Effect of adding different levels of vitamin B7 to the diet on some blood for the parameters of Japanese quail (Coturnix coturnix japonica) exposed to oxidative stress

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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 vitamin B7 to the diet on some blood for the parameters of Japanese quail (Coturnix coturnix japonica) 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: a negative control treatment that is free of vitamin B7 or H2O2. The second treatment: a positive control treatment that was free of adding vitamin B7 + water with H2O2 added by only 0.5%. The third treatment: adding vitamin B7 at a concentration of 500 micrograms/kg feed + water added with H2O2 at a concentration of 0.5%. The fourth treatment: adding vitamin B7 at a concentration of 600 micrograms/kg + water with H2O2 added at a concentration of 0.5%. The fifth treatment: adding vitamin B7 at a concentration of 700 micrograms/kg + water with H2O2 added at a concentration of 0.5%. The treatment lasted from 8 to 42 days. The experiment included the study of the following traits: red blood cell count, white blood cell count, percentage of Haematocrit (%), Hemoglobin concentration, percentage of the heterophile cells (%), percentage of lymphocyte cells (%) and H/L ratio. The results indicated that the fourth and fifth treatment (adding of 600 and 700 micrograms of vitamin B7 / kg feed) showed a significant improvement (p≤0.05) in the numbers of red blood cells compared to the first treatment (negative control) and the second treatment (positive control), as for the Haematocrit and haemoglobin concentration. The adding treatments (third, fourth, and fifth) significantly differed (p≤0.05) on the first treatment (negative control) and the second treatment (positive control) at the age of 42 days. All treatments of adding vitamin B7 recorded a significant decrease (p≤0.05) in the numbers of white blood cells, percentage of the Heterophil cells (%) and increase in Lymphocytosis cells and decrease in the percentage of H/L cells as compared with the second treatment (positive control). It is concluded from the present experiment that adding of the vitamin B7 at a concentration of (600, 700 μg /kg feed) to the diet can lead to improving some blood parameters for Japanese quail.

Keywords: vitamin B7, blood parameters, Japanese quail, Oxidative stress

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 [1,2], 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 [3]. 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. 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 [4]. Among these vitamins is vitamin (B7), which is one of the types 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 [5] 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 [6]. 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 [7]. And to vitamin B7 has an important role in Histological immunity, as [8] indicated that vitamin B7 is distributed regularly within tissues in poultry and works to participate in the production of antibodies, 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 vitamin B7 to reduce the effect of experimentally induced oxidative stress using hydrogen peroxide H2O2 in Japanese quail on some blood parameters.

2. Materials and Methods

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 vitamin B7 to the diet on some blood for the of parameters Japanese quail (Coturnix coturnix japonica) 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 chicks were managed in two periods: First, before the treatment: The chicks were raised for 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 treatment: a negative control treatment that is free of vitamin B7 or H2O2. The second treatment: a positive control treatment that was free of adding vitamin B7 + water with H2O2 added by only 0.5%. The third treatment: adding vitamin B7 at a concentration of 500 micrograms/kg feed + water added with H2O2 at a concentration of 0.5%. The fourth treatment: adding vitamin B7 at a concentration of 600 micrograms/kg + water with H2O2 added at a concentration of 0.5%. The fifth treatment: adding vitamin B7 at a concentration of 700 micrograms/kg + water with H2O2 added at a concentration of 0.5%. The treatment lasted from 8 to 42 days. This experiment included the study of the following traits: red blood cell count, white blood cell count, percentage of Haematocrit (%), Hemoglobin concentration, percentage of the heterophile cells (%), percentage of lymphocyte cells (%) and H/L ratio. Blood was collected from 6 birds (2 birds from each replicate) through the humeral vein at the age of 28 days and by cutting the jugular vein at the age of 42 days where it was randomly selected from each treatment and the blood was placed in tubes containers on the anticoagulant substance EDTA to prevent blood clotting. Haematocrit was calculated using special capillary tubes containing an anticoagulant according to the method indicated by [9]. Hemoglobin concentration was estimated according to the method indicated by [10]. The red and white blood cell counts were estimated according to the method indicated by [11]. The heterophil/lymphocyte ratio was estimated using glass slides where a drop of blood was placed on the glass slide and spread very carefully with another glass slide placed above the blood drop and pulled over the first slide at an without being pressed firmly and left to dry at a rate of 10 min. Thereafter, the slices are stained with a mixture of Gamsa stain dyes according to The counting is done using an optical microscope under a magnification power (1000) by placing an oil drop on the slide according to the method of [12]. 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 [13], and the SAS [14] was used to analyze the data.
Table 1. Ratios of fodder materials and the calculated chemical composition of the feed used in the study.

