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Original Research

Effect of vitamin C and natural antioxidants on the production performance and antioxidant status of laying hens through heat

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ABSTRACT:

An experiment was carried out to investigate the effect of using ascorbic acid (vitamin C) and Dried Tomato Pomace (DTP) as natural antioxidants on the production performance and antioxidant status of laying hens through heat stress. Two hundred laying hens (Lohman brown layer) were reared at 34 weeks with a mean weight of 1750 g (± 150 g), distributed randomly and divided into five treatments with four replicates per treatment and 10 hens per replicate (40 hens / treatment). The treatments were T_1 basal diet (control), T_2 basal diet with 300 mg/kg of ascorbic acid (vitamin C) in addition to treatments T_3, T_4, T_5 were given 1, 2 and 3 % of DTP respectively. The temperature of the breeding chamber was 38°C for all treatments. The control treatment showed a significant deterioration in most production characteristics, such as a significant decrease in egg production and egg mass and significant deterioration in Feed Conversion Ratio (FCR) and mortality percentage. Enzymatic activity of catalase, glutathione peroxidase, and a significant increase in the level of malondialdehyde (MDA) in the liver tissue. Additionally, a significant increase in the enzyme activity of AST, ALT with a significant decrease in the activity of the ALP enzyme in blood plasma compared with birds fed on antioxidants during hot weather which indicated the susceptibility of heat stress to form oxidative stress in laying hens. Furthermore, a significant improvement in the productivity traits was detected, whereas significantly, an increase in egg production and egg mass in addition to a significant improvement in FCR and mortality percentage were demonstrated. There was also a significant improvement in the anti-oxidation status against heat stress by a significant increase in the activity of plasma glutathione peroxidase and catalase enzymes with a significant decrease in the level of MDA in the liver tissue. Moreover, a significant decrease in ALT activity, with a significant increase in ALP enzyme activity in blood plasma compared with control treatment, indicating the role of treatments in protecting against the effect of heat stress and its ability to improve the studied traits.

Keywords:
Tomato pomace, Antioxidant status, Heat stress, Laying hens.

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INTRODUCTION

Heat stress is one of the most stressful factors that cause economic losses in the poultry industry in most of the world's hot regions. It also causes a decrease in growth, production performance, immune degradation and high mortality percentage. Heat stress is a major cause of increase free radicals for Reactive Oxygen Species (ROS) that cause oxidative damage in the cells through lipid peroxidation and oxidative damage to protein and DNA (Mujahid *et al*., 2007a; Mohammed, 2012). Several studies have shown that increased oxidative damage in laying hens is associated with increased environmental warming (Altan *et al*., 2003; Mujahid *et al*., 2007b). The oxidative damage caused by high temperatures is the main source for ROS formation by losing electrons from the respiratory chain during the process of reduction of molecular oxygen in the later stages of oxidative phosphorylation (Mujahid *et al*., 2007b).

Mujahid *et al*. (2005) demonstrated that heat stress leads to the oxidative damage of mitochondrial membranes in the skeletal muscle of hens meat, leading to an imbalance in the mitochondrial antioxidant defense system, leading to a decrease in mitochondrial function and a disorder in the formation of protein during the phase of transmission of electrons, which results in incomplete reduction of oxygen and, if reduced one electron, causes a free radicals the negative oxide. If reduced two electrons, it causes hydrogen peroxide, which is a cytotoxic compounds and causes oxidative damage to proteins and phospholipids and DNA. Therefore, the use of natural antioxidants to reduce the oxidation caused by heat stress was required. The DTP is a secondary product obtained after the manufacture of the juice or tomato paste and contains the skin, seeds and pulp (Rezaeipour, 2012), which is a good source of natural antioxidants that include α-Tocopherol and lycopene (carotenoid), the main content in DTP, (8600-3100 µg/100g DTP). Lycopene is one of the most powerful antioxidants and its antioxidant activity was ten times as much as tocopherol and twice as much as the activity of beta carotene (Palozza, 2012). Chemically, lycopene is a ring carotene consisting of a straight chain of forty carbon atoms interconnected by eleven of the unsaturated double bonds, two non-binding binary bonds and the chemical formula for lycopene was $C_{40}H_{56}$ (not soluble in water). Lycopene acquires antioxidant strength by bilateral bonds (John and Marc, 2000; Omoni and Aluko, 2005), (Figure 1). The lycopene cannot be manufactured in humans and animals' bodies, therefore they are dependent on lycopene from its primary sources, especially the foods rich in it (Willis and Wians, 2003). The mature red tomatoes and its products, watermelon, red grapefruit, red guava, and red pepper were rich sources of lycopene (John and Marc, 2000). Tomato also contains natural water-soluble antioxidants such as ascorbic acid, caffeic acid, rutin and quercetin (Vallverdú-

