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Original Article

The Effectiveness of Biotin (Vitamin B_7) Added to the Diet in Improving the Efficiency of Productivity, and Some Physiological Traits for Broiler Chickens (Ross-308) Exposed to Oxidative Stress

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Abstract

Biotin (B_7) acts as an antioxidant, as it inhibits the effect of many free radicals that are naturally formed within the organism's body. This study aimed to determine the effect of adding different concentrations of biotin to the diet of broilers exposed to oxidative stress in improving productive and physiological performance. 180 unsexed Ross-308 one-day age chicks of broiler chickens were used, and they were reared together until 7 days. Then the chicks were randomly distributed into 5 treatments; each treatment included 3 replicates, 12 chicks for each replicate. Oxidative stress was induced by adding 0.5% H_2O_2 to drinking water. The first treatment, T1 (control), was free from adding B_7 or H_2O_2, and the second treatment (T2): was a positive control treatment free from adding vitamin B_7 + water added to it H_2O_2 at an average of 0.5%. The third treatment (T3): adding 550 micrograms of B_7/kg of feed + water added H_2O_2 at an average of 0.5%. Fourth treatment (T4): 650 micrograms/kg vitamin B_7 + water with 0.5% H_2O_2 added on average. Fifth treatment (T5): 750 micrograms of vitamin B_7/kg of feed + 0.5% H_2O_2 added to the water. The results of adding vitamin B_7 to the diet of T4 and T5 birds exposed to oxidative stress showed a significant increase (P<0.05) in the average live body weight, total weight gain and cumulative feed consumption average, and the best cumulative feed conversion ratio for treated birds (T3, T4, T5). In comparison to the other therapies, all additional vitamin B_7 treatments demonstrated a significant decrease (P<0.05) in the concentrations of glucose, cholesterol, triglycerides, as well as Malondialdehyde (MDA), and low-density lipoproteins (LDL) and a significant increase (P<0.05) in the concentrations of high-density lipoproteins (HDL) and total protein. The concentration of glutathione peroxidase (GSH-Px) was significantly elevated (P<0.05) in the serum of T5-treated birds compared to all other treatments. We conclude that adding vitamin B_7 in different concentrations improved the productivity and some physiological traits of broilers exposed to the induced oxidative stress compared with the negative and positive control treatments.

Keywords: Biotin, Vitamin B_7, Physiological traits, Broiler chickens, Ross-308, Oxidative stress

1. Introduction

Many researchers working in the field of public health and nutrition have been interested in studying the biological importance of vitamins of all kinds because of their importance in maintaining the permanence and continuity of living organisms (1). Recent studies emphasized the importance of poultry food containing substances such as vitamins that help improve digestion and help in vital metabolic processes within the bird's body. Some vitamins are included in the composition of many vital compounds, such as enzymatic chaperones that act as carrier factors in cellular respiration. Others work in maintaining bodily structures such as connective, epithelial, bone, and endothelial tissues (2). Some vitamins prepared industrially play a protective role against the oxidative processes that can damage the vital molecules inside the living cell (3, 4). The increase in free radicals in the body leads to a state known as Oxidative Stress, where free radicals are constantly produced in the body, and quantities of these components are necessary for critical physiological functions. However, when their production excelled normal levels, they cause damage to the cell membrane (5). Therefore, antioxidants are divided into two categories: Antioxidants of the first type that are self-generated or of somatic origin, such as glutathione (GSH), as well as a group of enzymes such as glutathione peroxidase, superoxide dismutase, and Catalase, and of them as antioxidants of the second type, which are food antioxidants that are added in diets (6).

Biotin (B_7) acts as an antioxidant, as it inhibits the effect of many free radicals that are naturally formed within the organism's body (6). It acts as an enzymatic companion for many of the metabolic processes that occur in the body. It plays a vital role in transporting and stabilizing the carbon dioxide group CO_2. It also acts in the metabolism of proteins and fats, gluconeogenesis, and other vital activities that occur inside the body (7). Therefore, the current study aimed to know the ability of biotin to protect against oxidative stress caused by adding 0.5% hydrogen dioxide in broiler chickens.

