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EFFECT OF ADDING DIFFERENT LEVELS OF BIOTINTO THE DIET IN SOME BIOCHEMICAL CHARACTERISTICS OF THE BLOOD SERUM OF JAPANESE QUAIL EXPOSED TO OXIDATIVE STRESS

Ibrahim Hamza YASSER[1] & Nihad Abdul-Lateef ALI [2]

Abstract

This experiment was conducted at the poultry farm of the college of Agriculture, University of Al-Qasim Green for the period from 26/9/2019 to 8/11/2019. The study was aimed to the effect of adding different levels of Biotin to the diet in some biochemical characteristics of the blood serum of Japanese quail exposed to oxidative stress . in the experiment, 300 unsexed Japanese quail birds were used. The eggs were obtained from the Agricultural Research Department in Abu Ghraib and the eggs hatched in Al-Nasr hatchery in Babylon province. They were raised together until the age of 8 days and then were randomly distributed to 5 treatments with 3 replicates each treatment (20 birds / replicate). The oxidative stress was induced by adding 0.5% H2O2 to drinking water and the experimental treatments were as follows: The first treatment: Negative control treatment is free of any addition. The second treatment: a positive control treatment adding water with H2O2 at just by only 0.5%. The third, fourth and fifth treatment: adding biotin at a concentration of 500, 600 and 700 micrograms / kg feed + water with H2O2 added at a concentration of 0.5%, respectively. The treatment lasted from 8 to 42 days. The results are as follows:The results of the experiment showed a significant improvement ($p\leq0.05$) for the adding of Biotin (third, fourth and fifth) in the total protein and albumin concentration, and a significant decrease in the concentration of glucose compared to the first treatment and the second treatment, as for the concentration of liver enzymes ALT and AST, the results showed a significant decrease in the first treatment and adding treatment (Biotin) compared to the second treatment (positive control), while the results showed a significant increase ($p\leq0.05$) in the enzyme Glutathione for the adding treatment Biotin (third, fourth and fifth treatment) Compared to the first treatment and the second treatment .Biotin adding treatment (third, fourth and fifth) showed a significant decrease ($p\leq0.05$) in the level of cholesterol and triglycerides concentration as well as low-density lipoproteins and the level of MDA and an increase in the level of high-density lipoproteins in the Serum blood of bird compared to the first treatment and the second treatment.

Keywords: Biotin, Biochemical Characteristics, Japanese Quail,Oxidative Stress.

[1] Al-Qasim Green University, Iraq
[2] Al-Qasim Green University, Iraq, dr.nihad@agre.uoqasim.edu.iq, https://orcid.org/0000-0002-2684-9243

1. Introduction

Oxidative stress is a difference in the balance between the free radicals resulting from the vital activities and defensive ability of antioxidants in the body accompanied by an increase in fat oxidation which results in subversive damage to different tissues and a decrease in immune susceptibility to diseases, and antioxidants work in the treatment of tissues affected by damage also works to prevent the generation of free radicals that are generated as a result of various vital activities in the body or slow them down, so it constitutes a defensive line against the subversive activity of free radicals in terms of their generation or chain of reactions (Bartosikova et al , 2003; Prakash and Joshi, 2004) and the opportunity increases infection with free radicals with organ activity. The organs and tissues that are highly effective, their chances of infection with free radicals are higher (Loven and Oberley , 1985). There are physiological and structural factors that increase the chance of exposure of tissues and biomolecules to oxidative damage, and among them the amount of it contains Long-chain polyunsaturated fatty acids(Salami et al.,2015). There are two types of antioxidants, Natural ones, such as medicinal grass, and processed ones, such as some vitamins that have been observed to have a protective role against oxidative processes induced by free radicals (McDonald et al, 1973). Among these vitamins is biotin which is one of the B group and works as an anti-Oxidation as it inhibits the effect of a number of free radicals that are naturally formed within the body of the organism (Combs,2008)which are irritant, unstable and have high energy and are very familiar to interactions with the biomolecules in the body. What distinguishes free radicals is their ability to Starting a chain of reactions leads to amplification The reality of the activity of free radicals, leading to the destruction of the necessary large molecules and cell components in biological systems (Matkovics , 2003). Biotin is an essential coenzyme for all organisms. Its physiologically active form is linked to enzymes of great metabolic importance like carboxylase and decarboxylase that stimulate metabolism and play an important role in biochemical processes like gluconeogenesis and fatty acids and protein synthesis, this vitamin contributes to such important processes as growth, skin regeneration, bone development and reproduction, increasing feed conversion in animals (McMahon , 2002).Biotin increases the body's immunity, as it is one of the vitamins that have a great role in preventing diseases and pathogens and works to participate in the production of antibodies (Gürel and Kuşçu, 2008). and in light of the availability of these factors, the idea of this study has crystallized and is a statement of the extent of The role of biotin to reduce the effect of experimentally induced oxidative stress using hydrogen peroxide H_2O_2 in Japanese quail onsome biochemical characteristics of the blood serum.

