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EFFECT OF SUPPLEMENTATION OF ZINC AND VITAMIN-E AND THEIR COMBINATION ON PHYSIOLOGICAL TRAITS OF BROILER CHICKENS UNDER OXIDATIVE STRESS CONDITION

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Abstract

This study was carried out to evaluate the effect of adding zinc and vitamin E and their combination to the broiler chicken diet on physiological traits of broiler chickens under oxidative stress condition. Oxidative stress was introduced by adding hydrogen peroxide (H_2O_2) to drinking water from the age of 10 days with 0.5% for all treatments except the negative control treatment (T1). Two hundred eighty two, unsexed one day old broiler chicks (Ross 308) were reared at average body weight (BW) of 38 g, were randomly allotted into 8 treatments with 3 replicates per treatment (each replicate represents one pen) and 12 birds per replicate for 42 d. The treatments were included T1 free of any addition (negative control), T2 basal diet + 0.5% of H_2O_2 (positive control), T3 basal diet+ 80 of mg/kg of vitamin E, T4 basal diet + 160 mg/kg of vitamin E, T5 basal diet + 40 mg/kg of zinc, T6 basal diet + 80 mg/kg of zinc, T7 basal diet + 40 mg/kg of zinc + 80 mg/kg of vitamin E, and T8 basal diet + 80 mg/kg of zinc + 160 mg/kg of vitamin E. The obtained results of blood characteristics of broilers in 42 days showed insignificant differences between different experimental treatments in the value of hemoglobin and the number of red blood cells while T6 was significantly ($P < 0.05$) exceeded in the percentage of hematocrit compared to all experimental treatments. In regard to the number of white blood cells, the percentage of heterophils, rate of sedimentation velocity of blood cells, it was observed a highly significant ($P < 0.01$) in T2 compared to all experimental treatments and with respect to the percentage of lymphocytes T6 treatment was recorded a highly significant ($P < 0.01$) compared to all experimental treatments, as well as for the characteristics ofbiochemical blood plasma, T2 recorded a highly significant ($P<0.01$) in the glucose of blood plasma compared with other experimental treatments in addition to a highly significant ($P < 0.01$) in T1 in the total protein rate was observed which was not significantly different with T5, T7 and T8. In terms of the albumin level, it was highly significant ($P < 0.01$) in T2 compared to all experimental treatments, while the level of globulin was significantly ($P<0.05$) exceeded in T1 compared to T2 and T6. For the level of cholesterol, T2 was significantly exceeded compared to the other treatments, and with regard to the level of zinc, T8 did not differ significantly with T1, T3 and T6. In relation to the efficacy of the AST enzyme, T2 was significantly ($P<0.05$) exceeded compared to other treatments, and with regard to the effectiveness of the enzyme ALT a significant decrease ($P<0.05$) in T2 compared with other experimental treatments was observed. Additionally, T2 showed a significant increase in the level of Malondialdehyde (MDA), the Peroxide Value (P.V) and Free Fatty Acid (FFA) in liver tissue compared to the other treatments.

Key words : Malondialdehyde (MDA), broiler chicken, oxidative stress, blood cells.

Introduction

The broiler breeding has been of great interest by many researchers and educators. Many methods have been applied to reduce diseases resulting from metabolic processes, as well as trying to find solutions that are not cost-effective, reduce the economic losses of breeders and improve the productive performance of meat breeds to meet the market needs of poultry meat. In poultry, oxidative stress can be defined as an imbalance between the production of free radicals and their elimination by antioxidants, causing tissue damage and health problems for birds (Pand and Cherian, 2014). The absence of antioxidants in the diet leads to an increase in the level of free radicals within the body and then increase the oxidation processes offset by weakness in the defense system, which leads to the accumulation of antioxidant fat peroxidation in the liver cells (Lipid peroxidation). These factors result in damage in the cell membrane ofhepatocytes, internal structures of the cell and its vital components (Christaki, 2012). Antioxidants are substances that prevent oxidation and chemical reactions that use oxygen and thus protect vital components in living cell casings from damage and reduce damage to cells by free radicals within the body (Balakumer *et al*., 2010).

The aim of adding antioxidants is not only to reduce stress, but also to be safe and economical. It is noticed recently that international companies have adopted several ways to modify the diet of poultry and in line with productivity and health. Among these substances as antioxidants in poultry diets are vitamin E and zinc (Zang *et al*., 2014 and Lopes *et al*., 2015). Zinc is of great importance for its important role in the metabolic processes that are involved in building the body and strengthen its immunity. It is also an important metal element added to poultry diets, which plays a large role as antioxidant and improve the rate of growth and immune response. Some studies suggest that zinc should be added to the diet by 40 ppm, according to the National Research Council's recommendations of 40 mg/kg and 75 mg/kg of diet (NRC, 1994). Vitamin E is one of the most important types of vitamins that are soluble in fat. It is an antioxidant in the body of the bird and is important for the maintenance of unsaturated fatty acids. Vitamin E also plays an important role in destroying the free radicals that attack the cell membrane.The plasma lipoproteins provide protection for the cell by interacting with the peroxid roots (Robert *et al*., 2006,). Due to the lack of studies on the use of vitamin E and zinc in broiler diets, the aim of the present study was to estimate their effect on the physiological characteristics of broiler chickens and determine the best level to add them under oxidative stress condition.

