Plant Archives Vol. 18 No. 2, 2018 pp. 2834-2841 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ADDING THE ANTIOXIDANTS ON SOME BIOCHEMICAL BLOOD PLASMA TRAITS OF BROWN LAYER DURING HOT SEASON IN IRAQ

Th. T. Mohammed[1]*, Z. J. Mohammed Saeid[1], Z. T. M. Al-Dhanki[1] and F. M. H. AL-Khalani[2]

[1]College of Agriculture, University of Anbar, Anbar, Iraq.
[2]State Board of Agricultural Research, Ministry of Agriculture, Iraq.

Abstract

During the hot season in Iraq (June, July and August) the ambient temperature inside the Layer house reached 39 Celsius, this condition put the layer in heat stress condition which resulted decreasing layer performance by increasing the oxidative damage of some biological traits of blood, so , adding antioxidants (synthetic and natural) could moderate this condition, this hypothesis was tested in field experiment lasted for 12 weeks (84 days), by using 200 Lohman brown layer at 34 wks of age , the hens were distributed into 5 treatments (40 hens/treatment) with 4 replicates / treatments and 10 hens/ replicate , the first treatment was the control with no antioxidant added to ration, whereas, synthetic antioxidants (vit. C and E) were added at rate of 300 mg/kg of feed in 2nd and 3rd treatment, in 4th and 5th treatments the natural antioxidants (grape seeds and rosemary leaves) were used at rate of 5 g/kg of feed.

The results of the experiment revealed that adding both types of antioxidants significantly decreased the glucose, triglycerides, total cholesterol and Non HDL in blood plasma, whereas, a significant increase in the total protein, albumen and HDL in blood plasma of hen consumed diet having antioxidant as compared with the control treatments. It can be concluded that adding antioxidant leads to protect the glucose, lipid profile and proteins in blood plasma from damaging by antioxidant during heat stress.

Key words : Natural antioxidants, Grape seeds, Rosemary leaves, Laying hens.

Introduction

There are many factors that increase the oxidative damages that occur in laying hen, including increased oxidation processes resulting from heat stress and the consumption of unsaturated fatty acids in the diet and the weakness of the anti-oxidant defense system, which leads to the accumulation of lipid peroxidation products in liver cells and thus damage may occur in the covers of Hepatocytes, membranes and internal structures of different body cells (Christaki, 2012), free radicals formed during metabolic processes and energy production from oxidation of nutrients act to damage and destroy biomolecules in body cells such as proteins, lipids, carbohydrates and nucleic acids (Pham-Huy and Pham-Huyc, 2008). There is a balance between the production of free radicals and the resistance to oxidation by the naturally occurring antioxidants in the body. If there is a large amount of produced free radicals offset by a small amount of antioxidants found naturally in the body will develop to so-called oxidative stress, which destroys DNA, proteins, carbohydrates, vitamins and also oxidize unsaturated fatty acids in cell membranes (Sandhya *et al*., 2010). Here are several types of antioxidants, including naturally occurring in the body due to biological processes such as Glutathion, Glutathion peroxidase, Catalase and Superoxide Dismatase (Christaki, 2012). In the absence of these antibiotics, it is necessary to add industrial antioxidants to eliminate free radicals, including industrial antioxidants, which are added to the feed, such as vitamin E and vitamin C, as vitamin E works to protect

***Author for correspondence :** Department of Animal production, College of Agriculture, University of Anbar, Anbar, Iraq
E-mail: ag.thafer.thabit@uoanbar.edu.iq

the unsaturated fatty acids in the cell wall of free radicals oxidation, as for vitamin C is considered as one of the most powerful antioxidants in extracellular fluid and has the potential to re-generate and renewal vitamin E after equalizing the free radicals (Egan *et al*., 1981; Shit *et al*., 2012), or it comes from natural sources found in foods eaten or added to them, such as grape seeds and rosemary leaves, which contain polyphenols, which has antioxidant properties as a free radicals sweeper and restricted to oxidizing metal ions such as iron (Fe+2) and copper (Cu+2). They also contain a number of hydroxyl groups that provide hydrogen atoms to the free radicals and thus stop oxidation (Veskoukis *et al*., 2010; Yesilbag *et al*., 2011). Proanthosyanidine is a phenolic compound found in grape seeds and it considered as one of the most powerful natural antioxidants. Grape seeds also contain active substances such as Catene and Ibecatene (Hogan *et al*., 2010), the leaves of rosemary react with antioxidant lead to form two active compounds, carnosol and carnosol acid, also rosemary leaves contain phenolic compounds such as rosemannol and rose marc acid (Polat *et al*., 2011), due to the large problem in raising laying hens and not being able to raise them under the circumstances of high temperatures during the summer (June, July and August), which may reach the temperature of the environment to 50^{o}, especially in semi-dry areas so the current study aimed to know the effect of the addition of antioxidants as vitamins E and C and grapes seeds and rosemary leaves to the diet to reduce the impact of heat stress on the productive and physiologic performance and the status of antioxidants in laying hens during the summer.