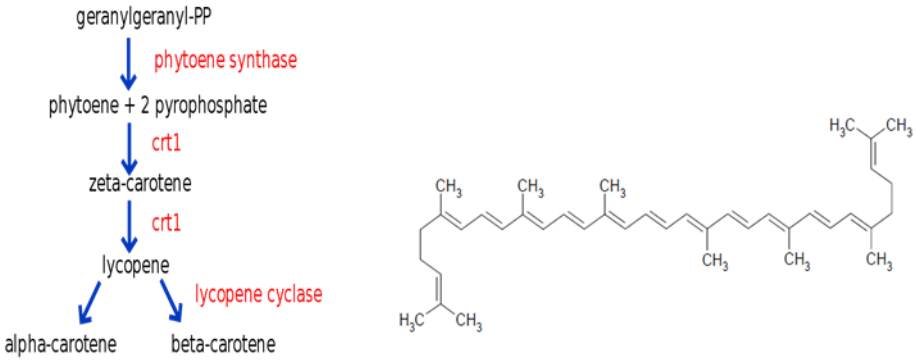

Figure 1. Cyclic chemical composition and lycopene metabolism (John and Marc, 2000; Omoni and Aluko, 2005)

Table 1. Composition and calculated nutrient content of the experimental diets (%)

S. No	Ingredients	Diets treatments			
		Control	DTP_1	DTP_2	DTP_3
1	Yellow corn	49	49	49	49
2	Wheat grain	5	5	5	5
3	Barley	12.29	11.39	10.49	9.69
4	soybean meal	19	18.9	18.8	18.6
5	DTP	0	1	2	3
6	Proteins concentration (40%)*	5	5	5	5
7	Hydrogenated plant fat	1	1	1	1
8	Calcium diphosphate	1.8	1.8	1.8	1.8
9	Limestone	6.82	6.82	6.82	6.82
10	Salt NaCl	0.09	0.09	0.09	0.09
11	Total	100 %	100 %	100 %	100 %
	Calculated nutrient content				
12	Metabolism Energy (kcal / kg)	2742.8	2739.6	2736.3	2733.4
13	Crude protein (%)	16.452	16.459	16.466	16.44
14	Lysine%	0.89	0.89	0.89	0.89
15	Methionine%	0.38	0.38	0.38	0.38
16	Cysteine %	0.28	0.28	0.28	0.28
17	Methionine + cysteine %	0.66	0.66	0.66	0.66
18	Arginine %	0.93	0.93	0.93	0.93
19	Calcium %	3.46	3.46	3.46	3.46
20	Available phosphorus%	0.55	0.55	0.55	0.55
21	Linoleic acid %	1.85	1.85	1.85	1.85
22	Sodium	0.164	0.164	0.164	0.164
23	Chlorine	0.164	0.164	0.164	0.164
24	Vitamin E (mg / kg)	25	25	25	25

*Proteins level for the feeding of poultry (Breedcom-5 special) produced by the Dutch company WAFI, Metabolism energy (kilo = 2100, raw protein 40%, raw fat 5%, raw fiber 2%, calcium 8%, phosphorus 2%, lysine 3.75% methionine 2.85%, methionine + cystine 3.20%, sodium 2.20%, 500 mg/kg vitamin E per kg protein,

**According to the values of the chemical composition of the feed materials found in the composition of the diet according to the American Research Council (NRC, 1994).