Materials and Methods

This study was conducted in the poultry field of the Department of Animal Production, Faculty of Agriculture, Anbar University in the alternative site (Abu Ghraib) for the period from 6th of October 2016 to 16th of November 2016 (42 days). Zinc and vitamin E were added to the diet individually and as a combination at the age of one day old until the end of the experiment, and developed oxidative stress by adding hydrogen peroxide (H_2O_2) to drinking water from the age of 10 - 42 days, at a concentration of 0.5% of drinking water for all treatments except the treatment of negative control, twice daily as water was replaced at 8 am and 2 pm to ensure the continued effect of hydrogen peroxide. In this experiment, 288 non-natural broiler chickens (308 Ross) at one day, with an average weight of 38 g of Belgian origin, who received the chick from the Rafidain hatch in Abu Ghraib. The chicks were randomly distributed into 8 treatments with 3 replicates per treatment and per replicate contained 12 birds. The experimental treatments were included: Second treatment: T1 free of any addition (negative control), T2 basal diet + 0.5% of H_2O_2 (positive control), T3 basal diet+ 80 of mg/kg of vitamin E, T4 basal diet + 160 mg/kg of vitamin E, T5 basal diet + 40 mg/kg of zinc, T6 basal diet + 80 mg/kg of zinc, T7 basal diet + 40 mg/kg of zinc + 80 mg/kg of vitamin E, and T8 basal diet + 80 mg/kg of zinc + 160 mg/kg of vitamin E. Birds were fed on the starter, grower and finisher diet as shown in Table 1 and follow the health and preventive program recommended by the veterinarian specialist for the duration of breeding.Zinc was added in the form of $ZnSO_4.7H_2O$ ($ZnSO_4.7H_2O$) as obtained from Zidane Scientific Office located in Baghdad Governorate /BDH England in the form of crystalline white powder color purity of 99.5%, while vitamin E and hydrogen peroxide obtained from the Office of Bashir scientific in the Baghdad Governorate, Bab Al-Ma'adam, where the vitamin in the form of alpha-tocopherol (α- tocopherol) produced by the company Asure Biotech Chinese powder white color purity of 95%.One way analysis was carried out. The trend included the effect of the eight coefficients using the General Linear Model and the SAS statistical software version 9.1 (7) was used. Morphological differences between mean values were tested using the Duncan Multiplicity test (11) at the mean of 0.05 and 0.01.

Results and Discussion

Effect of experimental factors in Blood Cell characteristics

Table 2 shows the effect of the experimental treatments on cellular blood characteristics. The level of hemoclobin was highest in T1 (negative control), followed by T3, T6, T5, T7, T8, T4 and T2 respectively, without any significant differences between treatments.A significant difference ($P <0.05$) in blood mass was noted in T6 compared to T2, while no significant differences were found between T6 and T1, T3, T4, T5, T7 and T8 in the average of blood mass rates . As for the number of red blood cells, the results showed that the highest value was in T5. The results showed that different experimental factors have given different values of red blood cells can be arranged in ascending order T5, T1, T3, T6, T4, T7, T8 and T2 respectively, while no significant differences were found between all experimental parameters. The mathematical superiority of the number of red blood cells in T5 may be due to the importance of zinc in preventing cell damage while acting as an antioxidant and protecting plasma membranes from damage caused by oxidative stresses as well as zinc associated with many enzymes

Table 1 : Percentages and calculated chemicalcomposition of the components of the rations used in the experiment.

Feed composition %	Starter diet (1-11 day)	Grower diet (12-22 day)	Finisher diet (23-42 day)
Yellow corn	54,7	59,7	61
Wheat	7	7	7
Soybean meal 48% protein	30	24	21,5
* Protein concentration	5	5	5
Corn oil	1	2	3,5
Dicalcium phosphate	0,55	0,4	0,2
Limestone	1,1	1,2	1,2
Methionine	0,16	0,17	0,14
Lysine %	0,19	0,2	0,16
Salt	0,3	0,3	0,3
Total	100%	100	100
Chemical composition			
kcal/kg	2978	3089	3207
Crude protein %	21,9	19,4	18,3
Lysine %	1,39	1,24	1,13
Methionine +Cysteine	1,02	0,96	0,90
Crude fiber	2,7	2,6	2,5
Calcium	0,90	0.88	0,83
Phosphorus	0,45	0,42	0,38
Zinc ml/kg	41,56	38,34	36,98
VitaminE (ml/kg)	43,94	44,84	45,01