| 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 (%)    | 22.05    |
| Metabolized Energy (kcal/kg) | 2954.19 |
| Methionine (%)       | 0.474    |
| Choline mg / kg      | 499.08   |
| Cysteine (%)         | 0.3459   |
| Claysine (%)         | 0.8343   |
| Lysine (%)           | 1.1839   |
| Calcium (%)          | 0.6772   |
| Phosphorus (%)       | 0.3478   |
| Energy ratio: protein| 133.97   |

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 [15].

3. Results

Table 2. shows the effect of adding different levels of vitamin B7 to the diet in some of the cellular blood traits of Japanese quail and exposed to oxidative stress at the age of 28 days, where we note the second treatment (positive control) recorded the highest number of white blood cells (10³ mm⁻³ blood) and reached (26.33 x 10³ mm⁻³ blood) and a significant difference (p≤0.05) from the rest of the trial treatments. As for the number of red blood cells (10⁶ mm⁻³ blood), the fifth treatment recorded the highest number of red blood cells, with a significant difference (p≤0.05) from the first and second treatment, where it recorded (3.57 x 10⁶ mm⁻³ blood), followed by the fourth treatment, which was recorded (3.28 x 10⁶ mm⁻³ blood). While the first and second treatment recorded the following values (2.52 and 2.29 x 10⁶ mm⁻³ blood), respectively. We also notice through the results that the fifth treatment recorded the highest percentage of Haematocrit with a significant difference (p≤0.05) from the first, second and third treatment and reached (43.00%) followed by the fourth treatment and recorded the percentage of Haematocrit and reached (41.33%). The same table shows that the concentration of hemoglobin significantly increased the fifth treatment (p≤0.05) over the second and third treatment, which recorded the highest concentration of hemoglobin and reached (14.33 g / 100 ml blood), while the second treatment (positive control) recorded the lowest concentration of hemoglobin and reached (11.00 g / 100 Ml of blood).
### Table 2. The effect of adding different levels of vitamin B7 to the diet in some of the cellular characteristics of the blood of Japanese quail and exposed to oxidative stress (mean ± standard error) at the age of 28 days

| Treatments               | White blood cell count \((\times 10^3 \text{mm}^{-3} \text{blood})\) | Red blood cell count \((\times 10^6 \text{mm}^{-3} \text{blood})\) | Haematocrit (%) | Hemoglobin concentration \((\text{g/100 blood})\) |
|--------------------------|-----------------------------------------------------------------|-------------------------------------------------|-----------------|------------------------------------------------|
| First treatment          | 1.20±20.33                                                      | 1.51±2.52                                       | 0.88±39.66      | 0.33±13.33                                    |
| Second treatment         | 1.45±26.33                                                     | 2.28±2.29                                       | 0.57±33.00      | 0.00±11.00                                    |
| Third treatment          | 6.66±21.33                                                     | 1.73±3.03                                       | 1.45±39.66      | 0.57±13.00                                    |
| Fourth treatment         | 5.77±22.00                                                     | 3.74±3.28                                       | 0.88±41.33      | 0.33±13.66                                    |
| Fifth treatment          | 3.33±22.33                                                     | 3.44±3.57                                       | 0.57±43.00      | 0.33±14.33                                    |
| Significant level        | *                                                                | *                                               | *               | *                                              |

- Averages with different letters within one column indicate significant differences \((p \leq 0.05)\).
- The first treatment = negative control treatment (basic diet + H2O2 free drinking water).
- The second treatment = positive control treatment (basic feed + drinking water containing 0.5% H2O2).
- The third treatment = (added 500 micrograms B7 / kg of basic diet + drinking water containing 0.5% H2O2).
- The Fourth treatment = (added 600 micrograms B7 / kg of basic diet + drinking water containing 0.5% H2O2).
- The fifth treatment = (added 700 micrograms B7 / kg of basic diet + drinking water containing 5.0% H2O2).

