69.73±0.40	4.58±0.13	1.60±0.10	2.54±0.55	0.44±0.03	1.32[ab]±0.14
T11	2296.25±148.01	1657.04±148.18	71.84±1.72	4.34±0.40	1.57±0.28	2.44±0.15	0.32±0.07	1.68[a]±0.15
T12	2227.50±183.23	1566.74±152.25	70.06±1.18	4.39±0.22	1.41±0.11	2.58±0.11	0.40±0.05	1.43[ab]±0.14

[1] Least squares means ± pooled standard error.

a,b, Means having different letter exponents among columns are significantly different (P≤0.05).

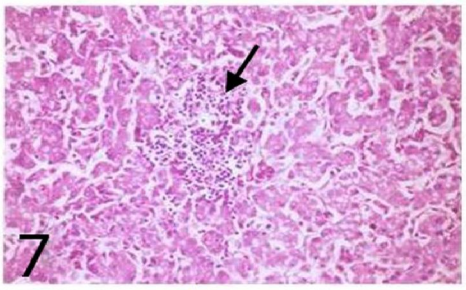

Fig. (7): Liver showing cytoplasmic vacuolization of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration, (T4). H&E.X 400

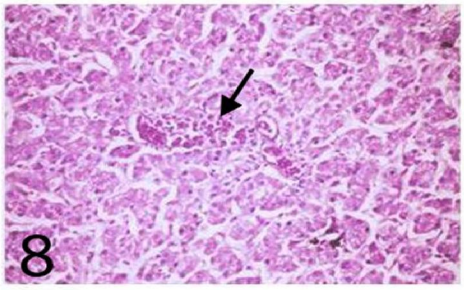

Fig. (8): Liver showing cytoplasmic vacuolization of and portal infiltration with heterophiles.(T11) H&E.X 400

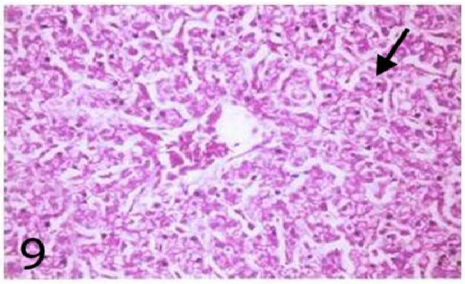

Fig. (9): Liver showing vacuolar degeneration of hepatocytes. (T12) H&E.X 400

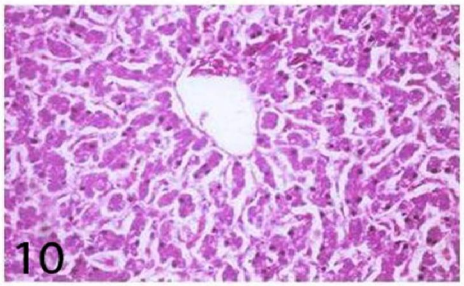

Fig. (10): Liver showing normal (T6) hepatocytes. H&E.X 400

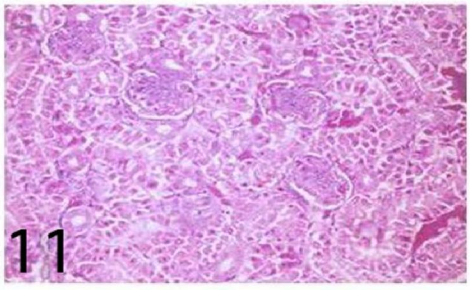

Fig. (11): Kidney showing control (normal) H&E.X 400

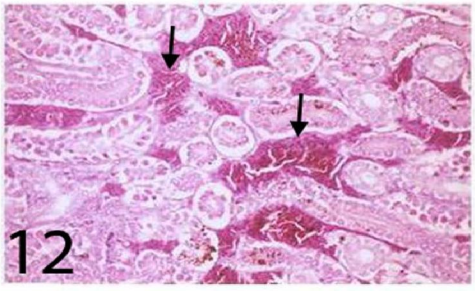

Fig. (12): Kidney showing congestion of interlobular BVS.(T4). H&E.X 400

Table (4): Effect of different levels of EDTA, VC and their combination in diets on absolute immune org weights (g) of broiler chicks at 6th weeks of age.