Queralt, 2011) as well as carotene, α-carotene, β -carotene and γ-carotene (Seybold *et al*., 2004). Furthermore, vitamin C (water-soluble vitamin) is considered as one of the strongest and most important antioxidants in extracellular fluid and have the potential to regenerate vitamin E after equating free radicals (Englmaierová *et al*., 2011; Shit *et al*., 2012). Due to the lack of studies on the possibility of using vitamin C and DTP as antioxidants to prevent the effects of high environmental temperatures on the performance of hens so this study aimed at the potential use of antioxidants to prevent the effect of environmental stress by supporting and raising the activity of the antioxidant defense system for mitochondria and the inhibitor of losing electrons from the respiratory chain and their effect on improving the productive performance of laying hens.

Table 2. The effect of antioxidants on cumulative production performance (48 days) under heat stress

S. No	Traits	Treatments					*SEM*	P- value
		T_1 Control	T_2 (300mg/kg) Vit c	T_3 (1%DTP)	T_4 (2%DTP)	T_5 (3%DTP)		
1	Egg production	Egg production Percentage HD %	68.06^{C}	81.85^{B}	88.36B^{A}	93.99^{A}	93.39^{A}	10.6	0.05
2		Accumulative egg production HD	57.17^{C}	68.75^{B}	74.23B^{A}	78.95^{A}	78.45^{A}	7.47	0.05
3	Egg weight		66.29	65.86	64.64	65.63	65.83	1.27	N.S
4	Egg mass		3792.8^{C}	4529.4^{B}	4797.9^{BA}	5181.0^{A}	5164.7^{A}	391.92	0.05
5	Feed conversion ratio(g feed / g egg /84d) FCR1		2.58^{A}	2.12^{BA}	2.06^{B}	1.90^{B}	1.93^{B}	0.03	0.05
6	Feed conversion ratio (g feed / egg /84d FCR2)		171.16^{A}	139.66^{B}	133.74^{B}	125.21^{B}	127.27^{B}	115.32	0.05
7	Feed conception		9717.0	9600.3	9926.3	9885.5	9984.4	49629.04	N.S
	Mortality%		12.5^{B}	0.0^{A}	0.0^{A}	2.5^{A}	0.0^{A}	25	0.05

The different litters in the column referred to significant differences between treatments at rate of 0.05

MATERIALS AND METHODS

This study was conducted at the poultry research station of the livestock research department of the general authority for agricultural research of the ministry of agriculture (Baghdad - Abu Ghraib) for 12 weeks (84 days) during the summer (June, July, August). Two hundred hens were employed at the age of 34 weeks at a mean weight of 1750 g (± 150 g), randomly distributed into five treatments, with four replicates per treatment and 10 hens per replicate (40 hens/ treatment). T_1 basal diet (control), T_2 basal diet with 300 mg/kg of ascorbic acid (vitamin C) in addition to treatments T_3, T_4, T_5 were given 1, 2, 3 % of DTP respectively. Every day, hens were fed with experimental diets *ad libitum* according to Lohman layer guide (120 g/hen) at 8 O'clock in the morning, with free water access for all treatments according to the recommendations of the National Research Council of the US (NRC, 1994) as in Table 1.

