



## Effect of dietary nano- selenium, astaxanthin and their combination on antioxidant status and immune function in Ross-308 broiler chicks

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### Abstract

The aim of this study was to evaluate the effect of adding nano-selenium and astaxanthin and their mixture to broiler rations on oxidation and immunity indicators. Unsexed Ross-308 broiler chicks were used. The results of the study showed that there was a significant ( $P \leq 0.05$ ) decrease in the concentration of ALT enzyme in the treatments of T2 and T5 compared to the control treatment T1. Also, there was a significant decrease ( $P \leq 0.05$ ) in the concentration of AST enzyme in the blood serum of the experimental treatments compared to the control treatment T1, and the two treatments T2 and T7 outperformed significantly ( $P \leq 0.05$ ) in the concentration of glutathione peroxidase enzyme compared to the control treatment T1, and the increase in the concentration of catalase enzyme in the T6 treatment compared to the control treatment T1, while the concentration of malondehyde decreased in the experimental treatments compared to the control. There was a significant increase ( $P \leq 0.05$ ) for treatment T4 and T7 in the weight bursa of Fabricia compared with the control treatment T1, as well as a significant increase ( $P \leq 0.05$ ) in the value of the relative weight and index of bursa of Fabricia for treatments T7, T4, T2 when compared with the control treatment. T1. There was a significant increase ( $p \leq 0.05$ ) for treatment T3 and T6 in the volumetric criterion of antibodies to Kamboru disease when compared with the control treatment T1, while the volumetric ratio of antibodies to Newcastle disease decreased in all experiment treatments when compared with the control treatment T1.

**Keywords:** Nano selenium , Astaxanthin , Oxidation , Immunity

### Introduction

Natural antioxidants play an important role in maintaining poultry health, productivity and reproductive performance, and there is a large group of antioxidants in the form of particles that are either present in body tissues such as ascorbic acid, coenzyme COQ, carnitine and antioxidant enzymes or They are provided to birds in the

ration such as vitamin E, carotenoids, and selenium. All the antioxidants in the body work together to create an antioxidant network called “antioxidant systems [1].

Selenium is an essential element that has a significant impact on a number of biological functions in poultry and its most important action is an antioxidant effect because it forms selenocysteine and is part of the active center of glutathione peroxidase [2]. This enzyme has an antioxidant effect and contributes to defense oxidative stress by reducing hydrogen and lipid peroxide stimulation to less damage [3], and the level of activity of this enzyme is in the liver and plasma. With the recent development of nanotechnology, nano-selenium (Nano-Se) has attracted attention because nanometric particles show many properties, including large surface area, high catalytic efficiency, strong absorption and low toxicity [4, 5]. The addition of nano-selenium in broiler diets led to an improvement in physiological and productive characteristics [6].

As for astaxanthin, it is a natural red carotenoid pigment classified among the xanthophylls found in algae, shrimp and salmon. The main feature of astaxanthin is its high ability to scavenge free radicals and the active types of oxygen present in biological systems [7] and it has been described as the king of all antioxidants, With its distinctive and unique chemical properties because it contains effective groups of both oxygen and hydroxyl at the end of the molecule chain at each aromatic ring, and it is more polar than the rest of the carotenoids [8]. The use of astaxanthin in poultry feed led to an improvement in production performance and achieved high results in rates of weight gain [9, 10], as well as the use of food additives from mineral elements in the form of nanoparticles that can be better absorbed by the animal which improves the quality of the obtained products [11]. Therefore, the current study aims to find out the effect of adding to the feed with two different levels of nano-selenium and astaxanthin and their mixture to the broiler diet on oxidation and immunity indicators.

## **Materials and Methods**

This experiment was conducted in the fields of Al-Anwar Company in Babylon Governorate for a period of 35 days, from 11/8/2020 to 12/12/2020, where 315 unsexed one-day-old chicks were used, which were randomly divided into 7 treatments with 3 replications for each treatment. Each replicate contained 15 chicks, and the replicates were distributed within pens of dimensions 1.5 x 1 m. Nano selenium and astaxanthin dye were added to the diet since the first day, according to the following treatments:

T1 control treatment / standard diet without addition

T2 basic diet supplemented with nano selenium at a concentration of 0.3 mg / kg feed

T3 basic diet supplemented with nano selenium at a concentration of 0.5 mg / kg feed

T4 is a basic diet to which the astaxanthin dye has been added at a concentration of 60 mg / kg of feed



T5 is a basic diet supplemented with astaxanthin dye at a concentration of 70 mg / kg of feed

T6 is a basic diet supplemented with a mixture of nano-selenium at a concentration of 0.3 mg / kg of feed + and astaxanthin at a concentration of 60 mg / kg of feed

T7 is a basic diet to which a mixture of nano-selenium at a concentration of 0.5 mg / kg of feed is added + and astaxanthin at a concentration of 70 mg / kg of feed

The chicks were fed on a starter diet (protein content 23.04% and energy quantity 3021.45 kilo calories/kg of feed) from the age of one day until the third week of the birds' life, after that it was replaced with a growth diet (protein ratio 20.06 and energy quantity 3194.92 kilocalories/ kg of feed ) until the end of the fifth week , and the feed with its additives of nano selenium and astaxanthin mixed in the concentrations shown above and water were provided freely . The feed used is as shown in Table 1.