Treatment	Spleen weight	Bursa weight	Thymus weight	Cecal tonsil weight
T1	2.11[1]±0.43	1.96±0.30	7.98±0.82	0.68[ab]±0.08
T2	2.67±0.18	2.58±0.87	10.99±1.29	0.89[a]±0.08
T3	2.09±0.16	2.14±0.33	8.61±1.18	0.60[ab]±0.09
T4	2.46±0.27	3.09±0.41	11.90±2.08	0.62[ab]±0.11
T5	2.77±0.84	2.30±0.22	10.50±0.73	0.82[ab]±0.09
T6	2.70±0.66	2.63±0.78	10.10±1.49	0.75[ab]±0.11
T7	3.32±0.32	2.18±0.46	8.29±0.98	0.62[ab]±0.10
T8	2.32±0.34	3.22±0.47	8.52±1.74	0.76[ab]±0.14
T9	2.73±0.22	3.02±0.34	10.50±1.03	0.68[ab]±0.05
T10	2.62±0.44	2.48±0.36	9.90±1.37	0.60[ab]±0.06
T11	2.25±0.23	1.65±0.38	7.49±2.08	0.86[a]±0.06
T12	2.37±0.24	2.15±0.29	7.58±0.85	0.53[b]±0.09

Least squares means ± pooled standard error.

,b, Means having different letter exponents among columns are significantly different (P≤0.05).