The lighting was about 16 h (8 - 12 pm). The room temperature was 38°C and for all treatments, birds were reared on the floor and each pin represents one replicate with an area of 3 m x 2 m (long x wide). The pins were provided with nipples system drinker and hanging feeder. Vitamin C was added as a powder from Juvedco Jordan Company based on the active ingredient of vitamin C (L-ascorbic acid), which was concentrated in 98%. The DTP was collected from the tomato paste industry and cleaned from impurities, dried by electric oven at a temperature of 38°C, then kept in nylon bags in the refrigerator (until the use), then the samples were grinded and added directly to the feed. Egg collection was done twice daily at 8:30 am and at 1 pm throughout the experiment, the production performance of laying hens was calculated on the basis of hen day production and egg production as an egg production percentage (HD%) and accumulative egg production (HD egg / hen/84d) for 12 weeks (84 days), the average of egg weight was taken at the end of each week of the experi-

Table 3. The effect of addition of antioxidants on the antioxidant status of laying hens in heat stress

S. No	Traits	Treatments					SEM	P- value
		T_1 Control	T_2 (300mg/kg) Vit c	T_3 (1%DTP)	T_4 (2%DTP)	T_5 (3%DTP)		
1	GSH-Px U/ML	188.000^{B}	222.000^{A}	$217.50B^{A}$	223.000^{A}	223.000^{A}	31.25	0.05
2	CAT U/ML	220.000^{D}	288.000^{BC}	286.000^{C}	300.000^{A}	295.000^{BA}	2	0.05
3	MDA Mlg/Kg Liver	1.0360^{A}	0.2481^{B}	0.1794^{B}	0.1558^{B}	0.1999^{B}	0.071743	0.05

The different litters in the column referred to significant differences between treatments at rate of 0.05

ment and each replicate of the experiment. Egg mass (g of eggs / hen / 84d) for each replicate of the experiment by multiplying the cumulative number of eggs with egg weight. Additionally, feed consumption (g feed / hen / 84d) was weekly calculated for each replicate of the treatments by dividing the amount of feed consumed per replicate on the number of birds multiplied by seven. Feed Conversion Ratio (g feed to g egg /84d) (FCR1) and Feed Conversion Ratio(g feed to egg /84d) (FCR2) was calculated by dividing the feed rate per bird on egg mass or the cumulative number of eggs, respectively, in addition to the mortality percentage was recorded when they occurred and calculated as a weekly percentage and for 84 days according to Al – Fayyad and Naji (1989).

Blood samples were collected from the bronchial vein by taking three birds from each replicate (12 birds / treatment). The blood samples were placed in tubes containing potassium ethylene diamine tetra acid, then blood plasma was isolated by using the centrifuge (3000 cycles/min for 15 min) and froze at -20°C for the determination of the activity of ALT, AST (Reitman and Frankel, 1957) and ALP (King and Armstrong, 1934) as well as the activity of plasma antioxidant enzymes, which include: Glutathione peroxidase and catalase activity (Wheeler *et al*., 1990). At the end of the experiment (84 days), three birds of each replicate (12 birds / treatment) were slaughtered and the liver was stored directly at -18°C to estimate the MDA level according to the method of Witte *et al*. (1970). The experimental data was calculated using one way ANOVA analysis. All data obtained in the present study were analyzed by SAS software 9.1 (SAS, 2012). A comparison between the mean values was done by using Duncan's Multi-Border Tests in addition, $P<0.05$ was considered statistically significant.

RESULTS

This study was conducted to compare the protective effect of vitamin C and various levels of DTP on the production performance and antioxidant status of laying hens from heat stress in the summer at 38°C during 84 days.