Materials and Methods

200 Lohman brown layer at age of 34 wk (1800 ± 160 g) were used in one way experiment lasted for 12 weeks (84 days) during hot season in Iraq (June, July and August), they were distributed in 5 treatments (40 hens/treatment) with 4 replicate per treatment and 10 hens per replicate, the first treatment was the control with no antioxidant added to ration, whereas, synthetic antioxidants (vit. C and E) were added at rate of 300 mg/kg of feed in 2nd and 3rd treatment, in 4th and 5th treatments the natural antioxidants (grape seeds and rosemary leaves) were used at rate of 5 g/kg of feed.

Every day hens were offered 120 g according to Lohman layer guide at 8 o'clock in the morning, water supply *ad libitum* for all treatments.

The percentage of components of basal diet was calculated to provide requirements according to NRC (1994), as follows; corn (49%), wheat (5%), barley (12.29%), soybean meal (19%), commercial protein concentrate (5%), hydrogenated plant fat (1%), calcium diphosphate (1.8%), NaCl (0.09%) and Limestone (6.82%).

The chemical analysis of basal diet as follow: ME (2724.65 kcal/kg), CP (16.55%), Lysine (0.89%), Methionine (0.38%), cysteine (0.28%), Met.+cys. (0.66%), Arginine (0.93%), calcium (3.46%), available phosphorus (0.55%), sodium (0.164%), chlorine (0.614%) and vti. E (25 mg/kg).

Proteins concentrate that used in basal diet produced by the Dutch company WAFI, contained ME (2100 kcal/kg), CP (40%), raw fat (5%), raw fiber (2%), calcium (8%), phosphorus (2%), lysine (3.75%), Methionine (2.85%), methionine + cystine(3.20%), sodium (2.20%), and 500 mg vitamin E per kg.

The illumination provided for 16 hours (from 8 am to 10 pm) during the day light with 2 hours at midnight, to allowed more feed consuming during this moderate period of day which inside house temperature reached 39^{0}C.

Floor Pens (L 3 m v W 2m), were used to raise the birds, each pen represent replicate of the experiment treatment, the pens were provided with nipples system drinker and hanging feeder.

Synthetic antioxidant (vitamin E and C) were added in the form of powder (Juvedco Jordan), based on the active ingredient of vitamin E (alph-tochopherol acetate) and the active ingredient of vitamin C, which were 50% and 98%, respectively. The spent grape seeds (Naturally antioxidant) used in the current experiment were collected from grape juice making shops. The seeds were separated from pulp and skin and washed with tap water and dried in an electric oven at 30°C until they dried, then stored in nylon bags in the refrigerator until grinded and added directly to the feed.

Naturally antioxidant Rosemary leaves (*Rosemarinus offcinalis*) was purchased from local markets and was confirmed to be classified according to the National Herbsand Seed Inspection which belong to Certification Authority of the Ministry of Agriculture (Iraq) , after cleaning it from impurities, it was grinded weekly by an electric mill and added to the diets immediately after grinding.

The blood samples were collected from bronchial vein, for biochemical blood plasma parameters. The collected blood samples,during cervical dislocation, were distributed in tube have anticoagulant (EDTA). Blood samples in the tube were centrifuged (at 2000×rpm for 10 min) and plasma was separated and then stored at -20^{0}C until assayed for measuring blood parameters; total

protein concentration (Varley *et al*., 2008), concentration of albumin protein and globulin (Henry *et al*., 2074), glucose (Asatoor and King, 1954), cholesterol and Triglycerides (Toro and Ackermann, 1975), HDL (Warnick and Wood, 1995), Non-high density non-HDL (Grundy *et al*., 2004) calorimetrically determined using appropriate laboratory kits and following the same steps as described by manufactures.