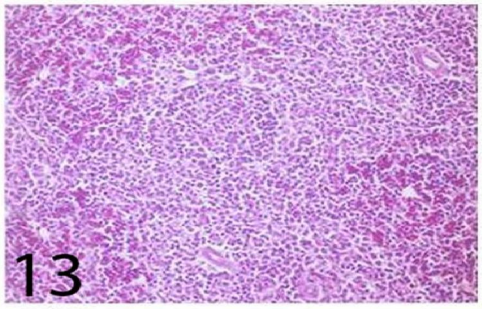

Fig. (13): Spleen showing control (normal). H&E.X 400

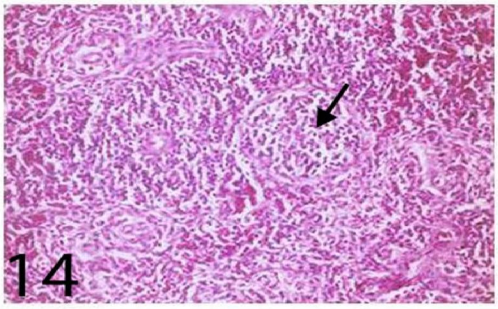

Fig. (14): Spleen showing atrophied lymphoid Follicle (T4). H&E.X 400

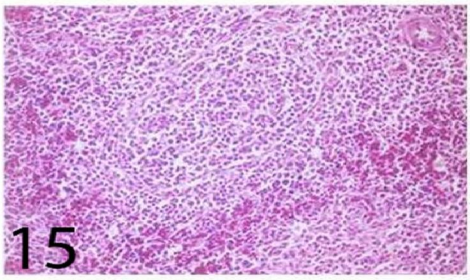

Fig. (15): Spleen showing heterophilic cells infiltration (T11) H&E.X 400

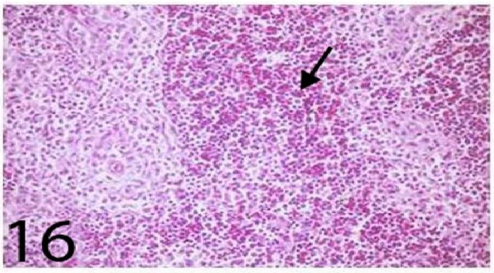

Fig. (16): Spleen showing heterophilic cells infiltration (T3) H&E.X 400

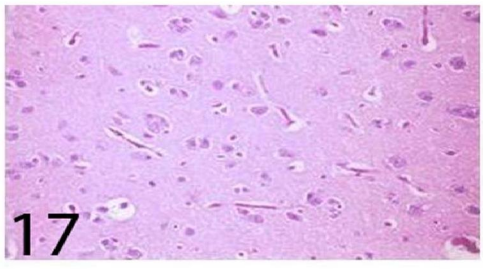

Fig. (17): Brain showing control (normal). H&E.X 400

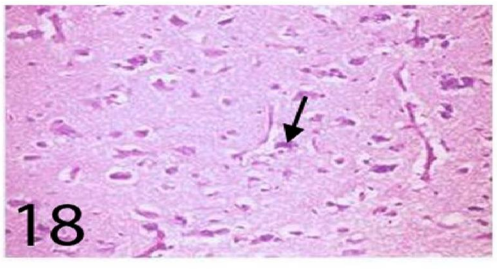

Fig. (18): Brain showing necrosis of neurons (T4&T11) H&E.X 400

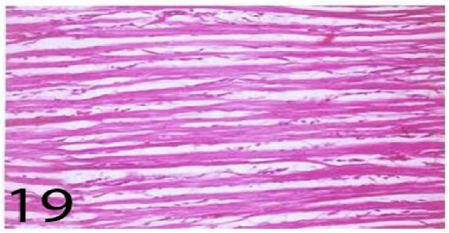

Fig.(19): Heart showing control (normal) H&E.X 400

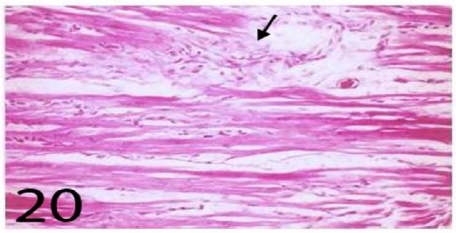

Fig.(20): Heart showing myolysis of focal myocytes (T4) H&E.X 400

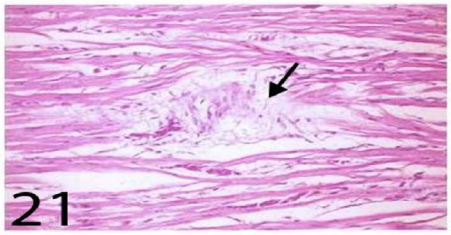

Fig. (21): Heart showing focal myocytes associated with intermuscular edema (T11) H&E.X 400

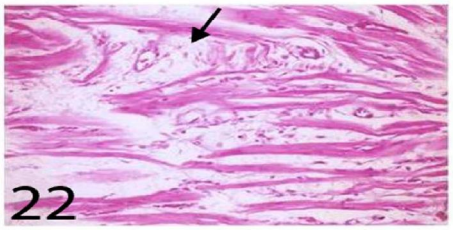

Fig. (22): Heart showing intermuscular edema (T12) H&E.X 400

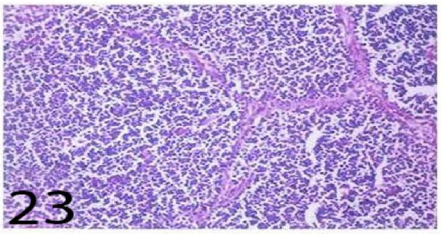

Fig. (23): Thymus gland control (normal) H&E.X 400

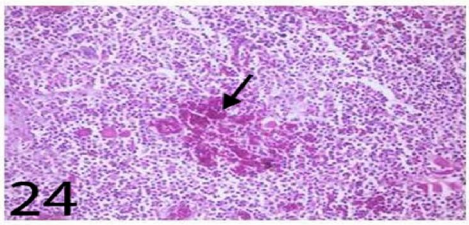

Fig. (24): Thymus gland showing focal hemorrhage (T4) H&E.X 400

Table (5): Effect of different levels of EDTA, VC and their combination in diets on cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiration rate (Rr) of broiler chicks during the experimental period (6 weeks).