* Brocorn-5 special W is produced by WAFI BV ALBLASSERDAM HOLLAND, which contains 40% raw protein, 5% crude fat, crude fiber 2,20%, moisture 7.13%, ash 28,32 , Calcium 4,50%, phosphorus 2,65%, phosphorus available 4,68%, lysine 3,85%, methionine 3,70%, methionine + cystine 4,12%, tryptophan 0.42%, threonine 1,70% , Energy represented 2107, selenium 2.30%, copper 4%. ** According to the chemical composition values by N.R.C.

that are essential for maintaining the integrity of the cells involved in the immune response (Khan *et al*., 2016). In addition, it contributes to the increased absorption of the mineral elements such as iron, which is the basis of the hemochlorine synthesis, (Sanchez *et al*., 2009; Kendall *et al*., 2012). Zinc deficiency is also affected by many vital functions of the organism, including the effectiveness of thyroid gland and its secretion to thrombocytopenia (T3) and thiroxine (T4) (Sahin *et al*., 2011).

Morley *et al*. (1980) reported that the low serum zinc level was accompanied by a reduction in the level of thyroid hormone stimulating hormone TSH from the pituitary gland, which reduced the level of thyroid hormones such as Triyodothronine (T3) and Thiroxne (T4) which playedan important role in formation of red blood cells by increasing the rate of gene expression of the bio-synthesis of Erythropoietin, a substance of proteins and carbohydrates that directly affects the division of the red blood cells (Erythroid) and their transformation into red-cell aromas containing hemoglobin Hb (Yluan *et al*., 2004). Therefore, the reduction in the number of red blood cells in T1 may be due to the decrease of Erythropoietin, which is associated with the level of thyroxine, that affected by the effectiveness of the thyroid correlated with the level of zinc in the blood, which is reflected directly on the levels of Hb and PCV. Since these traits behave in different conditions, the addition of zinc to birds' diet under stress conditions can compensate for the decline or maintain the normal level of blood. The reason for the significant increase in the other experimental treatments compared with the T2 may be due to the individual or combined effect of adding zinc and vitamin E to the diet and their apparent role as antioxidants in the diets. Saadi (2008) demonstrated that vitamin E plays a major role in maintaining red blood cell membranes from the process of oxidation occurring in the body, reducing its degradation and protecting the plasma membranes from the damage caused by the oxidizing factors therefore, the height of the blood mass is observed. Al Khatib (2000) detected that an increase in blood mass of the broilers with increasing the level of vitamin E supplementation was noted due to the role of this vitamin in the preservation of the membranes of oxidation processes and reduce its degradation as this leads to an increase in hemoglobin and blood mass rates, the result of this study with the findings of Abdul Wahid and Zuhairi (2009), who included the addition of vitamin E in broiler chicken diets, significantly raised the proportion of Hb and PCV compared with control diets. The results of this study were also consistent with the findings of Al Khuzaie (2013), who observed a significant improvement ($P < 0.05$) in Hb and PCV for 42-day-old broilers for the treatment of vitamin E supplementation.

The cause of the reduction in T2 in the number of erythrocytes, hemoglobin and blood mass may be due to oxidative stress, which leads to damage in the cells and living tissue and thus affects the overall health of the bird (Salami, 2015). The addition of zinc to the diet was agreed with the findings of Jawad *et al*. (2014), who noted that the addition of zincat the level of 30 and 45 mg / kg was fed to broiler chicken for a period of 42 days, resulting in significant superiority ($P < 0.05$) in RBC, Hb and PCV compared with other experimental treatments.