Data of percentage, biochemical parameters , were first transfer to arcsine; then all transformed data were analyzed using OneWay ANOVA analysis to assess the effect of Naturally and Synthetic antioxidant treatments on selected parameter of Layer Hens. The analysis were applied by using General Linear Model (GLM) procedure of statistical software package SAS version 9.1 (SAS Institute, 2004), P-values less than 0.05, 0.01, and 0.001 were considered to be significant between treatments and the significant differences were examined using Duncan Multi Border Test, and theresults were presented as mean/SEM (pooled).

Results

Concentrations of glucose and proteins in blood plasma

Using antioxidants (synthetic and Natural) significantly decreases glucose plasma, hens layer, there were no significant differences between antioxidant treatments, whereas, significant increases were happened in the plasma concentrations of total proteins, albumin, and globulins, without any differences between antioxidant treatments (table 1).

Untreated birds in the control group significantly decreased the concentrations of total protein, albumin, and globulin in plasma, without any differences between antioxidant treatments (naturally and synthetic).

The results of table 1 showed a highly significant effect ($P < 0.05$) for all antioxidant treatments in the total protein, albumin, and globulin level in blood plasma laying hens. All the treatments were superior to the control treatment in this feature with no significant differences between the different levels for industrial and natural antioxidant treatments in total protein and globulin levels for blood plasma of laying hens. There was significant superiority ($P<0.05$) in albumin level in blood plasma in the treatment of rosemary leaves (5 g / kg feed) compared to vitamin C (300 mg / kg feed) treatment. However, this superiority was not significant compared with vitamin E (300 mg/kg feed) and grape seed treatments (5 g/kg feed). The treatment of vitamin C (300 mg/kg feed) recorded the lowest values in the level of globulin.

Lipid profilein blood plasma of laying hens

Using antioxidants (naturally and synthetic) in diets causes significant decreases in the concentration of triglycerides, cholesterol, non-HDL and significantly increase HDL concentration in plasma compared to untreated birds in the control group (table 2). The results showed no significant differences in the level of triglycerides in the blood plasma of birds of antioxidant treatments. On the other hand, the treatment of vitamin E has the lowest a significant in cholesterol concentration compared to other antioxidant treatments except for the treatment of grape seeds which did not have a differ significantly in Cholesterol concentration. The results also showed a significant increase in the concentration of HDL in blood plasma of the treatments of grape seeds and rosemary followed by treatment of vitamin C and then vitamin E, respectively.

Discussion

The results in table 2 were consistent with Abdul-Rahman and Alkatan (2009), which indicated that the addition of vitamin C (300 mg/kg feed) and vitamin E (600 mg / kg feed) to laying hens diets caused a significant decrease on glucose level in serum, as well as with Aldabaj (2010), which concluded that the oral dose of male rabbits with a dose of 0.35 ml of grape seed oil/kg of body weight caused a significant decrease on glucose level in blood plasma. These results were consistent with Ghazalah and Ali (2008), who observed a significant decrease in the concentration of serum glucose compared with control treatment when adding 0.5 and 1% of rosemary leaves.

The reason for the ability of vitamin C to lower the glucose level in serum may be caused by the decreasing concentration of the corticosterone hormone, which is produced by the adrenal cortex, hence, increases its secretion during the exposure of birds to heat stress, working on the construction of glucose from non-carbohydrate sources (Gluconeogenesis), so the addition of this vitamin inhibits the operation of this process (Panda *et al*., 2008).

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 resulting from the exposure of birds to heat stress, which activates the work of the body cells, including pancreatic beta cells, which work on the secretion of insulin hormone which reduces serum glucose (Abdul-Rahman and Alkatan, 2009), as for the role of grape seeds in lowering blood glucose is due to its effective antioxidant role in protecting pancreatic cells from oxidation by the

free radicals of effective oxygen types formed by heat stress, as well as its role in activating the pancreatic antioxidant defense systems and thus protecting the pancreatic cells from damage or Oxidative Injury, which regulates the release of the insulin hormone to maintain the level of glucose within the normal range in blood.

As well as grape seed extract plays an important role in stimulating the release of insulin by pancreatic beta cells (Montagut *et al*., 2010). Some studies have shown that grape seed extracts have an inhibitory effect on the efficacy of intestinal-alpha-glucosidase and Pancreatic alpha-amylase and thus reduce carbohydrate digestibility (Adisakwattana *et al*., 2010).