Treatment	Tc (°C)	Ts (°C)	Tf (°C)	RR (r.p.m)
T1	40.62[bc]1±0.08	40.03[abcd]±0.23	34.45[abc]±0.57	76.33±3.95
T2	40.57[bc]±0.12	39.37[de]±0.28	33.83[abc]±0.91	74.00±5.73
T3	40.62[bc]±0.07	39.98[bcde]±0.24	33.03[bc]±0.56	83.00±5.88
T4	40.40[bc]±0.09	40.25[ab]±0.21	34.67[abc]±0.74	77.00±4.75
T5	40.70[ab]±0.10	40.40[ab]±0.19	35.30[ab]±0.58	79.00±2.86
T6	41.00[a]±0.21	40.75[a]±0.23	36.15[a]±0.57	87.00±2.05
T7	40.57[bc]±0.80	39.92[bcde]±0.19	35.07[abc]±0.85	84.33±2.85
T8	40.48[bc]±0.80	39.82[bcde]±0.17	33.78[abc]±0.88	80.00±4.00
T9	40.50[bc]±0.13	39.25[e]±0.38	34.65[abc]±0.83	85.33±1.84
T10	40.55[bc]±0.13	40.15[abc]±0.18	33.00[bc]±0.75	75.00±3.71
T11	40.62[bc]±0.08	40.03[abcd]±0.23	34.45[abc]±0.57	76.33±3.95
T12	40.30[c]±0.09	39.42[cde]±0.21	32.75[c]±0.75	74.00±4.29

[1] Least squares means ± pooled standard error.

a,b,c,d,e, Means having different letter exponents among columns are significantly different (P≤0.05).

Table (6): Effect of different levels of EDTA, VC and their combination in diets on chemical analysis of breast muscles of broiler chicks at 6th weeks of age.

Treatments	Moisture%	Crud protein %	Ether extract %	Ash%
T1	72.00[1abc]±0.55	20.00[c]±0.55	2.15[a]±0.08	1.50±0.37
T2	72.80[ab]±0.77	23.00[a]±0.40	1.78[b]±0.07	1.45±0.05
T3	71.25[bc]±0.66	22.20[ab]±0.37	1.44[c]±0.05	1.44±0.05
T4	71.36[bc]±0.62	22.20[ab]±0.33	0.37[h]±0.01	1.40±0.04
T5	72.70[ab]±0.55	21.50[b]±0.40	0.56[g]±0.01	1.45±0.04
T6	71.40[bc]±0.51	22.00[ab]±0.44	0.91[f]±0.03	1.43±0.04

T7	71.13^{bc}±0.55	22.20^{ab}±0.40	0.51^{g}±0.01	1.48±0.04
T8	71.50^{bc}±0.58	23.20^{a}±0.44	1.29^{d}±0.04	1.46±0.03
T9	70.25^{c}±0.44	23.00^{a}±0.33	0.55^{g}±0.02	1.43±0.05
T10	71.27^{bc}±0.44	22.30^{ab}±0.33	1.07^{e}±0.03	1.38±0.04
T11	71.45^{bc}±0.62	22.50^{ab}±0.37	0.86^{f}±0.02	1.45±0.04
T12	73.50^{a}±0.47	22.60^{ab}±0.29	0.38^{h}±0.01	1.45±0.03

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f,g,h, Means having different letter exponents among columns are significantly different (P≤0.05).

Table (7): Effect of different levels of EDTA, VC and their combination in diets on serum constituents of broiler chicks at 6th weeks of age. (ALT, AST, AP., Creat., and UA).