2. Materials and Methods

This experiment was conducted at the Poultry Farm Agriculturefaculty,Al-Qasim Green University durinthe period,which lasted from 26/9/2019 to 8/11/2019 toevaluate the effect of adding different biotin levels to the diet on the Productive performance of Japanese quail exposed to oxidative stress. A total of 300 unsexed Japanese quail birds produced from eggs were purchased from the Agricultural Research Department in Abu Ghraib which incubated in Al-Nasr hatchery in Babylon province. They were raised together until the age of 8 days and then were randomly distributed to 5 treatments with 3 replicates each treatment (20 birds / replicate).The chicks were managed in two periods : The period before treatment: The chicks were raised from the age of one day until a week floor breeding without treatment with biotin for the purpose of habituating the chicks to the ambience of the hall, where the hall was divided by reservations and on a cardboard floor mat. Water and fodder were provided free of charge ad-libitum and fed on a diet indicated in Table (1) with the use of a continuous lighting system before and after the treatment.Treatment period: This period extended from 8 daysof ageup to 42 daysof age, as it used 300 quail birds, and after weighing a week old at a rate of primary weight, the chicks were transferred to batteries manufactured locally. Each battery consists of 3 floors, the size of each floor 1 x 1 M, at a height of 40 cm, contains fountains, plastic fountains, and a wooden floor. The chicks were randomly distributed on 5 treatments. Each treatment included 3 replicates, at 20 birds per repetition (20 birds per floor). And the experimental treatments were as follows: The first group: a negative control treatment that is free of biotin or H_2O_2. The second group: a positive control treatment that was free of adding biotin + water with H_2O_2 added by only 0.5%. The third group: adding biotin at a concentration of 500 micrograms/kg feed + water added with H_2O_2 at a concentration of 0.5%. The fourth group: adding biotin at a concentration of 600 micrograms/kg + water with H_2O_2 added at a concentration of 0.5%. The fifth group: adding biotin at a concentration of 700 micrograms/kg + water with H_2O_2 added at a concentration of 0.5%. The experiment included the study of the following Total protein, albumin, globulin, glucose,liver enzymes (AST, ALT), and glutathione in the blood serumas well as studying the characteristics of the lipid profile of blood serum of Japanese quail birds exposed to oxidative stress, represented by cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, and the level of malondehide .all studied traits were estimated at the end of the experimentat the age of 6 weeks. The completely randomized design was used to study the effect of different treatments on the studied traits, the significant differences between the averages were compared using Duncan's Multiple Range Test (Duncan , 1955)and the SAS (SAS , 2012)was used to analyze the data.

Table (1) Ratios of fodder materials and the calculated chemical composition of the feed used in the study.

(1) The soybean coating used from an Argentinian source has a crude protein content of 44% and 2230 kcal / kg energy represented. (2) Concentrated protein produced by a Belgian company (importer) Intraco Containing 40% crude protein, 2100 kilograms of protein / kg representative energy protein, 3.5% raw fat, 1% raw fibers, 6% calcium, 7.5% phosphorous, 3.25% Lysine, 3.50% methionine, 3.90% methionine + cysteine. (3) chemical composition was calculated according to analysis of feed materials mentioned in (NRC, 1994)

3.

Feed material	Diet %
yellow corn	40
Local wheat	20
Soybean meal(1)	31.8
Concentrated Protein(2)	5
sunflower oil	2
Limestone	0.7
Vitamin mixture	0.2
Food salt	0.3
Calculated Chemical Analysis	
Crude protein (%)	19.69
(Metabolized Energy (kcal/kg	2913.14
Methionine (%)	0.434
Choline mg / kg	499.08
Cysteine (%)	0.3459
Claysine (%)	0.8343
Lysine (%)	1.021
Calcium (%)	0.910
Available phosphorous (%)	0.709
Energy ratio: protein	147.95

Results and Discussion

Data presented in table 2 shows the average biochemical characteristics of blood serum at the age of 6 weeks, where the results showed a significant superiority (p≤0.05) in the total protein concentration in the third fourth and fifthtreatments compared with first and second treatments. Thehighest concentration of albumin was recored in the fourth treatment significantlydiffered (p≤0.05) comparedwith first and second treatments, while the and thelowest value was recorded in the second treatment, The globulin concentration, wasno significant differences occurred among all experiment groups.The second treatment (positive control) recorded the highest glucose concentration with a significant difference (p≤0.05) from the rest of the trial treatments, while the lowest concentration was recorded in the fifth treatment .