The ability of rosemary leaves to lower glucose level in blood because it contains cellulose fibers, hemicelluloses and lignin at rates of 15.59, 6.79 and 5.94%, respectively, which inhibit enzymes from accessing the glucose by capsulizing them, thus delaying the absorption of glucose from the intestines forming complexes with digestible carbohydrates and making them indigestible and then exiting with waste (Ghazalah and Ali, 2008).

These results are consistent with those observed by Abdul-Rahman and Alkatan (2009), who pointed out that the addition of vitamin C (300 mg/kg feed) and vitamin E (600 mg/kg feed) to hen layer diets caused a significant decrease on glucose level in serum. This result, as well, is consistent with the findings of Ghazalah and Ali (2008) that the addition of rosemary leaves (0.5, 1%) to broiler diets caused an increase in total plasma protein concentration.

Exposure of birds to different environmental stresses, especially the heat stress, increases the secretion of corticosterone, which works to supply glucose from non-carbohydrate sources, especially protein. The effect of corticosterone on the metabolism of proteins during its effect in many enzymes in the liver, such as ALT and AST, which have a role in the process of glucose formation from 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).

The reason for the high level of blood plasma glucose which exposed to heat stress may be due to the control treatment due to its role in raising the level of free radicals , causing damage in the pancreatic cells, leading to irregular insulin secretion to maintain the normal level of blood glucose leading to increase its level in blood plasma (Montagut *et al*., 2010).

The total protein plays an important role in maintaining the balance of fluids volume between blood, tissue and basic acid. It is the transporter of many food compounds from tissue to another in the body, such as lipid, carbohydrates, vitamins, minerals, and hormones. As well, it plays an important role in the formation of enzymes and hormones and transports the genetic information (DNA) and immunity in addition to balance the exhaustive blood pressure in tissues (Nelson and Cox, 2004).

The total plasma protein concentration is positively related to egg production (Bunchasak *et al*., 2013). The high concentration of total protein in blood plasma (within normal limits) is a good indicator of health status and an evidence of egg production because most egg components are transmitted through the blood to the ovary and are linked to protein (Stadelman and Cotterill, 1986) and what confirm that is the accompanying of total protein concentration increase in blood plasma with increasing egg production in antioxidant-treated birds compared to control treatment (Mohammed *et al*., 2013a).

The low level of total protein, albumin, and globulin in Layer's blood plasma of control treatment may be due to the accumulation of lipid peroxidation products in liver cells, resulting in damage to hepatocytes membranes and reduced liver manufacturing function in protein manufacturing. The ability to manufacture protein in the liver is evidence of liver manufacturing function (Koller, 1984).

Heat stress also increases the production of free radicals of active oxygen species, which leads to increase in protein oxidation in the plasma, leading to protein loss and lower in blood plasma (Sharma *et al*., 2006). The oxidative stress resulting from heat stress leads to a change in the path of cells growth and limits the breakdown of lysosomes and the release of enzymes responsible for protein breakdown (Ferrari, 2001).

The role of vitamin E in increasing the concentration of globulin in blood plasma is due to its role in stimulating the humoral immune response in the body by increasing the number of lymphocytes ß which in turn lead to increase the immunoglobulin globulin and increase the activity of lymphocytes type T by that immune stimulation, which in turn increase lymphokines, which utilize nutrients when digested and metabolized inside the body, thereby increasing the process of protein formation in the body (Boa-Amponsem *et al*., 2000).

The increase in the total protein content in the treatment of vitamin C is due to its role in providing protection against the demolition reactions in the body, which reduces the secretion of corticosteroids (Panda *et al*., 2008), which provides glucose from non-carbohydrate sources, especially protein (Gluconeogenesis) through

demolition of amino acids and convert it to glucose in the liver (Nelson and Cox, 2004).

The grape seed extract increased the total protein level in the rat serum by stimulating protein formation and accelerating the process of regeneration and production of hepatocyte (Uma Maheswari and Rao, 2005). The role of grape seed extract in preventing the gluconeogenesis process thus prevents protein demolition and convert it to glucose (Bujanda *et al*., 2006). This may lead to an increase in total protein concentration in plasma. The beneficial effects of proanthocyanidins in grapes and other polyphenols found in the grape seed extract occur first in the liver and then in the blood (Ahmad and Fatani, 2007).