Treatment	ALT(GPT) Iu/ml	AST(GOT) Iu/ml	AP. u/ml	Creat. g/dl	Uric A. mg/dl
T1	39.00^{bcde}±0.68	11.00^{bc}±0.54	10.00^{d}±0.15	1.25^{abc}±0.08	5.50^{a}±0.42
T2	39.00^{bcde}±0.89	11.00^{bc}±0.59	10.50^{cd}±0.24	1.30^{abc}±0.06	5.00^{ab}±0.48
T3	41.00^{abc}±0.78	13.50^{b}±1.16	12.50^{b}±0.24	1.38^{a}±0.06	4.85^{ab}±0.43
T4	43.50^{a}±0.93	19.00^{a}±1.37	17.03^{a}±0.88	1.40^{a}±0.05	4.50^{ab}±0.45
T5	37.00^{cdef}±1.43	11.00^{bc}±0.53	10.00^{d}±0.09	1.10^{cd}±0.07	4.50^{ab}±0.51
T6	33.50^{f}±1.01	9.00^{c}±0.48	8.00^{e}±0.12	1.02^{d}±0.06	3.75^{b}±0.28
T7	39.00^{bcde}±0.83	10.00^{c}±0.92	10.50^{cd}±0.25	1.15^{bcd}±0.06	4.50^{ab}±0.46
T8	38.00^{cde}±0.92	9.50^{c}±0.68	9.50^{d}±0.23	1.15^{bcd}±0.08	4.25^{ab}±0.34
T9	36.50^{def}±1.92	11.50^{bc}±0.70	11.50^{bc}±0.23	1.15^{bcd}±0.08	3.85^{b}±0.31
T10	35.00^{ef}±1.78	11.00^{bc}±0.52	11.50^{bc}±0.25	1.25^{abc}±0.05	3.75^{b}±0.26
T11	42.50^{ab}±1.49	17.50^{a}±1.18	16.50^{a}±0.68	1.35^{ab}±0.05	4.30^{ab}±0.38
T12	40.00^{abcd}±2.34	17.00^{a}±1.37	16.00^{a}±0.90	1.25^{abc}±0.05	4.10^{b}±0.42

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (P≤0.05).

Table (8): Effect of different levels of EDTA, VC and their combination in diets on serum Chol, Trig, TP, Alb, Glob and A/G ratio of broiler chicks at 6th weeks of age.

Treatment	Chol. mg/dl	Trig. mg/dl	Tp. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio
T1	106.00^{a1}±2.05	85.00^{a}±2.41	5.43^{f}±0.20	3.25±0.06	2.18^{e}±0.17	1.49^{a}±0.17
T2	102.50^{ab}±1.47	82.50^{a}±1.29	5.70^{ef}±0.11	3.25±0.07	2.45^{d}±0.04	1.33^{b}±0.02
T3	99.17^{abc}±2.32	72.50^{b}±1.41	5.90^{de}±0.09	3.25±0.11	2.65^{bcd}±0.03	1.23^{bcd}±0.05
T4	88.50^{bcd}±2.08	63.50^{cd}±1.08	6.05^{bcde}±0.16	3.55±0.18	2.50^{cd}±0.06	1.42^{ab}±0.44
T5	92.50^{abcd}±3.59	70.00^{bc}±1.21	5.95^{cde}±0.15	3.35±0.17	2.60^{bcd}±0.05	1.29^{bc}±0.04
T6	88.33^{bcd}±3.79	58.50^{def}±1.84	6.00^{bcde}±0.15	3.40±0.16	2.60^{bcd}±0.06	1.31^{b}±0.08
T7	94.00^{abcd}±2.32	63.00^{cd}±3.26	6.00^{bcde}±0.20	3.30±0.17	2.70^{bc}±0.19	1.22^{bcd}±0.12
T8	93.17^{abcd}±3.77	61.50^{cde}±3.07	6.20^{abcd}±0.12	3.40±0.11	2.80^{b}±0.05	1.22^{bcd}±0.05
T9	87.00^{cd}±3.31	57.50^{def}±3.48	6.20^{abcd}±0.16	3.40±0.08	2.80^{b}±0.11	1.22^{bcd}±0.04
T10	82.50^{de}±4.83	53.00^{ef}±4.14	6.45^{ab}±0.11	3.30±0.10	3.15^{a}±0.03	1.05^{d}±0.03
T11	70.00^{ef}±9.01	50.00^{f}±4.60	6.40^{abc}±0.13	3.55±0.10	2.85^{b}±0.05	1.25^{bcd}±0.03
T12	67.50^{f}±8.91	50.00^{f}±4.53	6.60^{a}±0.16	3.40±0.10	3.20^{a}±0.07	1.06^{cd}±0.02

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (P≤0.05).