**Table (1): The percentages of the components of the diet used in**

**Protein concentrate type Brocon-5 Special W: Chinese origin: Each kg of it contains (40% crude pro-**

Feed material	Feed startup	Feed growth
Yellow corn	30	40
wheat	28.25	24
(48% protein) soybean	31.75	24.8
Protein concentrate	5	5
Sunflower oil	2.9	4.4
limestone	0.9	0.6
Dicalcium phosphate (DCP)	0.7	0.9
A mixture of vitamins and minerals	0.2	0.2
Nacl	0.3	0.1
Total	100	100
Crude Protein ( % )	23.04	20.06
Calculated representative energy (kcal / kg feed)	3021.45	3194.92
Lysine %	1.27	1.07
Methionine%	0.41	0.38
cysteine %	0.35	0.30
Methionine+cysteine %	0.82	0.78
Phosphorous %	0.41	0.43
c/p Energy Ratio: Protein%	131.14	159.77

tein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorous, 3.25% leucine, 3.90% methionine + cysteine, 2.2% sodium, 2100 kcal / kg representative energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6, 300 mg B12, 10 mg folic acid, 100 micrograms butene, 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). Chemical analysis of the suspension was calculated according to [ 12]

## Preventive program

Use the preventive health program mentioned in Table (2) as follows:

**Table (2): Preventive Program**

Age / day	Vaccine or vitamins used	How to give the vaccine
1	Newcastle Vaccine + Kimboro Loaded on Merck	Subcutaneous injection into the neck
1	vaccine4.91 + 5 Ma for the prevention of IB . disease	instillation in the eye
	vitaminsB-Complex + C + AD3E + Anti-biotics	drinking water
14	Newcastle VaccineClone 30	drinking water 14

### **Management of experimental chicks**

Experimental chicks were prepared from the hatcheries of Al-Anwar Company in the province of Babil. The chicks were reared by ground breeding distributed in pens, the dimensions of which are 1.5 x 1 m. The floor was spread with sabus, with a thickness of 5-4 cm. The starter feed was provided in circular plastic dishes with a diameter of 38 cm, and at the age of seven days was carried out. They were replaced with cylindrical hanging feeders with a diameter of 45 cm. These feeders were raised weekly to the top to be at the level of the bird's back to facilitate feeding and reduce its scattering throughout the duration of the experiment. As for the water, it was served using inverted 1-liter manifolds in the first week and then replaced with a 5-liter manifold at the end of the experiment period.

### **The materials used in the experiment**

Nano-organic selenium was obtained from Nanosany corporation in Iran with a size of 30 nm and a purity of 99%. An industrial astaxanthin dye of American origin was used, with a purity of 100%, and the dye was in the form of a red colored powder.

### **Characteristics studied**

#### **Oxidation Index Measurements**

These measurements include each of the enzyme Alanin aminotransferase (ALT) and enzyme Aspartate aminotransferase (AST) and Glotathon Peroxidase enzyme (GSH-PX and enzyme Catalase (CAT) and Monaldehyde (MDA)

#### **Determination of Alanine Amino Transferase (ALT) Enzyme Activity**

This measurement was carried out using a kit supplied by the French company Or- phee and according to the method of [13] . and the examination was carried out based on “measuring the activity of the enzyme by colorimetric methods by measuring the pyruvic acid from alanine, as the pyruvic acid is reacted with the DNPH compound to form a complex of Red color measured at a wavelength of 546 nm and was estimated by the international unit / liter.

#### **Determination of Aspartate Amino Transferase (AST) enzyme activity**



For this measurement, a kit prepared by the French company Orphee was used, according to the method of [13] . This method is based on the ability of the enzyme to convert aspartic acid to oxaloacetic acid, which spontaneously converts to pyruvic acid, which in turn reacts with 2,4-dinitrophenyl hydrazine (DNPH) to form a red colored complex measured at a wavelength of 546 nm

#### **Determination of the activity of the enzyme Glutathion Peroxidase (GSH-PX)**

Glutathione was measured by following the method of [14] which is based on the use of a precipitation solution containing metaphosphoric acid (Na<sub>2</sub>EDTA) and the addition of sodium chloride (NaCl) and the solution was placed in a centrifuge at 4500 rpm for 10 minutes. The value of glutathione was estimated as the difference in the absorbance values of the samples in the presence or absence of DTNB and at a wavelength of 340 nm.