The increase in the levels of blood proteins and total albumin in blood plasma when using rosemary leaves in broilers diet is due to the protection of hepatocytes from oxidative damage and stimulates them to manufacture the necessary enzymes to build the protein, leading to a balance of nitrogen and thus leads to the ability of chicks to store the reserve protein in the body tissues even after the body reaches its maximum capacity in depositing the protein into the tissue (Ghazalah and Ali, 2008; Christaki, 2012). It is noticed through the results in table 2 that increasing the level of vitamin E in the diet increases the deposition of vitamin E in the body where it is stored with lipid in adipose tissue and this enhances its role in protecting cellular membranes as locations for lipids and cholesterol leakage through its ability of inhibiting the activity of free radicals resulting from lipid oxidation reactions (Mohammed *et al*., 2013b) in egg yolk, plasma and liver and thus reducing the concentration of cholesterol and lipid when exposing laying hens to the stress as vitamin E contains a hydroxyl group that gives the ability to give a hydrogen atom to the free radical of the fat, thereby inhibiting the oxidation of fat and this is reflected on providing protection to LDL (Low Density Lipoproteins) from oxidation (Puthpongsiriporn *et al*., 2001). As a result, vitamin E reduces hypolipidemic levels by inhibiting LDL oxidation and increasing HDL level in plasma (Pryor, 2000). The role of vitamin C in lowering cholesterol, triglyceride and non-HDL in blood plasma may be due to its important role in the representation of bile acid as well as its role in the stabilization of lipid profile and its protection from oxidation and also prevents the oxidation of lipoproteins and low density HDL and raises the levels of high-density lipoprotein (HDL) in blood plasma (Spritzer, 2007).

The ability to treat grape seeds (5 g/kg feed) may be due to a reduction in the percentage of liver lipids and its effect in lowering the level of cholesterol (hypocholeserolemic) and the level of hypotriglyceridemic (hypoglyceridemic) in blood plasma to the high antioxidant effectiveness of polyphenole compounds of grape seed (proanthocyanidin, citacin and ibecitacin) by inhibiting free radicals activity, nagative radical over the oxide and the hydroxyl radical (Faria *et al*., 2006) and activating the antioxidants in the body (glutathione enzyme, peroxidase and glutathione) and reducing lipid peroxidation (MDA) and its ability to restrict oxidation catalysts (associated iron) and reduce lipid decomposition (free fatty acids) and thus protect LDL from oxidation, as the table 2, Mohammed *et al*. (2013) refers to the ability of grape seed treatments to reduce the levels of non-high-density lipoproteins in laying hens blood plasma compared to the other treatments, thus, reduces cholesterol and triglycerides levels in blood plasma, in addition that grape seeds treatments (5 g/kg feed) recorded the highest level of HDL in blood plasma, reaching 52 mg/100 ml compared with control treatment and other treatments and that HDL is in an irreversible transfer operation for cholesterol and lipid from tissue and returns it to the liver convert it into bile acids (Van der Steeg, 2008) or discarded out of the body or is used in the process of lipid building (Criqui and Golomb, 1998).

The results in table 2 shows that all antioxidants additions, especially natural ones (grape seeds and rosemary leaves) have been highly effective as antioxidant material, significantly reducing the formation of free radicals in the body and thus protecting against the effects of heat stress and accumulation of lipid peroxidation products and this is done according to the two following mechanisms:

The first is the ability of the antioxidants to stop the series of free radicals reactions in the starting phase because it contains hydroxyl groups that act on giving the hydrogen atoms to the effective free radicals and make them ineffective, thus inhibiting the production of peroxide, inhibiting the formation of malondialdehyde and controlling the lipid stability by reducing the decomposition of free fatty acids, also its ability to restrict the metal ions stimulating oxidative reactions by inhibiting the release of iron from tincture of the liver tissue, as evidenced by the increased concentration of associated iron in the meat tissue (Mohammed *et al*., 2013 b).

The second is by the ability of antioxidants inhibition of the formation of free radicals in the body by promoting the activity of antioxidants of the body as increasing the activity of enzyme catalase and GSH-PX in blood plasma and increase the level of GSH in the liver tissue, which

Table 1 : Effect of adding Naturally and Syntheticantioxidants on some plasma biochemical traits of Lohman Layer during hot season in Iraq.