Production performance

Table 2 shows the effect of vitamin C and different levels of DTP in the production performance of the laying hens, including the percentage of egg production, accumulative egg production rate, egg weight, egg mass, feed conversion ratio, feed conversion ratio, feed consumption rate and percentage of mortality within 84 days. A significant increase ($P<0.05$) was shown in T_2, T_3, T_4, and T_5 in the percentage of egg production at 81.85, 88.36, 93.99 and 93.39%, respectively, compared with control treatment which reached 68.06% (Table 2). The results obtained in the present study showed a significant increase ($P<0.05$) for T_2, T_3, T_4 and T_5 in accumulative egg production and egg mass compared with the control treatment. T_5 and T_4 revealed the highest significant increase ($P<0.05$) in egg production percentage, accumulative egg production and egg mass compared to other experimental parameters, which signifi-

Table 4. The effect of antioxidants on the activity of liver enzymes in plasma blood of laying hens in heat stress

S. No	Traits	Treatments					SEM	P- value
		T_1 Control	T_2 (300mg/kg) Vit c	T_3 (1%DTP)	T_4 (2%DTP)	T_5 (3%DTP)		
1	ALT U/L	9.74^{A}	13.43^{C}	13.0^{D}	13.32^{C}	14.19^{B}	0.00045	0.05
2	AST U/L	39.85^{A}	28.23^{C}	26.86^{D}	25.89^{E}	29.46^{B}	0.00045	0.05
3	ALP U/L	25.24^{E}	38.19^{C}	37.62^{D}	40.48^{B}	41.89^{A}	0.00045	0.05

Different litters in the column referred to significant differences between treatments at rate of 0.05

cantly exceeded T_2 and T_1 while T_5 and T_4 were not significantly different from T_3. For FCR1 value, a significant improvement was noted in T_3, T_4, T_5 (2.06, 1.90 and 1.93 respectively) compared with T_1 at 2.85, while a significant improvement in FCR2 was shown in T_2, T_3, T_4, T_5 at 139.66, 133.74, 125.21 and 127.27 respectively, compared with T_1 at 171.16 . The highest significant increase ($P<0.05$) in the mortality rate (12.5%) was detected in T_1 compared with T_2, T_3, T_4, and T_5. Table 2 showed no significant differences between T_2, T_3, T_4, and T_5 in FCR1, FCR2 and mortality rate %. There were also no significant differences between T_1, T_2, T_3, T_4, and T_5 in the egg weight and feed consumption.

Antioxidant status

GSH-Px and CAT, and the concentration of MDA is shown in Table 3. The results from Table 3 indicated a significant difference between T_1, T_2, T_3, T_4, and T_5 in the activity of GSH-Px, CAT enzymes in plasma and MDA in liver tissue. Significantly, an increase in the activity of GSH-Px (222, 217.50, 223 and 223) and CAT (288, 286, 300 and 295) were detected in T_2, T_3, T_4, and T_5 respectively, compared with T_1, as the higher significant increase ($P<0.05$) was revealed in T_4, T_5 (300 and 295 respectively) in the activity of the CAT enzyme in blood plasma compared to other experimental parameters and significantly exceeded with T_3 at 268. The results indicated that the addition of different levels of DTP and vitamin C in laying hens diet caused a significant decrease in MDA concentration (0.2481, 0.1794, 0.1558 and 0.1999 respectively) in the liver tissue compared with T_1 at 1.0360 . Furthermore, an insignificant difference between T_2, T_3, T_4, and T_5 in the activity of GSH-Px and the concentration of MDA (0.2481, 0.1794, 0.1558 and 0.1999 respectively) was demonstrated (Table 2).

Activity of AST, ALT and ALP enzymes

Table 4 shows the effect of addition of vitamin C and different levels of DTP on the activity of ALT, AST and ALP enzymes in blood plasma. The results of the present study revealed that a significant decrease in the activity of ALT (13.43, 13.0, 13.32 and 14.19) and AST (28.23, 26.86, 25.89 and 29.46) enzymes was noted in T_2, T_3, T_4, and T_5 respectively compared with T_1 (19.47 and 39.58 respectively). Moreover, the results showed a significant ($P<0.05$) difference in the activity of AST and ALT between T_2, T_3, T_4, and T_5 in addition, T_4 (25.89) was the least significant activity in AST compared to other treatments followed by T_2, T_3, and T_5 respectively. The results showed a significant decrease ($P<0.05$) in ALT activity of T_3 (13) compared to T_2, T_4, T_5 at 13.43, 13.32 and 14.19 respectively. The results showed no significant differences in ALT activity between T_2 and T_4 which significantly decreased, compared with T_5. As well, the activity of ALP enzyme in blood plasma was significantly increased ($P<0.05$) in T_2, T_3, T_4 and T_5 (38.19, 37.62, 40.48 and 41.89 respectively) compared with T_1 at 25.24. The most significant increase in ALP activity was in T_5 followed by T_4, T_3, and T_2 respectively.