#### **Determination of Catalase (CAT) Enzyme Activity**

A kit supplied by the French company Orphee and based on was used [15] , and the method that supports spectrophotometry to estimate the activity of catalase, and this method depends on measuring the amount of hydrogen peroxide destroyed by the catalase enzyme and using Redox dye and a change in the intensity of the color at a wavelength 570 nm or fluorescence at a wavelength of 530/545 nm, which indicates the activity of the enzyme catalase in the sample.

#### **Determination of the level of Malondialdehyde (MDA)**

Its concentration was measured using a measuring kit from the French company Orphee based on [16] . where this method determines the amount of lipid peroxides by measuring aldehyde, which is one of the decomposition products of lipid peroxide and is done by the interaction of one molecule of Malondialdehyde and two molecules of Thiobarbituric acid to form a red colored MDA-TBA compound can be measured at a wavelength of 535 nm.

#### **Lymphoid organs weight measurements**

##### **Relative weight bursa of Fabricia**

This bursa was weighed using a sensitive scale and the relative weight of these internal parts to the body weight was calculated according to the equation mentioned by [17] .

Weight of the lymph organ (gm)

The relative weight of the lymphatic organ % =  $\frac{\text{Weight of the lymph organ (gm)}}{\text{live body weight (gm)}} \times 100$

##### **Fabricia Guide**

It was calculated based on the method [18] , and as in the following equation





Relative weight of the bursa  
in the experimental treatment

Fabricia Index % = \_\_\_\_\_ x 100

Relative weight of the bursa in  
the control treatment

### Examination of the volumetric criterion for antibodies to Kamboru and New-castle disease

It is the method of ELISA examination to find out the immunity of the bird to IBD Alcamboro disease, ND Newcastle. These tests are carried out in several steps, which are

#### Material preparation

- 1 - Preparing the washing. Add 20 ml of Washing solution to every 380 ml of distilled water (D.W)
- 2 - Prepare the STOP. Add 2.5 ml of Stop Solution for every 10 ml of distilled water (D.W) .

#### Mitigation

Dilute the sample. Dilution is done by adding 5 ml of serum to 250 ml of buffer solution in the special dilution plat and left for 5 minutes. Ease (- and +) Control. It is diluted by adding 5 mL of Control to 250 mL of buffer solution and placed in a special Eppendorf for each of the control (+ and - )

#### Statistical analysis

The data were analyzed using the complete random design (CRD) to study the effect of the studied transactions on the different traits, and the significant differences between the means were compared using the Duncan (1955 ) [19 ] . polynomial test, the SAS program (2012) was used in the statistical analysis

### Results and Discussion

#### Oxidation indicators

Table (3) shows the effect of adding two levels of nano-selenium and astaxanthin and their mixture to broiler ration on the concentration of alanin aminotransferase (ALT), aspartate aminotransferase (AST), Glotathon peroxidase (GPX), catalase (CAT) and malondehyde (MDA) in the blood plasma. The results showed that there was no significant difference in the concentration of ALT enzyme between treatments T7, T6, T4, T3, and T1, but it decreased significantly ( $P \leq 0.05$ ) in the serum of treated birds T2 compared to the control treatment.

In the same table, we notice a significant ( $P \leq 0.05$ ) decrease in the concentration of AST enzyme for the experimental treatments when compared with the T1 control treatment. Also, the two treatments T6 and T3 were significantly similar in the enzyme concentration, but they outperformed the treatments T7, T5, T4, and T2, while



the treatment T4 had a significant decrease ( $P \leq 0.05$ ) in the concentration of AST enzyme in comparison with treatments T2, T7, and T5, which did not show a significant difference between them in the same character.

As for Glutathione peroxidase (GPX), the birds of the two treatments T7 and T2 obtained the best concentration at a significant level ( $P \leq 0.05$ ) on treatment T4, and no significant difference was recorded in the table of statistical analysis between treatments T7, T6, T5, T3, T2 as well as treatments T3, T6, T5, T4 in the concentration of glutathione peroxidase in the blood serum, while the experimental treatments recorded a "significant" difference higher than the control treatment in the concentration of this enzyme. Treatment T6 recorded a significant increase ( $P \leq 0.05$ ) in the concentration of catalase enzyme on all treatments, followed by treatment T5, T2 where it did not show any significant difference with treatments T7, T4, but it outperformed treatment T3. On the other hand, we note that there is no significant difference between the treatment T7, T4 and the control treatment in the concentration of catalase enzyme, while the concentration of the enzyme decreased in the T3 treatment compared to the control treatment.