Table 2 :Effect of adding different biotin levels to the diet on some biochemical characteristics in blood serum of Japanese quail exposed to oxidative stress at 6 weeks of age(Mean ± SEM).

Treatments	Studied traits			
	Total protein (g / 100 ml)	Albumin (g / 100 ml)	Globulin (g / 100 ml)	Glucose (mg / 100ml)

First group	3.57±0.24 b	2.01±0.08 bc	1.56±0.18	304.95±2.04 b
Second group	3.34±0.05 b	1.77±0.14 c	1.57±0.09	374.78±14.51 a
Third group	4.37±0.07 a	2.35±0.29 ab	2.02±0.36	265.44±6.42 c
Fourth group	4.63±0.13 a	2.56±0.11 a	2.07±0.05	251.59±9.68 cd
Fifthgroup	4.47±0.07 a	2.32±0.05 ab	2.15±0.02	225.03±7.28 d

-Averages with different letters within one column indicate significant differences ($p \leq 0.05$).NS: Not significant.
-The first group = negative control treatment (basal diet + H2O2 free drinking water).
-The second group = positive control treatment (basal feed + drinking water containing 0.5% H2O2).
-The third group = (500 micrograms biotin / kg of basal diet + drinking water containing 0.5%. H2O2)
-The fourth group = (600 micrograms biotin / kg of basal diet + drinking water containing 0.5% (H2O2).
-The fifthgroup = (700 micrograms biotin / kg of basal diet + drinking water containing 5.0% H2O2).

From these results, it can be noted that the treatment with biotin led to an increase in the concentration of total protein and albumin, and this proves its role in acting as one of the most important antioxidants (Combs, 2008; A l- Qudah,2010), which was reflected in the health status of the herd and reducing stress. Resulting from oxidation of free radicals represented by an increase in the concentration of total protein and albumin, in addition to the role of biotin in increasing the number of lymphocytes white, which decreased its percentage in the index of the ratio of lymphocytes to heterozygous cells in the factors of adding biotin in this study, and that the significant improvement in the albumin concentration of the biotin treatments in the blood serum It may be due to the improvement of the total protein ratio, as an increase in the total protein concentration in the blood serum indicates an increase in protein synthesis and a decrease in protein catabolism (Patterson et al,1967).As for glucose concentration, it is known that biotin has an important role in maintaining normal levels of glucose in the blood (Bryden ,1991; Volker ,2007), through its work as an co enzymatic companion to carboxylases as it acts as a non-protein group accompanying the enzymes to transport the carboxyl group. Carboxy pyruvate is an enzyme of the Carboxylase group for which biotin acts as an co enzyme companion, and Carboxy pyruvate serves to determine the process of regenerating glucose (Chapman and Cronan,1999). This role of biotin was evident through the results of the current experiment, as it worked to maintain the level of glucose sugar in the treatments that were carried out in addition to the quail bird's diet (the third, fourth and fifth treatment), which was added to the hydrogen peroxide drinking water.The reason for the significant increase in glucose in the secondgroup (positive control) may be attributed to the role of hydrogen peroxide added to the drinking water of quail birds, which increased the level of free radical formation causing damage in pancreatic cells and thus the irregular release of the hormone insulin responsible for maintaining a normal level of glucose causing an increase in the level of glucose in the blood (Montagut et al ,2010).
The results in Table 3 indicated that the effect of adding different levels of biotin to the diet on the activity of liver enzymes AST, ALT, and glutathione in the blood of Japanese quail exposed to oxidative stress the results showed a significant increase ($p \leq 0.05$) in the second group in the activity of the AST enzyme compared to the first, third, fourth and fifthgroups, as for the activity of the ALT enzyme, we also note that the second group recorded the highest concentration of the enzyme, with a significant difference ($p \leq 0.05$) than the rest of the trial treatments, while the fourth group and the treatment were recorded Fifth, lowest concentration of ALT.The concentration of the enzyme glutathione, in the fifth group recorded the highest levelwith a significant difference ($p \leq 0.05$) from the rest of the experiment groups, followed by the third and the fourth group, while the lowest concentration of the glutathione enzyme was recorded in the first and second groups.