DISCUSSION

Production performance

Antioxidant treatments showed a significant increase ($P<0.05$) in egg production, the cumulative egg production rate, as well as the egg mass rate in addition to a significant improvement in the feed conversion coefficient compared with T_1 (Table 2). It can be attributed to vitamin C role of inhibition of corticosterone hormone secretion of the adrenal cortex in plasma blood, which leads to increase the egg production, as this hormone's role in inhibiting the secretion of FSH and LH. Moreover, a negative correlation coefficient between stimulates of hormones for sex hormones and corticosterone hormone was observed in plasma.

As well, vitamin E stimulates the secretion of the hormone LHT (LHRH) and thus stimulates the secretion of FSH and LH hormones in blood plasma and egg production as well as the positive effect of vitamin C in improving the digestibility of nutrients by improving the activity of digestive enzymes (trypsin, chymotrypsin, amylase and lipase) and thus lead to increase utilization of nutrients, which improve the efficiency of feed conversion of laying hens (Panda *et al*., 2008; Mohammed, 2012). The ability of antioxidants added to the treatments in the improvement of productive properties may be due to their ability to reduce the effect of heat stress, which leads to the increase of effective oxygen varieties, which break down the membranes of liver cells by lipid peroxidation and breakdown the polyunsaturated fatty acids in cell membranes which has an adverse effect on the activity of hepatocytes leading to reduce production and release of egg yolk components of the liver, so antioxidants play a role in catching the free radicals formed by heat stress in cell membranes and thus protecting cell membranes from oxidation that promotes the transmission of yolk components from the liver to the ovary (Puthpongsiriporn *et al*., 2001). Panda *et al*. (2008) noted that the positive effect of adding antioxidants to poultry diets on egg production and egg mass was associated with increased protein concentration in egg yolks. The egg yolk contains three major molecules composed of the yolk protein, Lipovitelline, phosvitin, and livetin in addition to VLDL (Bunchasak, *et al*., 2005). Antioxidants promote and accelerate the release of vitellogenin from the liver to the yolk in the ovary by protecting it from hepatocytes of oxidative damage caused by free radicals (OH^{-}, O^{-}) and thereby preventing the damage that occurs in liver cell membranes from oxidation and maintenance of the regular metabolic functions which increases the speed of the deposition of yolk in the developing oocytes and thus increase the mass of the egg and maturation in a faster time and therefore reflected on the production of eggs.

Safamehr *et al*. (2011) found that using levels at 0, 4, 8 and 12% of DTP in laying hens diets increased egg production, egg weight, egg mass, feed consumption improved eggshell thickness, eggshell weight and increased yolk concentration and low yolk cholesterol ratio compared to the control treatments. Akdemir *et al*. (2012) reported that a significant improvement was detected in egg production, egg weight, feed conversion efficiency and feed consumption efficiency of laying hens, while there was no effect on eggshell weight and eggshell thickness in tomato powder compared with the control treatment. Furthermore, adding antioxidants to the laying diets showed a significant improvement of all performance traits such as increase the egg production, feed intake, egg mass, and feed conversion ratio (Mohammed *et al*., 2013).