As for malondialdehyde (MDA), the table showed that there was no significant difference ( $P \leq 0.05$ ) between treatment T5 and T7 compared to the control treatment. T2 compared to the control treatment, and there was no significant difference between treatment T3 and T4, which outperformed the two treatments T2 and T6 in the same concentration.

Through the table of statistical analysis, we notice a significant decrease in AST enzyme for treatment T7, T2 and it may be due to the role of nano-selenium antioxidant, or the cause may be the relationship between thyroid hormones and AST enzyme activity, and this explains the decrease in AST enzyme activity accompanied by an increase in Thyroid activity and then an increase in protein synthesis under the influence of nanoparticles of selenium [20]. When reviewing the results of the effect of adding astaxanthin in the AST enzyme, it can be said that the reason for the decrease in the level of this enzyme in the treatment T4 and T5 (60 and 70 mg / kg), respectively, may be due to the role of astaxanthin in enhancing antioxidant activity, reducing oxidative stress and maintaining the manufacturing function of the liver.

As for the high concentration of glutathione peroxidase enzyme in T7 and T2 treatment, the reason may be due to the role of selenium in regulating the antioxidant defense mechanism by controlling the glutathione peroxidase pathway in the bird's body [21, 22], and since GSH-PX It is one of the most important intracellular enzymatic antioxidants, and is the largest component of the internal cellular oxygen storage [23]. The elevated glutathione enzyme may be due to the role of selenium in regulating the expression of genes for selenoproteins, including the regulation of the GSH-PX gene [24, 25]. It is clear that the high concentration of glutathione peroxidase enzyme in the T2 treatment compared to the control treatment may be due to



the role of nano-selenium as an antioxidant, which may cause an increase in the activity of this enzyme in serum, tissues, liver and muscles of birds, i.e. when using nano-selenium at a concentration of 0.3 mg / kg [26] . And the results of our current experiment also indicate that adding astaxanthin to broiler diets in the T5 treatment at a concentration of 70 mg / kg had a positive role in improving the balance of antioxidant status by being able to enhance the role of antioxidants in the cell. The activity of this GSH-PX enzyme in the serum of treated birds to which astaxanthin was added to their diets and the reduction of lipid peroxides, and then a decrease in MDA resulting from fat oxidation, so malondehyde can be considered an indicator of cell fat oxidation[ 27 ] .

Carotenoids, including astaxanthin, have a high ability to protect fatty acids from oxidation and prevent oxidative stress from living cells. This may explain the reason for the low level of MDA in astaxanthin addition treatments and evidence of its ability to resist oxidative states [ 28 , 29 ] The bird's body systems can adapt to oxidative stress by increasing their production of antioxidants, repairing enzymes and restoring the natural balance. The positive correlation of lipid oxidation and cholesterol concentration can be observed with glutathione peroxidase activity. This enzyme can be used as an indicator to increase the intracellular oxidation process [30] .

The high concentration of catalase enzyme in T6 treatment may be due to the antioxidant effect of the enzyme, as it is within the first line of defense of the defensive antioxidant network. The Other Free [ 31 ] .

**Table (3): The effect of adding two levels of nano selenium and astaxanthin and their mixture to broiler ration Rose 308 on the concentration of alanin aminotransferase (ALT), aspartate aminotransferase (AST), Glotathon peroxidase (GPX), catalase (CAT) and malondehyde (MDA) in blood plasma.**

transaction	Standard Error ±Averages				
	U/L ALT	U/L AST	U/L GSH-PX	CAT U/L Catalase	MDA malondehyde U/L
<b>T1</b>	± 440.33 0.17 <b>a</b>	0.66 ±400.67 <b>a</b>	1.75 ± 43.34 <b>c</b>	0.60 ± 30.06 <b>c</b>	± 17.30 0.62 <b>a</b>
<b>T2</b>	0.93 ± 313.47 <b>c</b>	2.30 ± 261.30 <b>c</b>	2.08 ± 54.43 <b>a</b>	2.05± 39.90 <b>b</b>	0.08 ± 9.96 <b>c</b>
<b>T3</b>	0.79 ± 433.27 <b>a</b>	3.97 ± 323.47 <b>b</b>	1.22± 49.56 <b>ab</b>	1.49 ± 22.50 <b>d</b>	0.91 ±13.63 <b>b</b>
<b>T4</b>	6.42 ± 407.00 <b>ab</b>	3.90 ±176.77 <b>d</b>	1.15 ± 47.00 <b>bc</b>	1.32 ± 33.43 <b>bc</b>	1.53 ±13.43 <b>b</b>