2. Materials and Methods

2.1. Animals and Study Design

In this study, 180 unsexed Ross-308 broiler chicks of one day age with an average weight of 40g were used for the period from 1/3/2020 to 11/4/2020, during which the effect of adding different levels of biotin to the diet was studied to know its effect on productive and some physiological traits of broiler chicks. The chicks were fed on a starter diet at the age of 1-21 days and a final diet at the age of 22-42 days. Table 1 shows the composition of the starter and final diet used for feeding chicks throughout the experiment and the calculated chemical composition. The chicks were managed in two periods:

Table 1. Shows the composition of the starter and final diet used for feeding chicks throughout the experiment period

Feed material	Starter diet %(1-21 days)	Final diet %(42-22 days)
yellow corn	51	54
wheat	12.5	11.5
Soybean meal 44% protein	24	22
Protein Concentrate(1) *	10	10
Vegetable oil	1.5	1.5
limestone	0.7	0.7
salt	0.3	0.3
total summation	100%	100%
crude protein%	21.49	20
Represented energy (kilocalories/kg of feed)	2990.5	3150
Energy to Protein Ratio	139	158
Lysine	1.22	1.14
methionine + cysteine (%)	0.62	0.58
Calcium (%)	1.43	1.26
Total phosphorous (%)	0.90	0.81
availability phosphorous (%)	0.67	0.57

*Protein concentrate BROCON-5 SPECIAL W: Chinese origin, each kg contains: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorous, 3.25% lysine, 3.90% methionine + cysteine Sodium, 2.2% Sodium, 2100 kcal/kg Energy Representation, 20,000 IU Vitamin A, 40,000 IU Vitamin D_3, 500 mg Vitamin E, 30 mg Vitamin K_3, 15 mg Vitamin B_1+B_2, 150 mg B_3, 20 mg B_6, 300 B_{12} mg, folic acid 10 mg, biotin 100 mcg, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT). The chemist for the liquor according to the NRC (1994).

1) The period before treatment: the period from one day to seven days of age without treatment with vitamin B_7 to accustom the chicks to the atmosphere of the hall, Water and feed were provided free of charge with the use of a continuous lighting system, before and after the treatment.

2) The treatment period: This period extended from the age of 8 days until the age of 42 days (end of the experiment); the chicks were raised in a prepared hall and divided into cages, the dimensions of each cage x 1.5) 1 (m² according to the ground breeding system.

The chicks were randomly distributed to 15 cages with 5 treatments. Each treatment included 3 replicates, and each replicate included 12 birds. The experiment treatments were as follows:

The first treatment, T1 (control), was free from adding B_7 or H_2O_2, and the second treatment (T2): was a positive control treatment free from adding vitamin B_7 + water added to it H_2O_2 at an average of 0.5%. The third treatment (T3) is the addition of 550 micrograms of B_7 per kg of feed with water containing an average of 0.5 percent H_2O_2; the Fourth treatment (T4): adding 650 micrograms/kg of vitamin B_7 and 0.5 percent H_2O_2 in water, on average. Fifth treatment (T5): 750 mg of vitamin B_7 per kg feed with water containing an average of 0.5 percent H_2O_2.

2.2. Productive Traits and Physiological Assays

The following productive traits were estimated at 42 days of age: live body weight, total weight gain, total feed consumption rate, and cumulative feed conversion ratio, in addition to some physiological traits at the end of the experiment, which included: glucose, high-density lipoproteins, Low-density lipoproteins, total protein, cholesterol, triglycerides, malonaldehyde MDA and glutathione peroxidase GSH-Px in blood plasma. Blood was collected randomly from 6 birds from each treatment (2 replicates). The blood was collected from the brachial vein in tubes that do not diagonally contain anticoagulants and then placed in a centrifuge at a speed of 3000 rpm to separate the blood plasma. Then the serum was transferred to special sealed test tubes, and the containers were kept inside the freezer at -20°C until the above traits were measured. Where the concentration of glucose was measured using a measuring kit from the German business Roche, and the technique (8) and the concentration of cholesterol and high-density lipoproteins was measured using a measuring kit from the German company Roche and the method of Franey and Amador (9). As for low-density lipoproteins, they were estimated according to the method of (10) and measurement of the level of triglycerides in the blood serum using a ready-made kit; then, the samples were read at wavelength (546) nm using a spectrophotometer according to the method (11). The total protein concentration was measured using a kit from the German company Roche, based on the Biuret method and Wotton (12). As for the enzyme glutathione peroxidase, its concentration was measured using a kit (Kit) from Roche company based on Al-Qudah and Ismail (13) and malonaldehyde; its concentration was measured using a kit (Kit) from Roche company based on (14). A completely randomized design was used to study the effect of different treatments on the studied traits, and the significant differences between the means were compared using Duncan's polynomial test (15), and SAS (2012) (Version 9.1) was used to analyze the data.