Antioxidant status

The results Table 3 showed a significant increase ($P<0.05$) in the GSH-Px and CAT plasma activity in blood plasma of T_2, T_3, T_4, and T_5 compared to the T_1. Significantly, a decrease in the value of MDA was noted in T_2, T_3, T_4, and T_5 compared with T_1 which reflect a high efficacy in the control of lipid oxidation and inhibition of lipid peroxidation in the liver. Due to heat stress with low levels of antioxidants in the diet, the

activity and concentration of antioxidants were reduced in blood plasma and tissue. Therefore, the addition of vitamin C and natural antioxidants (DTP) to the diets that leads to increase antioxidant activity in tissue and plasma, which increases the effectiveness of GSH, CAT, SOD, and GSH-Px in addition, reduces the activity of oxidative enzymes that stimulate fat oxidation such as Xanthine oxidase and dehydrogenase NADH.

Additionally, increasing antioxidant activity reduce fat peroxidation and accumulation of their products in plasma and tissue in addition to prevent cell membrane damage by taking free radicals, thus provide the first line of protection against fat peroxidation, which results in increasing the concentration of antioxidants inside blood plasma of laying hens, hence it have an important role in the reduction and inhibition of lipid peroxidation (Panda *et al*., 2008; Nelson and Cox, 2004). A significant increase of the GSH level in T_2, T_3, T_4, T_5 compared to T_1 may be attributed to their ability to stimulate GSH formation of oxidative glutathione (GSSG) by re-reducing the oxidized form GSSG to GSH form through the enzyme Glutathione Reductase (GSH-RD) and Nucleotide Amide Adenine Dye Nucleotide Phosphate (NADPH), which has a reduction role produced by the pentagram sugar pathway HMP-Shunt (Nelson and Cox, 2004).

Table 3 showed that T_1 had a significant decrease ($P<0.05$) in GSH-Px and CAT plasma activity and accompanied by a highly significant increase of MDA in liver tissue compared with other treatments. The obtained results can be explained by heat stress has led to the initiation of a series of chemical reactions leading to internal oxidative stress created by increasing the production of oxygen in the gastrointestinal tract, which in turn enters the blood leading to high oxygen pressure in cells, leading to an excessive increase in the production of active oxygen compounds, including H_2O_2. Offset by a weakness in the internal antioxidant system, resulting in an imbalance in the antioxidant / antioxidant system (Loven and Oberley, 1985). The antioxidant enzymatic system plays an important role in dealing with the oxidative stress resulting from an increase in the free radicals. The CAT enzyme deals only with hydrogen peroxide, which decomposes it into water and oxygen. Therefore, the reduced activity of the CAT will increase the H_2O_2 concentration which can be removed with other types of free radicals by the GSH-Px enzyme that converts the glutathione from the effective form to the inactive oxidant GSSG (In this interaction the GSH-Px is converted into an effective form by withdrawing a selenium atom) and this enzyme can interact with other free radicals (Nelson and Cox, 2004).

The significant increase in the activity of catalase enzymes, glutathione peroxidase in blood plasma and decrease in MDA level in liver tissue compared with T_1 may be due to the ability to increase the activity of GSH-Px in blood plasma compared with T_1. The GSH-Px enzyme inhibits oxidation of PUFA, prevents the breakdown of lipid formation in the mitochondria, helps in the synthesis of vitamin C and the metabolism of sulfur-containing amino acids such as methionine, which has antioxidant properties as well as important in the manufacture of cysteine that is incorporated into GSH synthesis. Methionine may act as a sweeping agent for effective oxygen classes, particularly, (O_2), (H_2O_2), and (OH^-) as well as its ability to regenerate and release vitamin E within the body (Panda *et al*., 2008, Lee *et al*. 2006). The DTP is a high source of antioxidants such as phenolic compounds, lycopene, folate, vitamin C, β-carotene, α-tocopherol, phenolic and flavonoids, which are sweeping free radicals as roots above the negative oxide (O^-), hydroxyl radical (OH^-) and peroxyl radical (RO_2) and have beneficial properties for metals, especially copper, iron and free hydrogen ion stopping the oxidation process also helps to replenish vitamin E by giving a hydrogen atom to the radical of α-tocopheroxyl and converting it into tocopherol. Lycopene is a strong antioxidant among dietary carotenoids