The results of the statistical analysis of white blood cells (WBC) showed a significant superiority ($P<0.01$) in T2 compared with the other treatments. T7 was significant in the number of WBC and did not differ significantly with treatments T1, T3 and T6. The percentage of heterozygous cells was significantly higher ($P <0.01$) in T2 compared with the other treatments that did not differ significantly between them. The results of the lymphocyte ratio were significantly higher ($P <0.01$) in favor of T6 compared with T2 and no significant differences were observed between T1, T3, T4, T7 and T8. The reduction in the ratio of heterozygous cells and the proportion of heterozygous cells/lymphocytes in treatments supplemented with zinc and vitamin E compared to T2 may be due to the individual role or combination of zinc and vitamin E as effective agents against free radicals. Sun *et al*. (1993) and Kidd *et al*. 1996) revealed that zinc was an essential element in the growth, development and effectiveness of the immune system in animals through its association with enzymes necessary for the safety and effectiveness of immune cells, which plays an important role in increasing the numbers of T lymphocytes and antibodies, killer cells,and macrophage cells, hence the lack of zinc in the body leads to a decrease in the weights of the lymphatic organs (thymus, fabricia and spleen). This was accompanied by low initial and secondary immune response, antibody production and high heterozygous cells, which are an accurate indicator of the degree of stress in birds (Barlett and Smith, 2003; Mashaly, 2004; Cui *et al*., 2004). Several studies have shown the effect of zinc deficiency in lymphatic organs and their ability to produce lymphocytes through various mechanisms, including lymphatic atrophy and lack of lymphocyte production (Roy and Dwivedi, 2015). The decreased lymphocytes in T2 may be due to the high significant decline ($P <0.01$) obtained for the level of zinc in blood plasma birds of this treatment as in Table 13which may be due to depletion of the body by its role as an antioxidant and its absence of sufficient quantities in the regular diet provided to broiler, as well as the absence of other antioxidants. The results of this study were consistent with Hosseini *et al*. (2010), who detected that adding mixture of zinc and vitamin E (100 + 50 mg/kg feed) to the broiler chicken under normal conditions or under heat stress conditions, resulted in a significant decrease ($p<0.05$) in the proportionof heterozygous cells/lymphocytes compared to control treatments.

As for the individual role of vitamin E, this finding was consistent with the findings of Habibian *et al*. (2014), who indicated thatsupplementation of vitamin E at levels 125, 250 mg/kg to the 49-day broiler in normal and heat stress conditions, increased ($P <0.05$) the rate of lymphocyte ratio. There was also a significant decrease ($P <0.05$) in the ratio of heterozygous cells and the proportion of heterozygous cells /lymphocytes when vitamin E was added at both levels. As for the individual role of zinc, this finding was consistent with the findings of Jawad *et al*. (2014) who noted that the addition of zinc at 30 and 45 mg/kg to broiler diet at 42 days resulted in a significant decrease ($P <0.05$) in the ratio of heterozygous cells and the proportion of heterozygous cells / lymphocytes in addition,a significant superiority ($P <0.05$) was also observed in the proportion of lymphocytes.

Effect of experimental treatments in biochemical properties of blood

The results of the statistical analysis in table 3 showed the effect of the treatments on the biochemical blood characteristics, with a significant superiority ($P<0.01$) recorded in T2 which did not differ significantly with T3 as well as T1 and T8 had a significant decrease ($P <0.01$) with insignificant differences with T4, T5, T6 and T7 treatments. The current results can be attributed to the effect of zinc on regulating the secretion of insulin hormone, which helps in reducing blood glucose levels (Faure *et al*., 2007), as well as the role of zinc in the metabolism of carbohydrates, including glucose and reduce their high levels and then reduce the level of glucose in the serum (Alvarez *et al*., 2007). The effect of vitamin E in reducing serum glucose is due to the ability of vitamin E to enhance the role of antioxidants in the cell and reduce the effect of oxidative stress, which activates the work of the body cells, including pancreatic beta cells that activate the secretion of insulin, resulted in a reduceof blood glucose (Al-Qattan, 2006). Exposure of birds to different environmental stressors, especially oxidative stress, leads to increased secretion of corticosterone, which works on the processing of glucose from non-carbohydrate sources, especially protein. The effect of corticosteroids on the metabolism of proteins through its effect in many enzymes in the liver, such as ALT and AST, which have a role in the process of glucose synthesis of protein sources and relies on the provision of amino acids resulting from the demolition of protein in many tissues of the body (Panda *et al*., 2008). It was noted that the total protein level in blood plasma showed a high significant superiority ($P <0.01$) in T1 compared to T2, T3, T4 and T6, and no significant differences were observed in T5, T7, and T8. Furthermore, a significant decrease was noted in T2 compared with T1, T5, T7 and T8 with mean value of 3.43 g/100 ml, the significant

Table 2 : The effect of Individual and combination of zinc and vitamin E supplementation on blood characteristics of broilers at 42 days.

Traits	Treatments								L.S.D
	T1	T2	T3	T4	T5	T6	T7	T8	
Blood Hb (g/100Ml)	9.96±0.480*	8.76±0.491	9.90±0.435	9.23±0.290	9.73±0.202	9.90±0.346	9.46±0.352	9.36±0.088	N.S**
Blood mass %	32.00±1.52ab	28.00±1.73b	31.66±1.45ab	29.66±0.881ab	31.00±0.577ab	32.33±1.20a	30.33±1.20ab	30.00±0.577ab	0.05
RBC(10^6/µl)	2.80±0.316	2.30±0.121	2.73±0.208	2.66±0.137	2.81±0.063	2.68±0.139	2.51±0.132	2.48±0.025	N.S
WBC(10^3/µl)	18.96±0.240cd	28.76±0.480a	20.50±0.378bcd	22.06±1.03b	21.50±0.608b	19.20±1.28cd	18.56±0.272d	20.90±0.624bc	0.01
Heterozygous cells%	64.33±2.33b	75.66±1.45a	64.33±2.96b	65.33±0.666b	68.66±0.881b	63.00±2.08b	67.33±1.45b	63.66±1.20b	0.01
Lymphocytes %	34.00±2.64ab	22.33±1.85c	33.66±2.40ab	32.66±1.20ab	29.00±0.577b	35.00±2.08a	31.00±1.15ab	33.66±0.881ab	0.01