<b>T5</b>	0.25 ± 359.00 <b>bc</b>	2.96 ± 255.33 <b>c</b>	1.73 ± 51.10 <b>ab</b>	1.82 ± 40.73 <b>b</b>	0.33 ± 16.66 <b>a</b>
<b>T6</b>	7.81 ± 418.00 <b>a</b>	0.62 ± 327.37 <b>b</b>	2.25 ± 50.76 <b>ab</b>	3.62 ± 49.82 <b>a</b>	0.66 ± 10.66 <b>c</b>
<b>T7</b>	7.00 ± 463.00 <b>a</b>	3.69 ± 260.33 <b>c</b>	1.45 ± 54.33 <b>a</b>	4.15 ± 33.42 <b>bc</b>	1.28 ± 15.53 <b>ab</b>
<b>morale level</b>	*	*	*	*	*

\*Different small letters refers to significant differences at  $P \leq 0.05$  in same column.

### Measurement of the relative weight of the bursa of Fabricia and Fabricia Guide

The results of Table (4) indicate the effect of adding two levels of nano-selenium and astaxanthin to the broiler diet on the weight bursa of Fabricia and the relative weight of the bursa and its evidence, where a significant increase ( $p \leq 0.05$ ) was observed for treatment T4 in the weight bursa of Fabricia compared with the rest of the treatments. It shows any significant difference between it and treatment T7 and also "the superiority of treatment T2 over treatments T6, T5, T3, T1, but on the other hand it was similar with treatment T7 in the weight bursa of the Fabricia, while treatment T5 recorded a decrease" at a significant level ( $P \leq 0.05$ ). ) When compared with treatments T6 and T3, there were no significant differences between the last treatments and the control treatment.

In the same table, it was found that the best relative weight bursa of Fabricia and its guide was in favor of treatments T4, T2, T7 and at a significant level ( $P \leq 0.05$ ) on treatments T6, T5, T3, T1 and no significant difference appeared in statistical significance between treatments T7, T4, and T2 as well. We note the superiority of treatment T6 over treatments T5, but it is similar to treatment T3 in the same trait. On the other hand, we note that treatment T3 did not have any significant difference compared to the control treatment, but the relative weight and index of Fabricia pouch decreased in birds of treatment T5 compared to "the same treatment." From the table, there is an improvement and an increase in the relative weight and evidence of the Fabricia follicle in the treatment of T4 astaxanthin addition. The production of free radicals by phagocytes [32]. It is known that the weight of the Fabricia follicle is an indication of the extent of the development of humoral immunity, as well as representing the center of the development of B-lymphocyte immune cells, i.e. The development that takes place in the immune side will show its effect mainly in the increase in the relative weight of this pouch [33] We also note an increase in the relative weight and evidence of Fabricia pouch that occurred for birds with nano-selenium treatments, [34, 35] found that nano-selenium contributed to improving the immunity of birds under heat stress conditions, where the relative weight increase of immune organs is evidence of the effect of nano-selenium in them, but not in normal conditions. The significant increase that occurred in the weight of Fabricia pod in treatment



T2, which is the addition of nano-selenium at a concentration of 0.3 mg / kg, does not agree with what was stated by [36] . in terms of the lack of effect of selenium added to the diet on the weight bursa of Fabricia . As for the superiority of the treatment of the T7 mixture, it may be due to the role of the two substances in reducing the immunosuppressive peroxide and perpetuating the cell membrane receptors and protecting them from the damages of oxidative processes and free radicals

**Table (4): Effect of adding two levels of nanoscale selenium and astaxanthin and their mixture to Rose 308 broiler ration on bursa of Fabricia weight (gm) and the relative weight of the bursa and its evidence**

Transaction	Averages ± Standard Error		
	Fabricia Pod Weight (gm)	relative weight%	Fabricia guide%
T1	3.85 ± 0.05 c	0.16 ± 0.002 c	1.00 ± 0.01 C
T2	4.73 ± 0.13 b	0.21 ± 0.005 a	1.29 ± 0.03 a
T3	3.80 ± 0.14 c	0.18 ± 0.007 bc	1.07 ± 0.04 bc
T4	5.05 ± 0.06 a	0.22 ± 0.0008 a	1.33 ± 0.005 a
T5	3.21 ± 0.09 d	0.14 ± 0.003 d	0.84 ± 0.02 d
T6	4.06 ± 0.07 c	0.18 ± 0.003 b	1.11 ± 0.02 b
T7	4.93 ± 0.10 ab	0.21 ± 0.004 a	1.28 ± 0.02 a
morale level	*	*	*

Different small letters refers to significant differences at  $P \leq 0.05$  in same column