Treatments	Glucose (mg/100 ml)	Total protein (gm/100 ml)	Albumin (mg/100 ml)	Globulin (g/l)
Control	260.5a	3.07b	1.64c	1.43b
Vitamin E (300 mg / kg feed)	183.25b	5.10a	2.73ab	2.37a
Vitamin C (300 mg / kg feed)	185.22b	5.01a	2.65b	2.37a
Grape seed (5 g / kg feed)	184.0b	5.11a	2.81ab	2.3a
Rosemary leaves (5 g / kg feed)	160.50b	4.79a	2.87a	2.0a
SEM	44.2	0.36	0.11	0.47
Probability	0.05	0.05	0.05	0.05
General Mean	194.69	4.616	2.54	2.04

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

Table 2 : Effect of adding Naturally and Syntheticantioxidants on some lipid profile traits of Lohman Layer during hot season in Iraq.

Treatments	Triglycerides (mg/100ml)	Cholesterol (mg/100ml)	HDL (mg/100ml)	Non-HDL (mg/100ml)
Control	1116a	250a	24d	226a
Vitamin E(300 mg/kg feed)	641b	99c	41c	111b
Vitamin C(300 mg/kg feed)	819b	156b	45b	58c
Grape seed(5 g/kg feed)	754b	121bc	52a	69c
Rosemary leaves(5 g/kg feed)	633b	199b	50a	99b
SEM	174.6	53	1.4	51
Probability	0.05	0.05	0.05	0.05
General mean	592.6	165	42.4	113.2

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

works to remove the free radicals through giving the hydrogen and the increase of hydrolysis H_2O_2 and restricting it to metal ions, thus protecting the hepatocytes membranes and the cell's internal structures membranes (Hepatocytes) and thus their ability to maintain the function of manufacturing hepatocytes (Mohammed *et al*., 2013 b).

Antioxidants also play an important role in reducing the lipid profile in blood plasma through their ability to raise HDL levels, which removes cholesterol and lipid from cells and walls of the blood vessels in reverse transfer operation to the liver and thus convert it into yellow acids or discard it out of the body, as well as its ability to protect LDL from the oxidation of free radicals, LDL is the main carrier of cholesterol, triglycerides and all phosphorus-containing lipids, which displace these molecules from different body cells for use in the process of bio-building of lipid in the liver and using in the formation of bile acids, which consists of VLDL, triglyceride and cholesterol, and here comes the role of antioxidants in the stimulation of liver cells and membranes by protecting them from oxidation and encouraging them to release the predecessor of yolk from the liver to the ovarian follicles.

This can be summarized by the addition of antioxidants in laying hens diet on the performance production of the following points:

- Protect the lipid s and components of the diet from oxidation and thus increase the utilization of energy values for the diet lipids and other nutrients in the diet, thus providing the necessary nutrient requirements to meet the needs of egg production.
- The role of antioxidants in inhibiting the formation of free radicals and protection against damage resulting from the impact of oxidative stress in the tissues and liver cells. This leads to the maintenance of the metabolic functions of the hepatocyte in the representation of the biological molecules (glucose, proteins and lipids), which are necessary to form the components of the predecessor of yolk and albumen and components of the crust.

- Protecting the main carriers (LDL, HDL) for the transfer of lipid (triglyceride, cholesterol, lipid) from blood to the liver for manufacturing yolk components and the role of antioxidants in the rapid deposition of the predecessor of yolk from the liver to ovaries, which increases the egg mass and egg production rate.

References

Abdul-Rahman, S. Y. and M. M. Alkatan (2009). Effect of some antioxidants on some physiological and reproductive parameters in laying hens. *Iraqi J.vet. Sci*., **23(2)** : 377-384.

Adisakwattana, S., P. Jiphimai, P. Prutanopajai, B. Chanathong, S. Sapwarobol and T. Ariyapitipan (2010). Evaluation of alpha-glucosidase, alpha-amylase and protein glycation inhibitory activities of edible plants. *Int. J. Food Sci. Nutr*., **61(3)** : 295-305.

Ahmad, A. A. E. and A. J. Fatani (2007). Protective Effect of Grape Seeds Proanthocyanidines against Naphthalene-Induced Hepatotoxicity in Rats. *Saudi Pharmaceutical J*., **15(1)** : 38-47.

Aldabaj, A. M. A. (2010). Effect of grape seed oil on hepatic, thyroid and adrenal functions in adult male rabbits treated with sodium fluoride. *MSc thesis*, Collage of Veterinary Medicine, University of Baghdad.