Table 3. shows the effect of adding different levels of vitamin B7 to the diet in some of the cellular blood characteristics of the Japanese quail and exposed to oxidative stress at the age of 42 days. The results indicate that there are significant differences between all the characteristics, and it is observed with respect to the number of red blood cells \((10^6 \text{mm}^{-3} \text{blood})\) The fifth treatment recorded the highest number of red blood cells and reached \((5.33 \times 10^6 \text{mm}^{-3} \text{blood})\) with a significant difference \((p \leq 0.05)\) from the first, second and third treatment, which recorded the following values \((3.15, 3.16 \text{ and } 3.91 \times 10^6 \text{mm}^{-3} \text{blood})\), respectively Also, the fourth treatment was significantly superior \((p \leq 0.05)\) to the first and second treatment and recorded \((4.61 \times 10^6 \text{mm}^{-3} \text{blood})\). As for the Haematocrit characteristic\%, the third, fourth and fifth treatment significantly increased \((p \leq 0.05)\) over the first and second treatment, which recorded the lowest percentage of hematocrit and reached \((36.33 \text{ and } 32.00\%)\) respectively, while in the hemoglobin concentration we notice the superiority of the fourth and fifth treatment significantly \((p \leq 0.05)\) over the first and second treatment, which recorded the lowest percentage of hematocrit and reached \((36.33 \text{ and } 32.00\%)\) respectively, while in the hemoglobin concentration we notice the superiority of the fourth and fifth treatment significantly \((p \leq 0.05)\) over the first and second treatment, as well as the third treatment significantly increased \((p \leq 0.05)\) over the second treatment (positive control). From the same table, we note that the second treatment recorded the highest number of white blood cells with a significant difference \((p \leq 0.05)\) from the third, fourth and fifth treatment, which recorded the lowest number of white blood cells \((27.66, 22.66 \text{ and } 23.33 \times 10^3 \text{mm}^{-3} \text{blood})\), respectively. As for the heterophile cells \%, we note that the second treatment recorded the highest percentage of heterophile cells \((33.33\%)\) compared to the rest of the experiment parameters. While in of lymphocytes, we note the addition of the Treatments of (third, fourth and fifth), the best ratio of lymphocytes \((64.00, 68.33 \text{ and } 76.00\%)\), respectively, and a significant difference \((p \leq 0.05)\) from the first treatment and the second treatment, which recorded the lowest percentage of lymphocytes and reached \((54.66 \text{ and } 54.00\%)\), respectively. As for the H/L ratio, the results indicate, through statistical analysis, the third, fourth and fifth treatment recorded, the best ratio of H/L compared to the first and second factors, where the second treatment (positive control) recorded the highest percentage of H/L \((0.61\%)\).

### Table 3. The effect of adding different levels of vitamin B7 to the diet in some of the cellular characteristics of the blood of Japanese quail and exposed to oxidative stress (mean ± standard error) at the age of 42 days

| Treatments       | Red blood cell count \((\times 10^6 \text{mm}^{-3} \text{blood})\) | Haematocrit (%) | Hemoglobin concentration \((\text{g/100 blood})\) | White blood cell count \((\times 10^3 \text{mm}^{-3} \text{blood})\) | Percentage of the heterophile cells (%) | Percentage of lymphocyte cells (%) | H/L ratio |
|------------------|-------------------------------------------------|-----------------|------------------------------------------------|------------------------------------------------|----------------------------------------|----------------------------------|------------|
| Significant level| *                                               | *               | *                                             | *                                               |                                        |                                   |           |
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4. Discussion

The significant increase in the number of red blood cells Table (2) is due to the role of added vitamin B7 at a concentration of 600 and 700 micrograms / kg fodder [16], that the vitamin B7 has a role in increasing the production of the hormone thyroxin T4 and activating its role in the body, as thyroxine works To raise the rate of gene expression to manufacture Erythropoietin [17], the Erythropoietin is a growth factor that basically stimulates red blood cell production [18], and thyroxine is also involved in the formation of red blood cells. Accordingly, the increase in the red blood cell counts will be reflected in the increase in the percentage of Packed Cell Volume (PCV) and as shown in Table (2,3), where the factors that affect red blood cells affect the percentage of Haematocrit and hemoglobin [19]. From Table (3), a significant decrease in the number of white blood cells is observed in the fourth and fifth treatments, and that this decrease may be due to the decrease in the proportion of heterophile cells, and this is what the current study found, as for the decrease in the proportion of Heterophile cells and the high percentage of lymphocytes in B7 coefficients, as this may be a positive indication of a low inflammatory response, as B7 is an important factor in stimulating and stimulating the immune system and increasing its immune response [20]. The decrease in the H/L cells in the Vitamin B7 treatments gives a good impression of bird health. [21], mentioned that the H/L ratio is the best measure for detecting the general condition of birds and the level of stress they are exposed to.

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