### Volumetric criterion for antibodies against Newcastle and Camboro disease

The results of Table (5) indicate the effect of adding two levels of nanoscale selenium and astaxanthin and their mixture to the broiler ration on the volumetric criterion of antibodies specific to Kamboru disease (IBD) and Newcastle disease (ND). For all the studied treatments compared to “the control treatment, which recorded the lowest values of the compro antigens standard, which amounted to (6144.4), and it was noted in the table also that “nano selenium has the effect of raising the camboro antigens, as the T3 treatment recorded an increase” at a significant level ( $P \leq 0.05$ ) over the rest of the treatments. Treatment T6 was similar with it in terms of high morale over the other treatments, followed by treatment T4 with a significant difference ( $P \leq 0.05$ ) on treatments T7, T5, T2, T1 and also treatment T5 outperformed treatments



T7, T2, T1 and the two treatments T7 and T2 were similar in terms of size Kamburu disease antibodies. The results also showed a decrease in the experimental treatments compared to the “control treatment” as well as the superiority of the T2 treatment with a significant level ( $P \leq 0.05$ ) in the volumetric criterion of antibodies against Newcastle disease on the treatments T7, T5, T4, T3, and no significant difference was recorded with the treatment T6, but from On the other hand, treatment T6 outperformed treatments T7, T5, and T4, and the table did not record any significant differences between treatment T3 and T6. In the same table, we note the superiority of treatments T5 and T4 over treatment T7, but no significant difference appeared between T4 and T5.

The increase in the volume of anti-Kamporo antibodies in T3 treatment may be due to the effect of nanoparticles of selenium and its function in perpetuating the proliferative capacity of T and B cells [ 37 ] . and it also works to modulate the expression of (interleukin) IL-2R, which is a group of cytokines that stimulate The immune system on the cell surface leads to a change in the ability of lymphocytes to respond to antigens [38 ] .

Immune cells are particularly sensitive to oxidative stress due to the high content of polyunsaturated fatty acids in cell membranes and overproduction of reactive oxygen species (ROS) can upset the oxidative balance resulting in the destruction of cell membranes, proteins and DNA and thus affecting antigen receptors. [39 ] , this explains the decrease in the significance of antigens in the control treatment, which could be due to lipid oxidation and damage to the outer membrane of cells, and then damage to antigen receptors present on the cell membrane [ 38 ] .

As for the high antigenic significance in astaxanthin treatment with nano-selenium mixture, it may be due to the role of astaxanthin in promoting the production of T-cell antibodies to different antigens, as well as stimulating the function of immune cells and releasing the modified cytokine by cells of immune organs [ 40 . 41] Or, it may be due to the antioxidant activity of nano-selenium and astaxanthin in scavenging free radicals resulting from oxidative reactions, causing an increase in the immune response of lymphocytes to antigens [42 ] .

**Table (5): The effect of adding nanoparticles of selenium and astaxanthin and their mixture to the ration of broiler rose 308 on the volumetric criterion of antibodies to Kamburu disease (IBD) and Newcastle disease (ND) .**

Transaction	Averages ± Standard Error	
	komboro antigen	Newcastle antigen



<b>T1</b>	6144.4 ± 6.08 <b>e</b>	10113.4 ± 0.9 <b>a</b>
<b>T2</b>	8499.8 ± 8.34 <b>d</b>	8669.8 ± 6.86 <b>b</b>
<b>T3</b>	12271.0 ± 0.43 <b>a</b>	7927.0 ± 1.29 <b>c</b>
<b>T4</b>	10981.4 ± 7.25 <b>b</b>	6524.4 ± 3.11 <b>d</b>
<b>T5</b>	9651.2 ± 8.92 <b>c</b>	7129.2 ± 9.13 <b>d</b>
<b>T6</b>	12000.0 ± 2.73 <b>a</b>	8137.4 ± 0.65 <b>bc</b>
<b>T7</b>	8639.4 ± 3.44 <b>d</b>	5440.0 ± 8.08 <b>e</b>
<b>morale level</b>	*	*

\* Different small letters refers to significant differences at  $P \leq 0.05$  in same column.

The addition of feed of nano selenium at a concentration of 0.3 mg / kg of feed in the T2 treatment led to an improvement in the immunological characteristics represented by a high relative weight bursa of Fabricia and its guide, as well as the regulation of the antioxidant defense mechanism by controlling the glutathione peroxidase enzyme pathway, the most important indicator of intracellular enzymatic oxidation. Also, the concentration of 0.5 mg / kg of feed in the T3 treatment led to an increase in the proliferative capacity of immune cells (T and B) and an increase in the stimulation of the immune system and the response to antigens. Furthermore, addition of astaxanthin dye at a concentration of 60 mg/kg T4 to broiler ration of Rose 308 led to the protection of biomolecules from oxidation and spoilage, which led to an improvement in the immune characteristics. As well as, treatment of a mixture of nano-selenium and astaxanthin at a concentration of (0.5 + 70) mg / kg of T7-treated fodder gave good results for their effectiveness as antioxidants that have an effect on oxidative and immune indicators.