3. Results and Discussion

3.1. Productive Traits

The statistical analysis results in table 2 show significant differences ($P<0.05$) in the average live body weight, total weight gain, total feed consumption, and cumulative food conversion ratio among all treatments. A considerable rise was seen after the sixth week of the trial. ($P<0.05$) in the live body weight of the birds of the fourth and fifth treatments compared to the birds of the first, second, and third treatments, and the values were (2694, 2714) g/bird birds versus (2608, 2251, and 2664) g/bird birds, respectively. As for the total weight gain, a significant increase ($P<0.05$) was observed in favor of the birds of the fifth treatment, which recorded 2501 g/bird the paired with other treatments. The significant increase in the average live body weight in the treatment of adding vitamin B_7 may come through the role played by this vitamin as a non-enzymatic antioxidant. It removes free radicals naturally formed inside the body before entering the chain reaction (6).

In addition to its essential role in improving the metabolism of amino acids and proteins (16) and in the development of somatic cells that all contain this vitamin (17), vitamin B_7 can also lead to an increase in the live body weight of the bird, as evidenced by the same table's findings that there is a significant decrease in the body weight of the birds in the second treatment, which did not include the addition of vitamin B_7. Inside the body, the lipids present in the plasma membrane of cells are oxidized, causing tissue damage and destruction (2); this may be the reason for the second treatment's reduction in the weight of the birds. According to the close relationship between the rate of live body weight and weight gain, the significant improvement in the average total weight gain for the treatments of adding vitamin B_7 (fourth and fifth) is due to the improvement in the average live body weight as a result of the effect of adding B_7 to the diet. It had an apparent effect on improving the overall weight gain of the experimental treatment birds. This may be due to the fact that it is involved in the formation of tissues and organs, which leads to good growth and the building of the bird's skeleton correctly. It also works on the AcetylCoA enzyme, the source, and limiter of fat formation. It then leads to an increase in the percentage of fat, which in turn leads to an increase in body weight (17) and its role in increasing the representation of proteins and amino acids (18). As for the results of the cumulative feed consumption average, a significant increase (P<0.05) was observed in the total feed consumption average for the birds of the fourth treatment, where they recorded 4301 g/bird birds, followed by 4249 g/bird in the third treatment compared to the first, second and third treatments, which recorded (4180, 3614 and 4202 g/bird)respectively. Vitamin B_7's antioxidant function and the inclusion of a sulphur atom in its structural formula, one of the electron-donor atoms that operate to saturate the free radical's outer shell, may be responsible for the increase in the total feed consumption of birds treated with vitamin B_7. In addition to the critical role of vitamin B_7, which converts carbs and proteins into lipids, resulting in its stability and reduced damage to the body's cells, this causes an increase in the hunger of birds (19). In the same table, it was noted that the vitamin B_7 adding treatments (third, fourth, and fifth) provided the best cumulative food conversion ratio of (1.72, 1.76, and 1.76) g/bird weight gain, compared to the first (negative) and second (positive) treatments, which recorded (1.77 and 1.82) g/bird of weight gain, respectively. This may be due to the fact that vitamin B_7 acts as the enzyme Carboxylase, Decarboxylase, and Transcarboxylase, which stimulate metabolism and play an important role in biochemical processes such as gluconeogenesis. In addition to the favorable effect of this vitamin when given to feed and its role in resisting oxidative stress, it promotes the creation of fat and energy. This leads to an improvement in feed consumption and an increase in the utilization of various nutrients and consequently an improvement in the food conversion ratio, which leads to healthy and highly effective birds (20).