Asatoor, A. M. and E. J. King (1954). Simplified colorimetric blood sugar method. *Biochem J.*, **16** : 56(325th Meeting):xliv.

Boa-Amponsem, K., S. E. H. Price, M. Picard, P. A. Geraert and P. B. Siegel (2000). Vitamin E and Immune Responses of Broiler Pureline Chickens. *Poult. Sci*., **79** : 466-476.

Bujanda, L., M. García-Barcina, V. G. Juan, J. Bidaurrazaga, M. F. de Luco, M. Gutiérrez-Stampa, M. Larzabal, E. Hijona, C. Sarasqueta, M. Echenique-Elizondo and J. I. Arenas (2006). Effect of resveratrol on alcohol-induced mortality and liver lesions in mice. *BMC Gastroenterol*., **6** : 35.

Bunchasak, C., K. Poosuwan and R. Nukraw (2005). Effect of dietary Protein on egg production and Immunity responses of laying hens during peak production period. *Int. J. of Poult. Sci*., **4(9)** : 701-708.

Christaki, E. (2012). Naturally derived antioxidants in poultry nutrition. *Res. J. of Biotechnol*., **7(3)** : 109-112.

Criqui, M. H. and B. A. Golomb (1998). Epidemiologic aspect of lipid abnormalities. *Am. J. Med*., **105 (1A)** : 48s-57s.

Egan, H., R. S. Kirk and R. Sawyer (1981). *Pearson's chemical analysis of Foods*. Edinburgh, UK, Churchill Livingstone.

Faria, A., C. Calhau, V. de Freitas and N. Mateus (2006). Procyanidins as antioxidants and tumor cell growth modulators. *J. Agric. Food Chem*., **54** : 2392-2397.

Ferrari, C. K. B. (2001). Oxidative stress pathophysiology: Searching for an effective antioxidants protection. *Int. Med. J.*, **8** : 175-184.

Ghazalah, A. A. and A. M. Ali (2008). Rosemary leaves as a dietary Supplement for growth in broiler chickens. *Int. J. of Poult. Sci*., **7(3)**: 234-239.

Grundy, S. M., J. I. Cleeman, C. N. B. Merz, H. B. Brewer, Jr. L. T. Clark, D. B. Hunninghake, R. C. Pasternak, S. C. Smith and Jr. N. J. Stone (2004). Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. *Circulation*, **110** : 227-239.

Henry, R., D. C. Cannon and J. W. Winkelman (1974). *Clinical chemistry: principles and Techniques*. 12nded, Harper and row.

Hogan, S., L. Zhang, J. Li, S. Sun, C. Canning and K. Zhou (2010). Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. *Nutrition and Metabolism*, **7** : 1-9.

Koller, A. (1984). Total Serum Protein. In: *Clinical Chemistry*. Kaplan, A. et al The C.V. Mosby Co.St Louis. Toronto. Princeton., 1316-1324 and 418.

Mohammed, Th. T., F. M. H. AL-Khalani and Z. T. M. AL-Dhanki (2013a). The study of effect adding antioxidants in the diet to reduce the effect of heat stress on production performance and antioxidant status in brown laying hens. *Al-Anbar J. of Vet. Sci*., **6(1)** : 96-108.

Mohammed, Th. T., F. M. H. AL-Khalani and Z. T. M. AL-Dhanki (2013b.2013). Comparison effect of adding artificial and natural antioxidant on performance, physiological traits, antioxidant status and eggs quality of brown layer. *Al-Anbar J. of Agric. Sci.*, **11(1)**.

Montagut, G., C. Blade, M. Blay, J. Fernandez-Larrea, G. Pujadas, M. J. Salvado, L. Arola, M. Pinent and A. Ardevol (2010). Effects of a grape seed procyanidin extract (GSPE) on insulin resistance. *J. Nutr. Biochem*., **10** : 1016.

National Research Council (N. R. C.) (1994). *Nutrient requirement of poultry*. 9th revisited National academy press, Washington D. C., U.S.A.

Nelson, D. L. and M. M. Cox (2004). Lehninger principles of Biochemistry. Fourth ed. Copyright by: W. H. Freeman and Company.