* Values represent the average error.[a, b, c]: The different letters within one row indicate significant differences between the coefficients at the level of significance (P00.01) and (P00.05). T1: Control without any addition. T2: Control with the addition of H2O2 to drinking water. T3: Add vitamin E to the mixture at 80 mg / kg feed. T4: Add vitamin E to the mixture at a level of 160 mg / kg feed. T5: Add zinc to the mixture at 40 mg / kg feed. T6: Zinc supplement for the mixture with a level of 80 mg / kg feed. T7: Add zinc mixture + vitamin E (40 + 80 mg / kg feed, respectively). T8: Add the zinc mixture + vitamin E (80 + 160 mg / kg feed respectively).

decline in T2 may be due to oxidative stress within the body caused by hydrogen peroxide, which promotes increased free radical production for different types of active oxygen (ROS) which leads to increasing protein oxidation in plasma resulting in protein loss and reducing level of protein in blood plasma (Sharma *et al*., 2008). The natural concentration of the total protein in the blood plasma of birds ranges from 6-3 g/100 ml (Al Darragi *et al*., 2008). The total protein has an important role in maintaining the balance of fluid volume between blood and tissue in addition, basal acid balance. It is also a vector of many food compounds between tissues in the body such as carbohydrates, fats, vitamins, hormones and mineral salts. Moreover, it plays an important role in the synthesis of enzymes, Genetic and immune systems in addition to balancing blood pressure in the tissues of the body (Nelson and Cox, 2004). Therefore, increasing total protein concentration in blood plasma (within normal limits) is considered a good indicator of the health status which may be due to the high level of total protein in the addition of zinc and vitamin E compared to T2 to the individual role or the combination of zinc and vitamin E as antioxidants during the removal of free roots and maintenance of cells and tissues from damage and the persistence of metabolic processes in the body better than in T2, as zinc has a major role in regulating protein metabolism and protecting it from destruction by preventing oxidation in cellular membranes (Jia *et al*., 2008; Xiao *et al*., 2 011). In addition to the zinc inclination in the formation of complexes with amine groups, carboxylic and thiol in amino acids and proteins, approximately 300 enzymatic reactions depend on the presence of zinc (Bozalioglu *et al*., 2005 and Mertens, 2014).