Table (9): Effect of different levels of EDTA, VC and their combination in diets on trace elements in breast muscles and serum of broiler chicks at 6th weeks of age.

Treat.	Ca		P		Na		K	
	Breast M. ppm	Serum mg/dl	Breast M. ppm	Serum mg/dl	Breast M. ppm	Serum mEq/L	Breast M. ppm	Serum mEq/L
T1	12.60^{a1}±0.66	11.19^{ab}±0.030	4.30ab^{c}±0.16	4.80^{ab}±0.08	119.00^{d}±1.10	119.50^{abc}±0.71	5.40^{ab}±0.18	5.25^{ab}±0.25
T2	10.30^{c}±0.14	10.35^{bc}±0.47	4.20ab^{c}±0.12	4.80^{ab}±0.08	122.00^{cd}±0.73	117.50^{c}±0.81	4.20^{e}±0.14	5.10^{ab}±0.24
T3	9.80^{c}±0.26	9.05^{de}±0.27	4.00^{cd}±0.11	4.40^{bc}±0.18	125.00^{c}±1.10	112.50^{d}±1.74	4.00^{e}±0.11	4.90^{ab}±0.26
T4	7.60^{d}±0.10	8.100^{e}±0.37	3.70^{d}±0.25	4.12^{c}±0.21	133.00^{a}±0.73	109.00^{e}±1.07	4.00^{e}±0.10	4.60^{b}±0.22
T5	12.80^{a}±0.10	11.80^{a}±0.45	4.50^{ab}±0.05	4.50^{abc}±0.19	120.00^{d}±1.10	121.50^{ab}±0.89	5.40^{ab}±0.08	5.35^{a}±0.11
T6	13.10^{a}±0.29	12.00^{a}±0.38	4.60^{a}±0.09	4.95^{a}±0.12	122.00^{cd}±1.46	122.50^{a}±1.32	5.60^{a}±0.07	5.50^{a}±0.08
T7	11.65^{b}±0.31	11.15^{ab}±0.47	4.30^{abc}±0.12	4.85^{ab}±0.13	120.00^{d}±1.46	119.50^{abc}±0.97	4.70^{d}±0.05	5.22^{ab}±0.27
T8	12.60^{a}±0.33	11.35^{ab}±0.36	4.30^{abc}±0.07	4.85^{ab}±0.11	121.00^{d}±0.73	119.50^{abc}±0.62	4.80^{cd}±0.08	5.35^{a}±0.25
T9	12.30^{ab}±0.07	10.55^{bc}±0.24	4.20^{abc}±0.08	4.80^{ab}±0.15	122.00^{cd}±1.10	118.50^{bc}±1.06	4.60^{d}±0.11	5.15^{ab}±0.09
T10	12.60^{a}±0.26	10.70^{bc}±0.16	4.30^{abc}±0.15	4.85^{ab}±0.18	120.00^{d}±1.10	119.00^{abc}±1.33	4.70^{d}±0.11	5.20^{ab}±0.18
T11	10.20^{c}±0.07	9.70^{cd}±0.30	4.10^{bc}±0.11	4.40^{bc}±0.14	129.00^{b}±0.73	114.00^{d}±1.18	5.10^{bc}±0.12	4.95^{ab}±0.26
T12	10.50^{c}±0.11	9.75^{cd}±0.21	4.00^{cd}±0.08	4.55^{abc}±0.12	130.00^{ab}±1.46	113.00^{d}±0.74	5.20^{b}±0.08	4.95^{ab}±0.24

[1] Least squares means ± pooled standard error.

a,b,c,d,e, Means having different letter exponents among columns are significantly different (P≤0.05).

Table (10): Effect of different levels of EDTA, VC and their combination in diets on heavy metals in breast muscles and serum of broiler chicks at 6th weeks of age.