3.2. Biochemical traits

It is clear from the data in table 3, which includes the effect of adding biotin to the diet of broilers exposed to oxidative stress on total protein, glucose, cholesterol, and glycerides, a significant decrease (P<0.05) in the concentration of glucose, cholesterol, triglycerides and low-density lipoproteins (LDL) and a high Significant in the concentration of total protein and high-density lipoproteins (HDL) in the serum of birds of all vitamin B_7 supplementation treatments compared to the first and second treatments.The significant (P<0.05) decrease in glucose concentration may be due to the role of vitamin B_7 in maintaining the normal level of glucose in the blood (21) through its work as an enzymatic chaperone for carboxylases enzymes as it acts as a non-protein-accompanying group of enzymes on the transfer of the carboxyl group Carboxy pyruvate is one of the enzymes of the carboxylase group, to which vitamin B_7 acts as a coenzyme, and this enzyme determines the process of rebuilding glucose (22), The reason for the significant increase (P<0.05) of glucose level in the second treatment (positive control) free from adding vitamin B_7 is due to the role of hydrogen peroxides added to drinking water, which led to an increase in the level of free radical formation, causing damage to pancreatic cells, and consequently the irregular release of the hormone insulin. Responsible for maintaining the normal level of glucose in the blood, causing an increase in the level of this sugar in the blood serum (23), and the reason for the low concentration of cholesterol, triglycerides, LDL, and high HDL may be due to the role of vitamin B_7, which acts as a non-enzymatic antioxidant. It removes free radicals that are naturally formed inside the body before entering the reaction chain, thus reducing the peroxidative treatment of fat and enhancing the antioxidant status in the body (6), in addition to its role in determining the process of fat formation through its work as an enzymatic companion to the acetyl enzyme CoA (24). The ability of biotin to remove free radicals formed inside the body comes through the presence of a sulfur atom in its composition, which is the basis for its biochemical and biological activities, where this atom interacts directly with many free radicals such as hydroperoxide radicals and 2-O -OH, It is thus a scavenger of free radicals, especially the -OH radical (25). Shehata and Yousef (26) stated that the oxidation of low-density lipoproteins is caused by elevated levels of monoaldehyde (MDA) in tissues due to oxidative stress caused by hydrogen peroxide.It is known that the low-density lipoprotein molecule is formed when the triglyceride molecule is completely cleaved by the action of the enzyme lipoprotein lipase in the tissues outside the liver. Fat in its structure and composition (25). Through the results of the study, it can be said that treatment with vitamin B_7 led to a significant (P<0.05) increase in the concentration of total protein, and this proves its role in working as one of the most important antioxidants (13). This was reflected on the health of the herd and the reduction of stress caused by free radical oxidation, represented by an increase in the concentration of total protein, where the increase in the concentration of total protein in the blood serum indicates an increase in the process of building protein and a decrease in the process of protein catabolism (27). Table 4 showed significant differences between all treatments in the level of Malondialdehyde (MDA) and Glutathione peroxidase (GSH-Px).The level of MDA decreased significantly (P<0.05), and the level of GSHPx in the serum of birds increased significantly from the addition of vitamin B_7 compared to the first and second treatments. Interrupting the chains of chemical reactions thus will inhibit the formation of lipid peroxidation, so the concentration of triglycerides and MDA will decrease in the blood serum (6). It can be concluded from the results that adding Hydrogen peroxides to the drinking water of birds causes oxidative stress in the second treatment (positive control), to which vitamin B_7 was not added. It led to the deterioration of the physical antioxidant activity, inferred from the significant decrease in the activity of the enzyme glutathione in the blood serum, which was accompanied by a significant increase in the value of MDA compared to the added treatments of vitamin B_7 to the diet. The reason for this may be that the use of Hydrogen peroxides in drinking water has led to the start of a series of chemical reactions leading to endogenous oxidative stress, which occurs by increasing the production of oxygen in the gastrointestinal tract, which in turn enters the blood leading to a high oxygen pressure in the cells, which leads to an excessive increase in the production of active oxygen compounds (28), This is offset by a weakness in the endogenous antioxidant system, which leads to an imbalance between the oxidation and the antioxidant system, and it has been observed that hydrogen peroxides and other types of free radicals have a direct inhibitory effect on the activity of various endogenous antioxidant systems such as GSH-PX (Glutathione). Peroxidase) and others are responsible for expelling free radicals and peroxides, which will lead to an increase in the level of MDA (26). The enzyme glutathione peroxidase, whose action depends on the main substance, glutathione, converts glutathione from the active reduced form to the inactive oxidizer. This reaction converts glutathione peroxidase to the active reduced form by pulling out a selenium atom. Then this reducing enzyme can react with hydrogen peroxides and free radicals. Therefore, a decrease in the concentration of glutathione due to the effect of oxidative stress leads to a decrease in the activity of the enzyme glutathione peroxidase and then an increase in the concentration of free radicals and the continuation of the chain of destructive oxidation reactions in the cell (25).