Additionally, zinc can help build protein by increasing the level of thyroid hormones (Park *et al*., 2011). The results showed the effect of various treatments in the level of albumin, as it found that T1 has obtained a high superiority significantly (P <0.01), as it achieved 3.40 g/ 100 ml compared to the rest of the experimental treatments was also observed that T2 was decreased significantly compared with T1, T5, T7 and T8 and this may attributed to the oxidative stress in the body of the bird.Koller (1984) pointed that the albumin was the main part the total protein in the blood as it is synthesized by the liver and recognized as an indicator forprotein formation, and low Albumin ratio was evidence that the liver function has been reduced due to damage in liver tissue. The results obtained showed that T8 was significantly higher (P <0.05) than T2 and T6 were not significantly different with T1, T3, T4, T5 and T7. T8 was exceeded may be due to the combination effect of zinc and vitamin E, both of which contribute to the development and regulation of the immune response of broilers (Niu *et al*., 2009; Naz *et al*., 2016) as well as zinc has a structural and vital role in many peptides, proteins and growth factors in the body (Bozalioglu *et al*., 2005). The role of vitamin E in increasing the concentration of globulin in blood plasma was due to its function in stimulating the humoral immune response in the body by increasing the number of lymphocytes ß which in turn lead to increased immunoglobulin in addition to increasing the effectiveness of lymphocytes type T through this immune stimulation works to increase the production of lymphocytes (Lymphokines) as it works to increase the use of nutrients during the process of digestion and metabolism within the body, which leads to increased process of protein synthesis in the body (Boa-Amponsem *et al*., 2000). The present results agreed with results of Hosseini *et al.* (2010), who found that adding of mixture of zinc and vitamin E (100 + 50 mg/kg respectively) to the broiler diets under normal or heat stress conditions led to a significant increase (P <0.05) for the two treatments in the protein level of blood plasma compared to T1. Additionally, the current results consisted with previous work of ZEWeil *et al*. (2015), who observed a significant increasein total protein and globulin in blood plasma when vitamin E was added to broiler diets aged at 7 to 42 days compared to different experimental treatments. The results of the blood cholesterol level showed a high significant superiority (P <0.01) in T2 compared with the other treatments. It was also observed that T8 recorded a significant decrease in cholesterol level in blood plasma in addition to insignificant differences between T8 and T1, T4, T5, T6, and T7 were detected. The significant reduction in T8 may be due to the combined effect of increasing the level of vitamin E and zinc in the diet for their role in inhibiting free radicals and increasing the level of vitamin E in the diet may increase the deposition of vitamin E inside the body, which stored with fat in the fatty tissue. This process enhances its role in protecting cellular membranes from oxidative damage by maintaining the electoral permeability of the cell wall, because vitamin E does a complex job with unsaturated fatty acids and thus prevents leaking cholesterol and fat out of membranes cellular sites resulting from fat oxidation reactions (Mohamed, 2013 and Salami *et al*., 2015). As well as high significant exceeded(P<0.01) in T2 compared with other experimental parameters may due to the highlysignificant decline (P <0.01) in the zinc level in blood plasma of T2 (table 3) may be due to depletion of the body. As it works as an antagonist of free radicals and the absence of other antioxidants in the diet, as zinc deficiency in the body affects many of the vital activities of the organism, including the effectiveness of the thyroid gland and secretion of thyroxine, which leads to increased representation of cholesterol and utilization rate and then decrease the level of cholesterol (Iqbal *et al*., 2009 and Melesse *et al*., 2011). Vitamin E also plays a major role in lowering the level of cholesterol in blood plasma. Mazier *et al*. (2007) found that a significant decrease in serum cholesterol concentration in vitamin E-treated treatment compared to those with no vitamin E supplementation was detected. This result was consistent with Hosseini *et al*. (2010) who noted that Adding a mixture of zinc and vitamin E (100 + 50 mg/kg respectively) to broiler diet under natural conditions or under heat stress conditions (P <0.05) showed a significant decrease in cholesterol level in blood plasma compared with control treatments. As for the individual role of vitamin E, the results of this study were consistent with the findings of Al Khuzaie (2013), who observed a significant decrease in cholesterol level in the blood plasma of broilers at the age of 42 days in treatment supplemented with vitamin E to the diets contained rancid oil compared to the control treatment. As well, the results obtained of the present study was consistent with the findings of Habibian *et al*. (2014), who observed that adding vitamin E at levels 125 and 250 mg/kg to broiler diet at 49 days in normal and heat conditions, resulted in a significant decrease (P <0.05) in cholesterol level of blood plasma for both treatments compared with the treatment of control. Furthermore, adding vitamin E to broiler chickens aged 7 to 42 days, led to a significant decrease (P <0.05) in the cholesterol level ofblood plasma compared to other treatments(Zeweil *et al*., 2015). In contrast, this finding was not agree with finding of Fadaam (2016), who revealed that a significant increase (P <0.05) was noted in the concentration of cholesterol in the treatment of vitamin E supplementation compared with the other treatments. The level of zinc in blood plasma was significantly higher (P <0.01) in T8 compared with other treatments with insignificant differences with T1, T3 and T6. This significant reduction of zinc level in T2 may be attributed to zinc depletion of blood plasma due to its use as an antioxidant in the body. Normally, AST and ALT enzymes found in the cells of various tissues of the body, but when a crash of cells in a particular tissue happen,these enzymes will enter into the bloodstream and thus raise, it is level and this gives evidence of a defect either because of disease or bruising (Lu *et al*., 2014). Hence, the presence of AST and ALT levels in the bloodstream was evidence of the overall health of the body's tissues. The results of the statistical analysis showed the effect of the different treatments on the level of the AST enzyme. T2 showed a significant superiority ($P <0.05$) in AST activity compared withthe other treatments in addition no significant difference was noted between T2 and T1, T4, T5 and T6. Moreover, no significant difference was observed with T7 and T8. The results showed that the T2 was significantly higher ($P <0.05$) than the other treatments, with a value of 14.00 (IU/L) and no significant difference with T5, T4, T3 and T6 was revealed. In terms of T1, which achieved a value of 7.66 (IU/L) and did not differ significantly with T7 and T8. The significant reduction in the level of AST and ALT in T7 and T8 may be due to the combined effect of zinc and vitamin E in improving health status. Zinc has a role in protecting tissues from free radicals and stimulating immune response to different diseases (Yuan *et al*., 2010). With regard to the role of vitamin E, Abdul Wahid and Zuhairi (2009) demonstrated that vitamin E raise the vitality of the body and general health of birds as well as its role in stimulating the immune system, which can be concluded that zinc has a stronger effect as an antioxidant when added with vitamin E can be more effective in reducing the damage in the body cells resulting from the effect of free radicals and improve the state of health and immune response to various diseases. The ability of various experimentation to reduce the activity of ALT and AST may be due to its ability to enhance the activity of the enzyme ClotathionePyroxidase (GSH-Px) in blood plasma and reduce the oxidation catalysts such as metal ions by preventing their release from the tissuein the liver and then break down the free radicals reaction sequences and reduce their production and composition, especially the free radicals of the active oxygen species (ROS) and then protect the polyunsaturated fatty acids in the cellular membranes fromthe oxidation and protect the liver membranes from damage and maintain the properties of these membranes and the most important characteristic of the optional permeability, which prevent infiltration and leakage of these enzymes from the cell to the outside in the blood (Dani *et al*., 2008).