Treatment	Pb (ppm)		Cd (ppm)		Zn (ppm)		Cu (ppm)	
	Breast muscles	Serum	Breast muscles	Serum	Breast muscles	Serum	Breast muscles	Serum
T1	2.60^{a}±0.04	3.50^{a}±0.06	1.40^{a}±0.02	1.25^{a}±0.08	4.33^{ab}±0.05	6.65^{abc}±0.18	0.95^{c}±0.04	1.15^{bcd}±0.04
T2	1.40^{c}±0.10	3.15^{ab}±0.09	1.00^{c}±0.03	0.78^{bc}±0.07	4.40^{ab}±0.12	5.90^{cd}±0.33	0.98^{c}±0.07	0.90^{de}±0.06
T3	1.00^{e}±0.07	2.60^{cd}±0.18	0.80^{d}±0.03	0.40^{de}±0.09	4.40^{ab}±0.20	5.25^{de}±0.57	1.20^{b}±0.04	0.75^{ef}±0.07
T4	0.62^{f}±0.03	1.70^{e}±0.11	0.77^{d}±0.09	0.16^{e}±0.03	4.50^{a}±0.09	5.00^{e}±0.46	1.30^{ab}±0.07	0.55^{f}±0.07
T5	2.40^{b}±0.11	3.20^{ab}±0.06	1.20^{b}±0.09	0.95^{b}±0.05	4.50^{a}±0.11	6.95^{ab}±0.13	1.20^{b}±0.07	1.25^{b}±0.05
T6	2.30^{b}±0.09	2.70^{c}±0.12	1.20^{b}±0.03	0.68^{c}±0.03	4.60^{a}±0.09	7.15^{a}±0.19	1.30^{ab}±0.03	1.70^{a}±0.21
T7	1.20^{d}±0.04	3.00^{bc}±0.09	0.90^{cd}±0.08	$0.73b^{c}$±0.05	4.50^{a}±0.09	6.45^{abc}±0.11	1.20^{b}±0.03	1.20^{bc}±0.04
T8	0.90^{e}±0.06	2.95^{bc}±0.06	0.80^{d}±0.01	0.68^{c}±0.08	4.40^{ab}±0.12	6.50^{abc}±0.08	1.30^{ab}±0.04	1.20^{bc}±0.05
T9	0.60^{fg}±0.03	2.65^{c}±0.11	0.77^{d}±0.01	0.42^{d}±0.13	4.50^{a}±0.11	6.40^{abc}±0.13	1.29^{ab}±0.10	1.20^{bc}±0.13
T10	0.60^{fg}±0.05	2.25^{d}±0.14	0.41^{e}±0.03	0.37^{de}±0.10	4.50^{a}±0.11	6.50^{abc}±0.14	1.30^{ab}±0.03	1.32^{b}±0.11
T11	0.44^{fg}±0.03	1.60^{e}±0.20	0.40^{e}±0.03	0.30^{de}±0.09	4.40^{ab}±0.09	6.25^{bc}±0.10	1.40^{a}±0.04	0.95^{cde}±0.05
T12	0.41^{g}±0.03	1.50^{e}±0.23	0.21^{f}±0.01	0.23^{de}±0.08	4.10^{b}±0.08	6.30^{abc}±0.19	1.33^{ab}±0.08	1.10^{bcd}±0.06

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (P≤0.05).

4. Conclusion:

Based on these results, it is showed that the tested biochemical parameters, growth performance and feed conversion were improved significantly due to EDTA and VC application and they were more pronounced with supplementation of low level of EDTA with VC, that considered as an optimum levels which could improve the health status and growth parameters of broiler chicks in this experiment. Also, addition of EDTA and VC to diets of Ross 308 chicks, reduced significantly the Pb and Cd level in both of breast muscles and serum helped to eliminate heavy metals from the bird body and in turn improved the biochemical parameters (ALT, AST, AP, Chol, TG, Creatinine globulin and uric acid) as compared to non treated birds, but in certain levels to keep the body chemical parameters in balance without any adverse effects. Histopathological examination indicated that the examined organs appear normal in treated chicks with 1g EDTA in addition to 0.5g EDTA either alone or with 0.5 and 1g VC. But the treated chicks with 2g EDTA either alone or with 0.5 or 1g VC showed variable degree of lesion including slightly focal swollen and congestion in liver, spleen, kidney and heart.

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