It is concluded that adding vitamin B_7 in different concentrations improved the product and some physiological traits of broilers exposed to the induced oxidative stress compared with the negative and positive control treatments.

Table 2. Effect of feeding different levels of biotin to broiler diet exposed to oxidative stress on some productive parameters (body weight, total weight gain, total feed consumption, total feed conversion ratio. (mean ± standard error)

The cumulative food conversion ratio	Total feed consumption(g)	Total weight gain(g)	Live body weight(g)	Treatments
1.77 ± 0.012^{ab}	4180 ± 15.60^{c}	2361 ± 21.37^{c}	2608 ± 24.63^{b}	T1
1.82 ± 0.015^{a}	3614 ± 18.33^{d}	1981 ± 10.40^{d}	2251 ± 19.60^{c}	T2
1.76 ± 0.016^{b}	4202 ± 10.12^{C}	2389 ± 15.17^{cd}	2664 ± 23.07^{ab}	T3
1.76 ± 0.009^{b}	4249 ± 15.25^{b}	2415 ± 18.22^{b}	2694 ± 23.07^{a}	T4
1.72 ± 0.029^{b}	4301 ± 11.89^{a}	2501 ± 13.74	2714 ± 16.33^{a}	T5
*	*	*	*	Level of Significance

*The averages with different letters within the same column differ significantly from each other ($P<0.05$)

Table 3. Effect of feeding different levels of biotin to broiler diet exposed to oxidative stress on glucose, total protein and cholesterol and triglycerides, HDL and LDL of broiler chicken serum (mean ± standard error)

LDL (mg/100ml)	HDL (mg/100ml)	total protein (g/100ml)	triglycerides (mg/100ml)	cholesterol (mg/100ml)	glucose (mg/100ml)	Treatments
59.37 ± 5.69^{b}	71.40 ± 10.41^{b}	3.32 ± 0.15^{b}	97.29 ± 10.06^{b}	171.67 ± 4.63^{b}	269.68 ± 7.85^{ab}	T1
69.57 ± 4.24^{a}	73.55 ± 8.78^{b}	2.94 ± 0.11^{b}	115.40 ± 12.13^{a}	195.30 ± 3.22^{a}	282.07 ± 10.70^{a}	T2
43.40 ± 3.61^{c}	94.64 ± 3.54^{a}	3.95 ± 0.09^{a}	81.59 ± 10.88^{c}	148.10 ± 7.08^{c}	241.10 ± 11.23^{bc}	T3
33.63 ± 3.46^{c}	97.00 ± 4.73^{a}	4.01 ± 0.14^{a}	73.82 ± 3.03^{c}	139.92 ± 5.87^{c}	228.00 ± 8.89^{c}	T4
29.23 ± 4.23^{c}	98.67 ± 1.86^{a}	4.16 ± 0.13^{a}	70.00 ± 3.75^{c}	129.58 ± 7.56^{c}	217.37 ± 6.70^{c}	T5
*	*	*	*	*	*	Level of Significance

*Means of different letters within the same column differ significantly ($P<0.05$)

Table 4. Effect of feeding different levels of biotin in the diet on the level (MDA, GSH-Px) of broiler blood serum (mean ± standard error)

Level of Significance	T_5	T_4	T_3	T_2	T_1	Treatments
*	4.46 ± 0.23^{c}	4.67 ± 0.08^{c}	5.22 ± 0.06^{b}	6.04 ± 0.08^{a}	58.5 ± 0.20^{ab}	MDA µmol/ mol
*	195.61 ± 9.81^{a}	178.83 ± 5.34^{ab}	162.16 ± 5.18^{b}	121.13 ± 4.46^{d}	141.09 ± 3.45^{c}	GSH-Px µmol/ mol

*Means of different letters within the same column differ significantly ($P<0.05$)

Authors' Contribution

Study concept and design: A. H. K.
Acquisition of data: A. S. N.
Analysis and interpretation of data: M. A. A.
Drafting of the manuscript: A. H. K.
Critical revision of the manuscript for important intellectual content: A. H. K.
Statistical analysis: A. H. K.
Administrative, technical, and material support: A. S. N.

Ethics

All study protocols have been approved by the ethics committee of Al-Qasim Green University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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