Panda, A. K., S. V. Ramarao, M. V. L. N. Raju and R. N. Chatterjee (2008). Effect of dietary supplementation with vitamins E and C on production performance, immune responses and antioxidant status of White Leghorn layers under tropical summer conditions. *British Poult. Sci*., **49(5)** : 592-599.

Pham-Huy, L. A., H. He and C. Pham-Huyc (2008). Free radicals, antioxidants in disease and health. *Int. J. of Biomed. Sci*., **4(2)** : 89-96.

Polat, U., D. Yesilbag and M. Eren (2011). Serum Biochemical profile of broiler chickens fed diets containing rosemary and rosemary volatite oil. *J. Biol. Environ. Sci*., **5(13)** : 23-30.

Pryor, W. A. (2000). Vitamin E and heart disease: Basic science to clinical intervention trials. *Free Radic. Biol. Med*., **28**

(1) : 141-164.

Puthpongsiriporn, U., S. E. Scheideler, J. L. Shell and M. M. Beck (2001). Effect of vitamin E and C supplementation on performance in vitro lymphocyte proliferation and antioxidant status of laying hens during heat stress. *Poult. Sci*., **80**:1190-172:1118-1124.

Richmond, W. (1973). Preparation and Properties of a Cholesterol Oxidase from *Nocardia* sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. *Clin. Chem*., **19** : 1350-1356.

Sandhya, B., S. Manoharan, G. Sirisha Lavanya and Ch. Ratna Manmohan (2010). Lipid peroxidation and antioxidant status in prostate cancer patients. *Indian J. Sci. Technol*., **3 (1)** : 83-86.

Sharma, P., R. D. Senthilkumar, V. Brahmachari, E. Sundaramoorthy, A. Mahajan, A. Sharma and S. Sengupta (2006). Mining literature for a comprehensive pathway analysis: A case study for retrieval of homocysteine related genes for genetic and epigenetic studies. *Lipids in Health Dis*., **5(1)** : 2-19.

SAS (2004). SAS/TAT user's Guide Version 9.1st ed. SAS Institute Inc. Gary, NC.

Shit, N., R. P. Singh, K. V. H. Sastry1, R. Agarwal, R. Singh, N. K. Pandey and J. Mohan (2012). Effect of Dietary L-ascorbic Acid (L-AA) on Production Performance, Egg Quality Traits and Fertility in Japanese Quail (*Coturnix japonica*) at Low Ambient Temperature. *Asian-Aust. J. Anim. Sci*., **25 (7)** : 1009- 1014.

Spritzer, V. (2007). Vitamin basics.The facts about vitamins in nutrition. DSM Nutritional products. 3rd ed., printed in Germany.

Stadelman, W. J. and O. J. Cotterill (1986). *Egg Science and technology*. 3rd ed. AVI. Publishing company INC. West Port. Connecticut 13-Hafez, E. S.

Toro, G. and P. G. Ackermann (1975). *The practical clinical chemistry*. 1st ed., Little Brown and Co., Boston, USA. P. 354.

Uma Maheswari, M. and P. G. M. Rao (2005). Antihepatotoxic effect of grape seed oil in rat. *Indian J. Pharmacol*., **37(39)** : 179-182.

Van der Steeg, W. A. (2008). High- density lipoprotein cholesterol, high- lipoprotein particle size, and apolipoprotein A-I: Significance for cardiovascular risk: The IDEAL and EPIC-Norfolk studies. *J. A. C. C*., **51(6)**: 634-642.

Varley, H., A. H. Gowenlok and M. Ben (1980). *Practical Biochemistry*. 6th ed. William Heinemann Medical books Ltd., London.

Veskoukis, A. S., A. Kyparos, M. G. Nikolaidis, D. Stagos, N. Aligiannis, M. Halabalaki, K. Chronis, N. Goutzourelas, L. Skaltsounis and D. Kouretas (2010). The Antioxidant effects of a Polyphenol-Rich grape pomace extract *in vitro* do not correspond *in vivo* using exercise as an oxidant stimulus. Oxidative Medicine and Cellular Longevity, 1-14.

Warnick, G. R. and P. D. Wood (1995). National Cholesterol Education Program Recommendations for measurement of high-density lipoprotein cholesterol: Executive summary. *Clin. Chem*., **41** : 1427-1433.

Yesilbag, D., M. Eren, H. Agel, A. Kovanlikaya and F. Balci (2011). Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum SOD activity. *Br. Poult. Sci*., **52(4)** : 472- 482.