The effect of treatments on the level of Malondialdyhide (MDA) and the value of peroxide and free fatty acids in liver tissue

The results of the statistical analysis in table 4 showed that the addition of zinc and vitamin E alone or in combination has achieved high effectiveness as antioxidants in the control of lipid oxidation and inhibition of lipid peroxidation in liver, T2 was significantly higher ($P <0.05$) in the liver tissue and the mean value of 0.246 mg/kg wet tissue and T8 was lower in MDA in liver tissue followed by T7. This result was agreed with Hosseini *et al.* (2010), who pointed that adding mixture of zinc and vitamin E (100 + 50 mg/kg respectively) under natural or heat stress conditions had a significant decrease ($P <0.05$) in the level of MDA in blood plasma compared with control treatment which can be attributed to the role of antioxidants in cell protection, which prevents the production of MDA in the liver by increasing the production of the enzyme Clotathione peroxidase (GSH-Px), which protects the tissues from oxidative damage by removing the peroxides free radicals, especially in cases of stress. The value of peroxide (PV) in the liver tissue was significantly superior ($P <0.01$) in T2 with mean of 3.82 mm/kg wet tissue compared to the other treatments. This value was acceptable, according to Egan *et al*. (1981), the fat is unacceptable when the value of the peroxide 10 mm/kg wet tissue as confirmed by the Iraqi standards that reported the same value mentioned above (Central Organization for Standardization and Quality Control, 1978). The addition of zinc and vitamin E has been shown to be highly effective as antioxidants in controlling the oxidation of fat in liver by reducing the development of peroxide value in the liver and notes from the results obtained thatthe value of peroxide varies from one treatment to another as it can be arranged upward to the highest value, such as T1, T8, T7, T4, T6, T3, T5 and T2 respectively and this indicate the antioxidants activity. This results in the effect of reaching the highest peroxide value, which increases the duration of the accumulation of peroxides and thus reduces the value of peroxide. The level of fatty acids in the liver tissue was significantly higher exceeded in T2 with mean value of 1.43% compared with other treatments which had insignificant difference between them, hence the results showed that all the treatments were highly effective as antioxidant properties by reducing the decomposition and release of free fatty acids in the liver of the broiler, because the antioxidants provide protection to fat membranes through its interaction with free radicals and break the chain of lipid oxidation reactions and then reduce or prevent the release of free fatty acids (Akarpat *et al*., 2008). The reason for this high significant superiority recorded in T2 in the value of MDA, PV, and FFE in the liver tissue may due to the development of oxidative stress within the body of birds through the addition of hydrogen peroxide (H_2O_2). Loven and Oberley (1985) revealed that adding hydrogen peroxide to the drinking water of rats led to a series of chemical reactions leading to internal oxidative stress by increasing the production of oxygen in the gastrointestinal tract, which in turn enter to the blood resulting in high oxygen pressure (Oxygentension) in the cells and this leads to an excessive increase in the production of active

Table 3 : The effect of Individual and combination of zinc and vitamin E supplementation on the biochemical characteristics of broilers at age 42 days.