## References

- 1) Surai, P. F. (2017). Antioxidant defences: Food for thoughts. EC Nutrition 10:65–66
- 2) De Almeida J.N. Dos Santos GR Beteto FM De Medeiros LG ; Oba A. ; Shimokomaki M., Soares AL 2012: Dietary supplementation of chelated selenium and broiler chicken meat quality. Semina-Ciencias Agrarias, (33) 3117-3122.



- 3) Arthur, JR (2000 ). The glutathione peroxidase. *Cell. Mol. Life Sci.*1835- 57:1825
- 4) Wang, HL, JS Zhang, and HQ Yu.( 2007). Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. *Free Radic. Biol. Med.* 42:1524–1533
- 5) Zhang, JS, XF Wang, and TW Xu. (2008 ) . Elemental selenium at nano size (nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: Comparison with Se-methylselenocysteine in m.
- 6) Al-Khafaji. Fadhil RA and Mohammed Khalil Ibrahim Saidi . (2019 )the effectiveness of nano-selenium and vitamin e added to the diet in improving the productive efficiency for broiler chickens exposed to thermal stress. *College of Agriculture, University of Al-Qasim Green, Iraq.* 19 Supplement 2, 2019 p. 1239-1246.
- 7) Birben E. , UM Sahiner , C. Sackesen , S. Erzurum and O. Kalayci. ( 2012). Oxidative stress and antioxidant defense. *National Library of medicine.* 5(1):9-19
- 8) Tsuchihashi, H. ; Kigoshi, M. ; and Niki, E. (1995 ).Action of B-carotene as anti oxidant against lipid per oxidation , *Arc. Of Bio phys. ,* 323 (1) : 137 - 147
- 9) Jeong JS and IH kim .( 2014). Effect of Astaxanthin produced by *phaffia rhodozyma* on growth performance, meat quality, and fecal noxious gas emission in broilers, *Poultry Science*, Volume 93, issue 12, December 2014, pages 3138-3144.
- 10) Ulaiwi. G . H. and Fadhel Rasoul Abbas Al-Khafaji (2020). The effectiveness of astaxanthin added to the diet for improving productive efficiency, traits for broiler chickens exposed to oxidative stress. *Animal production Department, Agriculture college, Al- Qasim Green University, Iraq.* t 2, 2020 p. 1661-1666.
- 11) Damian K. , Wanda Górnjak, Marita Świniarska, Mariusz Korczyński (2018). *Algae Biomass: Characteristics and Applications, Algae Biomass in Animal Production*, 123-130.
- 12) Nelson , D. L. and M. M. Cox. (2004) .*Lehninger principles of Biochemistry .* 4th ed. copyright by : WH Freeman and C0mpany
- 13) Ritman, S. and S. Frankel. (1957). Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *AM . J. Clin. Path*, 28 : 56-63
- 14) Sedlak J, and Lindsay R..( 1968). Estimation of total protein bound and non-protein sulphhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* 25:192–205.