Table 3:Effect of adding different levels of biotin to the diet on the AST and ALTenzymes and glutathione in the blood serum of Japanese quail exposed to oxidative stress (mean ± SEM) at 6 weeks of age.

Treatments	Studied traits		
	AST (IU / liter)	ALT (IU / liter)	Serum glutathione enzyme (Mmol / mol)
First group	21.51±0.64 b	12.09±0.29 b	859.39±28.24 d
Second group	23.80±0.90 a	14.25±0.35 a	834.18±8.37 d
Third group	21.01±0.33 b	11.84±0.26 b	1020.73±67.64 c
Fourth group	20.87±0.38 b	10.60±0.35 c	1233.65±42.45 b
Fifth group	20.16±0.38 b	10.84±0.25 c	1382.75±4.87 a

-Averages with different letters within one column indicate significant differences ($p \leq 0.05$).
-The firstgroup = negative control treatment (basic diet + H2O2 free drinking water).
-The second group = positive control treatment (basic feed + drinking water containing 0.5% H2O2).
-The third group= (500 micrograms biotin / kg of basic diet + drinking water containing 0.5%. H2O2)
-The fourth group = (600 micrograms biotin / kg of basic diet + drinking water containing 0.5% (H2O2).
-The fifth group = (700 micrograms biotin / kg of basic diet + drinking water containing 5.0% H2O2).

Through the results mentioned in Table 3, it can be noted that the reason for the high levels of AST and ALT in the blood serum of the second group (positive control) may be due to the role of hydrogen peroxide root added to drinking water in increasing the induction of endogenous oxidative stress through its establishment. By stimulating the production of free radicals, especially the roots of active oxygen varieties, which act to attack the polyunsaturated fatty acids present in the membranes of the liver cells, and then the accumulation of lipid peroxide products represented by the compound malondehide in the blood serum, and all these changes will lead to a change in the permeability of cell membranes It causes an increased leakage of the ALT and AST enzymes into the blood (Samual et al, 2000) and thus an increase in their levels in the blood.As for the ability of adding biotin to reduce the activity of ALT and AST enzymes in the blood serum, this may be due to the role of this vitamin in enhancing the activity of antioxidants in the body and then working to reduce oxidative stress and maintain the manufacturing function of the liver (Samual et al, 2000).Referring to the results of glucose in the blood serum, Table (2), it can be said that the reason for its high level in the second treatment (positive control) birds in our current study may have occurred as a result of the high activity of the ALT and AST enzymes in the blood of these birds because these two enzymes encourage the process Manufacture of glucose from non-carbohydrate sources, especially from proteins, leading to an increase in protein catabolism to provide essential amino acids and then convert them into keto acids used to build glucose (Ray et al, 2001; Nelson and Cox,2004).It can be concluded from the results that adding H2O2 to bird drinking water for the purpose of inducing oxidative stress in the second group (positive control), to which biotin was not added, led to a deterioration of the physical antioxidant activity, which was inferred from the significant decrease in the activity of the glutathione enzyme in the blood which accompanied by a significant increase in the value of MDA compared to the treatments of adding biotin to the diet, and the reason for this may be that the use of hydrogen peroxide in drinking water led to the start of a series of chemical reactions leading to endogenous oxidative stress, which leads to an excessive increase in the production of oxygen compounds The activity (Loven and Oberley , 1985) is matched by a weakness in the endogenous antioxidant system, which leads to an imbalance between oxidation and the antioxidant system. It has been observed that hydrogen peroxide and other types of free radicals have a direct inhibitory effect of the activity of various antioxidant systems. Endogenous, such as GSH-PX (Glutathione Peroxidase) and others responsible for expelling free radicals and peroxides, then this will lead to a shelf at the level of MDA (Shehata and Yousef ,2010), the glutathione peroxidase enzyme whose action depends on the main