Traits	Treatments								L.S.D
	T1	T2	T3	T4	T5	T6	T7	T8	
Glucose (mg/dl)	172 ± 4.37c	217 ± 12.9a	200 ± 3.17ab	180 ± 5.54bc	181 ± 4.33bc	184 ± 5.13bc	178 ± 9.64bc	167 ± 4.04c	0.01
Total protein (g/100 ml)	4.86 ± 0.120*a	3.43 ± 0.145d	3.63 ± 0.260cd	3.76 ± 0.120bcd	4.36 ± 0.284abc	3.83±0.088bcd	4.46 ± 0.328abc	4.60 ± 0.529ab	0.01
Albumin (g/100 ml)	3.40 ± 0.057a	2.20 ± 0.057e	2.26 ± 0.120de	2.33±0.088cde	2.80 ± 0.321bc	2.70±0.152bcde	2.86 ± 0.066b	2.73 ± 0.218bcd	0.01
Globulin (g/100 ml)	1.46 ± 0.120ab	1.23 ± 0.088b	1.36 ± 0.185ab	1.43 ± 0.185ab	1.56 ± 0.088ab	1.13 ± 0.088b	1.60 ± 0.264ab	1.86 ± 0.317a	0.05
Cholesterol (mg/dl)	116.6 ± 4.63bc	161.6 ± 6.38a	128.0 ± 5.56b	121.3 ± 10.4bc	111.6 ± 4.70bc	107.3 ± 11.8bc	112.3 ± 4.25bc	97.0 ± 7.76c	0.01
Zinc(mg/dl)	88.33 ± 2.02abc	74.66 ± 2.90d	87.00 ± 1.52abc	80.33 ± 1.20cd	81.66 ± 3.17bcd	90.33 ± 3.84ab	80.66 ± 2.33cd	95.33 ± 3.28a	0.01
ActivityAST(IU/L)	6.00 ± 0.577c	12.66 ± 2.02a	11.33 ± 1.85ab	10.66 ± 1.45abc	9.33 ± 0.881abc	10.66 ± 2.60abc	7.33 ± 0.881bc	7.18 ± 0.881bc	0.05
ActivityALT(IU/L)	7.66 ± 0.881b	14.00 ± 2.08a	12.33 ± 0.881ab	10.00 ± 1.66ab	10.66 ± 1.73ab	11.66 ± 1.85ab	9.00 ± 0.577b	8.66 ± 0.666b	0.05

* Values represent the average ± standard error, [a, b, c]: different characters within a single row indicate that there are significant differences between the treatments at the abstract level (P≤0.01) and (P≤0.05), T1: Control without any addition. T2: Control with the addition of H_2O_2. T3: Addition of vitamin E to the mixture at the level of 80 mg / kg feed. T4: Vitamin E supplement for the level of 160 mg / kg feed. T5: Add zinc to the mixture with 40 mg / kg feed. T6: Zinc supplement for the mixture with a level of 80 mg / kg feed. T7: add a mixture of zinc + vitamin E (40 + 80 mg / kg feed respectively). T8: add a mixture of zinc + vitamin E (80 + 160 mg / kg feed respectively).

Table 4 : The effect of Individual and combination of zinc and vitamin E supplementation on the level of Malondialdihyde, peroxide and free fatty acids in the liver tissue of the 42-day-old meat.

Traits	Treatments								L.S.D
	T1	T2	T3	T4	T5	T6	T7	T8	
Malondialdihyde ml/kg (MDA)	0.149 ± 0.034ab	0.246 ± 0.025a	0.131 ± 0.027b	0.135 ± 0.013b	0.154±0.024Ab	0.149±0.041ab	0.130 ± 0.025b	0.107 ± 0.039b	0.05
Peroxide value (PV)ml/kg	1.02 ± 0.092e	3.82 ± 0.179a	2.41 ± 0.233bc	2.14 ± 0.104c	2.59 ± 0.107B	2.24 ± 0.061bc	1.64 ± 0.116d	1.11 ± 0.098e	0.01
Free fatty acids (FFA)%	0.353 ± 0.024b	1.433 ± 0.074a	0.580 ± 0.091b	0.493 ± 0.100b	0.513 ± 0.104B	0.553 ± 0.013b	0.420 ± 0.057b	0.373 ± 0.040b	0.01

* Values represent the average ? standard error, [a, b, c]: different characters within a single row indicate that there are significant differences between the treatments at the abstract level (Pd"0.01) and (Pd"0.05), T1: Control without any addition. T2: Control with the addition of H2O2 to drinking water at the age of 10 days. T3: Addition of vitamin E to the mixture at the level of 80 mg / kg feed. T4: Vitamin E supplement for the level of 160 mg / kg feed. T5: Add zinc to the mixture with 40 mg / kg feed. T6: Zinc supplement for the mixture with a level of 80 mg / kg feed. T7: add a mixture of zinc + vitamin E (40 + 80 mg / kg feed respectively). T8: add a mixture of zinc + vitamin E (80 + 160 mg / kg feed respectively).

oxygen compounds (ROS) including hydrogen peroxide (H_2O_2). In addition, the absence of antioxidants in the diet leads to increased processes of oxidation resulting from free radicals that destroy the biological molecules in the body cells and this imbalance in the system of balance existing in the body between the production of free radicals and resistance to oxidation by existing antioxidants (Lu *et al*., 2014), or because of the direct inhibitory effect of hydrogen peroxide and other types of free radicals in the activity of various antioxidant systems produced within the body such as GSH-PX, GSH, SOD and CAT Restrain free radicals and peroxides in the body (Shehata and Yousef, 2010).

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