and can prevent the production of ROS that associated undesirable effects, an inverse relationship between lycopene, tomato or tomato products (Palozza *et al*., 2011; Sahin *et al*., 2006). Antioxidant treatments caused a significant increase in the catalase, glutathione peroxidase activity in blood plasma, levels of heme-iron, in addition a significant decrease in the peroxide value, malondialdehyde concentration and free fatty acid percentage in liver tissue compared with T_1 was detected. The obtained results indicated the ability of antioxidant treatments to prevent heat stress or to remove the effects of oxidative stress in laying hens (Mohammed *et al*., 2013).

Activity of ALT, AST and ALP enzymes

Control treatment showed a significant increase ($P<0.05$) in the ALT and AST activity compared to the antioxidant treatments, while the antioxidant treatments resulted in a significant decrease in ALT and AST activity compared to control treatment with no significant differences between these treatments and treatment control of ALT and AST activity in blood plasma (Table 4).

The ability of the antioxidant treatments to reduce ALT and AST activity may be due to enhanced antioxidant status and reduced oxidative stress (Table 3) in addition to the ability of the antioxidant treatments of promoting the activity of glutathione peroxidase enzyme in blood plasma, reduce the level of MDA and reduce oxidation catalysts such as metal ions by preventing their release from the tissue (iron linked) in the liver and thus break the chains of free radical reactions and reduce their production and composition, especially free radicals of active oxygen varieties which leads to protect the polyunsaturated fatty acids in the cellular membranes of oxidation and protection of hepatocytes of damage and thus maintain the characteristics of this membrane and the most important characteristic of voluntary permeability leading to non-leakage the enzymes from inside the cell to the outside (Dani *et al*., 2008). The results of Table 4 showed that T_2, T_3, T_4 and T_5 significantly affected the activity of ALP enzyme in blood plasma and significantly exceeded ($P<0.05$) in the activity of ALP enzyme compared with T_1. Furthermore, a significant decrease in ALT and AST activity and a significant increase in ALP activity in blood plasma compared with T_1 were demonstrated in T_2, T_3, T_4 and T_5 (Mohammed *et al*., 2013).

It can be attributed that the increased activity of ALP in blood plasma for the experimental treatments may be due to the close association of ALP activity with bone metabolism where the bulk of the enzyme ALP in plasma blood comes mainly from the bone tissue and the liver, which leads to increase the effectiveness of the protein linked to calcium and increase its absorption as well as raising the calcium in the blood plasma (Weiser *et al*., 1990). The higher egg production in T_2, T_3, T_4 and T_5 can be due to the high activity of ALP in blood plasma, which leads to increase the calcium metabolism of the bones that necessary to form the eggshell.

CONCLUSION

The ability of antioxidants to inhibit the formation of free radicals in the body by enhancing the activity of antioxidants of the body as increasing the activity of the CAT and GSH-Px enzyme in blood plasma and reduce the level of MDA in the tissue of the liver which leads to maintain the function of manufacturing liver cells. The enhancement of the antioxidant status leads to the persistence of the metabolic functions of the liver cells in the metabolism of the biological molecules (glucose, proteins and fats) needed to form the components of the yolk, albumin and eggshell components. This can be inferred by increasing the activity of ALP enzyme and decreasing the activity of AST in blood plasma due to the addition of antioxidants in poultry diets, the high activity of the ALP enzyme in blood plasma is an indicator of the ability of the liver cells to increase the production of calcium and phosphorus from the bones to the blood, and then transferred to

the liver for the purpose of forming the eggshell and thus led to increasing egg production in addition to protect the fats and components of the diet from oxidation and which increase the utilization of energy values of fat and other nutrients in the diet. Hence, providing the necessary feed requirements for the egg production.

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