substance, glutathione, which converts glutathione from the reduced active form to the inactive oxidant, and with this reaction, the enzyme glutathione peroxidase is transformed into the active reduced form by withdrawing the selenium atom then this reduced enzyme can react with hydrogen peroxide and free radicals.
The results in Table 4 showed the effect of adding different levels of biotin to the diet in the lipids in blood of Japanese quail exposed to oxidative stress, where the second group recorded the highest concentration of cholesterol and triglycerides with a significant difference ($p \leq 0.05$) for the rest of the trial treatments, followed by the first group, while the third, fourth and fifth groups recorded the lowest concentration of cholesterol and triglycerides,as for high-density lipoproteins, the results indicated that the third, fourth and fifth group recorded the highest concentration of high-density lipoproteins, with a significant difference ($p \leq 0.05$) compared to the first and second treatments, which recorded the lowest concentration,while the concentration of low-density lipoproteins recorded the highest concentration the first and second groups with a significant difference ($p \leq 0.05$) from the rest of the experiment treatments, and from the same table we note that the second treatment recorded the highest level of malondehide with a significant difference ($p \leq 0.05$) The third, fourth and fifth groups recorded the lowest level of malondehide, compared to the experiment groups, followed by the first group (negative control).

Table 4: Effect of adding different levels of biotin to the diet in the lipid profile of Japanese quail blood exposed to oxidative stress (mean ± SEM) at 6 weeks of age

Treatments	Studied traits				
	Cholesterol (mg / 100mL)	Triglycerides (mg / 100mL)	High-density lipoproteins (mg / 100mL)	Low-density lipoproteins (mg / 100mL)	The level of malondehide (MDA) Micromol / mol
First group	205.62±3.34 b	216.64±11.06 b	99.25±2.28 b	63.03±6.81 a	5.27±0.22 b
Second group	227.77±5.17 a	254.17±4.57 a	101.85±3.66 b	75.08±8.78 a	6.75±0.02 a
Third group	190.85±1.51 c	186.21±2.35 c	136.05±3.15 a	17.55±3.90 b	4.66±0.34 c
Fourthgroup	185.60±5.19 c	152.92±5.79 d	134.38±2.13 a	20.63±8.07 b	4.15±0.04 c
Fifth group	187.19±1.54 c	156.56±6.00 d	135.73±1.19 a	20.14±2.83 b	4.09±0.02 c

-Averages with different letters within one column indicate significant differences ($p \leq 0.05$).
-The first group = negative control treatment (basic diet + H_2O_2 free drinking water).
-The second group = positive control treatment (basic feed + drinking water containing 0.5% H_2O_2).
-The third group = (500 micrograms biotin / kg of basic diet + drinking water containing 0.5%. H_2O_2)
-The Fourth group = (600 micrograms biotin / kg of basic diet + drinking water containing 0.5% (H_2O_2).
-The fifth group = (700 micrograms biotin / kg of basic diet + drinking water containing 5.0% H_2O_2).

The reason for the lower concentration of cholesterol, triglycerides, low-density lipoproteins, and the higher high-density lipoproteins in biotin addition treatments compared to the first group (negative control) and the second group (positive control) may be due to biotin, which acts as a non-enzymatic antioxidant, as it removes the free radicals formed in an image. Naturalness inside the body before entering the chain of reaction, thus reducing fat rancidity and enhancing the state of antioxidants present in the body (Combs, 2008)as well as its role in determining the process of fat formation through its work as an co-enzyme accompaniment to the enzyme Acetyl CoA (Sweetman , 2000).The ability of biotin to remove free radicals formed inside the body comes through its containment of a sulfur atom in its composition, which is the basis of its chemical and biological activity, as this atom interacts directly with many free radicals such as hydro peroxides, -2Oand - OHradicals. It is thus a free radical scavenger, especially -OH root (Nelson and Cox,2004).Shehata and Yousef (2010)noted that the oxidation of low-density lipoproteins comes from increased MDA levels in the tissues as a result of oxidative stress produced by hydrogen peroxide, and it is known that the low-density lipoprotein molecule is formed when the triglyceride molecule is completely split by the lipoprotein lipase enzyme in tissue outside the liver, and the molecule of low-density lipoproteins is the main carrier of cholesterol and triglycerides. This molecule is taken from various cells of the body for use in cellular construction and the manufacture of products that are involved in building and synthesizing fat (Nelson and Cox,2004).The decrease in the MDA concentration of biotin treatments compared to the first group (negative control) and the second group (positive control) may be due to the fact that biotin is an effective antioxidant as it works to prevent the oxidation of cell membranes lipids and suppress free radicals by cutting the chains of reactions, thus inhibiting from the formation of lipid peroxidation, therefore, the concentration of triglycerides and malondehide will decrease in serum (Combs, 2008).

Conclusion:

The addition of biotin to the diet, in different concentrations, improved the biochemical characteristics of Japanese quail compared to the negative and positive control groups.

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