- 15) Hadwan . M. , H.N. Abed. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity University of Babylon · Department of Chemistry Professor of Biochemistry
- 16) Buege, JA, and SDAust.( 1978 ). Microsomal lipid peroxidation. *Methods Enzymol* . 52:302 - 310 .
- 17) Al-Baghdadi, Rana Jaber Tarish. (2006) . Studying the effect of food addition of microbial phytase and jet leaf extract on the productive performance of broilers. Master's Thesis, College of Veterinary Medicine - University of Al-Qadisiyah
- 18) Lucio, B. and S. B. Hitchner .( 1979 ). Infection bursal disease emulsified vaccine ; Effect upon neutralizing – antibody levels in the dam and subsequent protection of the progeny. *Avian Dis.*, 23: 466-478
- 19) Duncan, D. B.( 1955). Multiple Rang and Multiple F-test .*Biometrics*. 11:4-42
- 20) Kaplan, MM, and PR Larson. (1985 ).the medical clinics of North America (thyroid disease ) Vol. 69 WB Saunders company. Philadelphia London Toronto Mexico city Riode Janeiro Sydney Tokyo .
- 21) Yoon, I., T. Werner, and J. Butler.( 2007). Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. *Poult. Sci.* 86:727–730
- 22) Jiang Z. Y . Lin YC Zhou GL LuoL . H. Jiang SQ Chen F.( 2009). Effects of dietary selenomethionine supplementation on growth performance, meat quality and antioxidant property in yellow broilers. *J. Agric. Food Chem.* 57: 9769-9772.
- 23) Storey KB Braz J.( 1996 ) .Oxidative stress : Animal adaptations in nature . *Braz. J. Med . Biol. Res.* 29: 1715 - 1733. 464 .
- 24) Wu, Q., K. Huang, and H. Xu.( 2003). Effects of long-term selenium deficiency on glutathione peroxidase and thioredoxin reductase activities and expressions in rat aorta. *J. Inorg. Biochem.* 94:301-306.
- 25) Zoidis, E., Pappas, AC, Georgiou, CA, Komaitis, E. and Feggeros, K. (2010 ).Selenium affects the expression of GPx4 and catalase in the liver of chicken . *Comparative Biochemistry and Physiology part B: Biochemistry and Molecular Biology* 155. 294-300.
- 26) Cai SJ, Wu CX, Gong LM, Song T., Wu H., Zhang LY.( 2012 ) . Effects of nano- selenium on performance, meat quality, immune function, oxidation resistance, and tis sue selenium content in broilers. *Poultry Science*, 91,2539-2532.
- 27) Miller, J.E. Brzezinska-Slebodzinska , and F. Madsen,( 1993). Oxidative stress antioxidants, and animal function. *Journal of dairy science* 76: 2812- 2823.



- 28) Shah, MM ; Y. Liang ; Cheng, J. J.; and Daroch, M., ( 2016 ) .Astaxanthin - Producing Green Microalga *Haematococcus pluvialis*: From Single Cell to High value commercial products. *Front Plant Sci.* 2016 Apr 28; 7 : 531
- 29) Jiang, S. ; and S. Tong .( 2019 ). Advances in Astaxanthin biosynthesis in *Haematococcus pluvialis*. *Sheng Wu Gong Cheng Xue Bao.* 2019 Jun 25; 35 (6): 988-997.
- 30) Maraschiello, C., Sarraga, C. and Regueiro, JAG,( 1999).Glutathione peroxidase activity, t-bars, and alpha-tocopherol in meat from chickens fed different diets. *J. Agr. Food Chem.* 47, 867-872 .
- 31) Nelson , D. L. and M.M. Cox.( 2004) . *Lehninger principles of Biochemistry* . 4<sup>th</sup> ed . copyright by : W. H. Freeman and Company
- 32) Chapple, I. (1997 ).Reactive oxygen species and antioxidants in inflammation diseases. *Journal of Clinical periodontology* (24). 287-296 .
- 33) S.A.S. 2012. *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS, Inst. Inc. Cary. NCUSA
- 34) Liao X. , Lu L. , Li S. , Liu S. , Zhang L., Wang G., Li A., Luo X.( 2012 ) . Effect of selenium source and level on growth performance , tissue selenium concentrations , antioxidation , and immune functions of heat-stressed broilers *Biological Trace Element Research* , 150, 158-165
- 35) Habibi M, Ghazi S, Moeini MM and Abdolmohammadi .( 2014). Effect of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions *International journal of Biometeorology*, 58:741-752.
- 36) Robert J, Edens FW, Ferket PR, (2003).The effects of selenium supplementation on performance and antioxidant enzyme activity in broiler chicken submitted to the graduate faculty of the North Carolina State University in partial fulfillment of requirements for the degree of Master of Science
- 37) Surai, P. F. (2000). Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. *World's Poultry Sci J.* 58:333-347
- 38) Larsen. G. , T Borén , P Falk, KA Roth , S Normark ,( 1993 ) .Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens
- 39) Yuan J. P ; J. Peng ; K. Yin, and J.-H. Wang, (2011).“Potential healthpromoting effects of astaxanthin: a high-value carotenoid mostly from microalgae,” *Molecular Nutrition & Food Research* vol. 55, no. 1, pp. 150–165, 2011
- 40) Chew, BP, Wong, MW, Park, JS and Wong, TS (1999). Dietary beta-carotene and astaxanthin but not canthaxanthin stimulate splenocyte function in mice. *Anticancer Research* 19:5223-5227



- 41)** Bennedsen, M., Wang X., Willén R., Wadström and Andersen LP (1999) .Treatment of *H. pylori* infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. *Immunology Letters* 70: 185-189
- 42)** Sumareva DDS, G. Ukrainsky DDS, L. Kiremidjian-Schumacher Ph.D., M. Roy Ph.D., HI Wishe Ph.D., AD Steinfeld MD, JS Cooper MD (1999). Effect of combined adoptive immunotherapy and radiotherapy on tumor growth . 10.1002/(SICI)1520-6823 7:1. Pages 22-29.