

## Effectiveness of nano-selenium and astaxanthin and their mixture added to the diet in the liver histological traits of broiler (Ross 308)

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### Abstract

This study was conducted at Al-Anwar Poultry Company , located Babil Governorate, for a period of 35 days 11/8/2020 until 12/12/2020, in order to demonstrate the effect of nano-selenium and astaxanthin and their mixture added to the diet on the histological characteristics of the liver of broiler . where 315 broiler chicks, one day old, unsexed, were used. The average weight of chicks was 40 g, divided randomly into 7 treatments, and each treatment consisted of 3 replicate, with 15 chicks for each replicate, the treatments were as follows: T1: control treatment without addition, T2, T3: addition ( 0.3, 0.5 ) mg nano-selenium/kg, T4, T5: add ( 60, 70 ) mg astaxanthin /kg, T6: addition (0.3 + 60) mg nano- selenium and astaxanthin/ kg. T7 : addition (0.5 + 70) mg nano- selenium and astaxanthin/ kg

The histological slide of the liver in the treatment of adding nano-selenium at a concentration of 0.3 mg / kg feed (T3) and astaxanthin treatment 60 mg / kg feed (T4) showed a normal appearance of the liver anormal appearance of the hepatic vein, hepatocytes and sinusoids when compared with the rest of the treatments.

. Treatments T1, T2, T5, T6, and T7 displayed congestion in the hepatic vein and the exudation of inflammatory cells (white blood cells) and fibrosis around the blood vessels in experience treatments .

### Introduction:

Natural antioxidants play an "important" role in maintaining the health, productivity, and reproductive performance of poultry birds. These antioxidants are either present in body tissues such as ascorbic acid, coenzyme COQ, carnitine, and antioxidant enzymes, or they are supplied to birds in the diet, such as vitamin E, carotenoids, and selenium. These antioxidants work in the body in a combined way, forming the "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, which is part of the active center of glutathione peroxidase) [2], this enzyme has an antioxidant effect and contributes to the oxidative defense of By reducing the catalysis of hydrogen and lipid peroxides to the least harmful [3], and the level of activity of this enzyme in the liver and plasma. With the recent development of nanotechnology, nanoselenium (Nano-Se) has attracted attention because nanoparticles exhibit many properties, including large surface area, high catalytic efficiency, strong adsorption, and low toxicity [4; 5] As for astaxanthin, it is a natural red carotenoid pigment that is classified among the xanthophylls found in algae, shrimp and salmon. The main feature of astaxanthin is its high ability to

capture free radicals and active oxygen species found in biological systems [6], and it has been described as the king of all antioxidants, with its properties distinctive and unique chemical properties because it contains active groups for 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 [7]. The liver, which is an accessory digestive gland, helps in the production of bile and the synthesis of many important proteins, enzymes and chemical compounds necessary for the digestion process [8], and also produces "many antioxidant enzymes that work to break down hydrogen peroxides such as catalase and glutathione peroxidase [9] It has an important and effective role in Destroying or removing toxins through a set of chemical processes that aim to bind these toxic substances and secrete them with bile to be disposed of with waste [10], And due to the lack of studies in Iraq that include the use of both nano-selenium and astaxanthin and their mixture in broiler diets, we decided to add two levels of nano-selenium and astaxanthin and their mixture in the diet of broiler chickens (Ross 308) and the extent of their effectiveness on the histological characteristics of the liver.

### Materials and methods:

This experiment was conducted in the poultry farm of Al-Anwar Company in Babylon Governorate for the period from 11/8/2020 to 12/12/2020, a total of 35 days. During this study 315 chicks of broiler (Ross-308) aged 1 day were used without gender separation, and were divided randomly into 7 treatment groups, with 3 replicates (15 chicks/ replicate) for each treatment that were raised in pens with the dimensions of 1 x 1.5 m.

Nano selenium and astaxanthin dye have been added to the diet since the first day according to the following division of treatments:

- T1 control treatment / standard diet without any additives
- T2 basil diet supplemented with nano selenium at a concentration of 0.3 mg / kg feed
- T3 basil diet supplemented with nano-selenium at a concentration of 0.5 mg / kg feed
- T4 basil diet supplemented with astaxanthin dye at a concentration of 60 mg / kg feed
- T5 basil diet supplemented with astaxanthin dye at a concentration of 70 mg / kg feed
- T6 basil diet to which a mixture of nano-selenium at a concentration of 0.3 mg / kg of feed was added + and astaxanthin at a concentration of 60 mg / kg of feed
- T7 basil 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 feed ration (protein content 23.04% and Metabolic energy 3021.45 kilo calories/kg of feed) from the age of one day until the third week of the birds' age, after that it was replaced with a growth feed ration (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 mentioned above and water were provided freely .

The feed used is as shown in ( Table 1).

**Table 1: Percentages of the components of the diet used in the study and their chemical composition**

Ingredients	Starter % day 1-22	Growth % day 22-35
Yellow corn	30	40
Wheat	28.25	24
Soybean meal (48% protein)	31.75	24.8
Protein concentrate	5	5
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
DCP . Dicalcium Phosphate	0.7	0.9
Mixture of vitamins and minerals	0.2	0.2
Salt	0.3	0.1
Total	100	100
Crude protein (%)	23.04	20.06
The calculated r Metabolic energy (kilo calories / kg of 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
Available phosphorous	0.41	0.43
c/p Energy Ratio : Protein %	131.14	159.77

\*\* Chemical analysis of the ration was calculated according to the [11]

As for the materials used in the experiment: The nano-organic selenium was obtained from the Nanosany corporation in Iran, with a size of 30 nm and a purity of 99%. Additionally, an industrial astaxanthin dye of American origin was used, with a purity of 100%, in the form of a red powder.

## Traits study

### 1. Preparation of tissue slide :

Slaughter of 2 birds from each Replicate (6 birds per treatment) at the age of 35 days. Tissue samples were taken in the shortest possible period of time to ensure that the bird's body tissues were not damaged. 2 cm of liver were cut, and all the sections were washed with tap water and placed in tubes containing formalin with a concentration of 10% g, the studies were performed on them according to what was mentioned by [12]

### 2. Examination of tissue slide

Histological slide were examined using a compound microscope, Olympus BH2 type of Japanese origin, and the tissue sections were photographed by a digital camera connected to a lenovo computer.

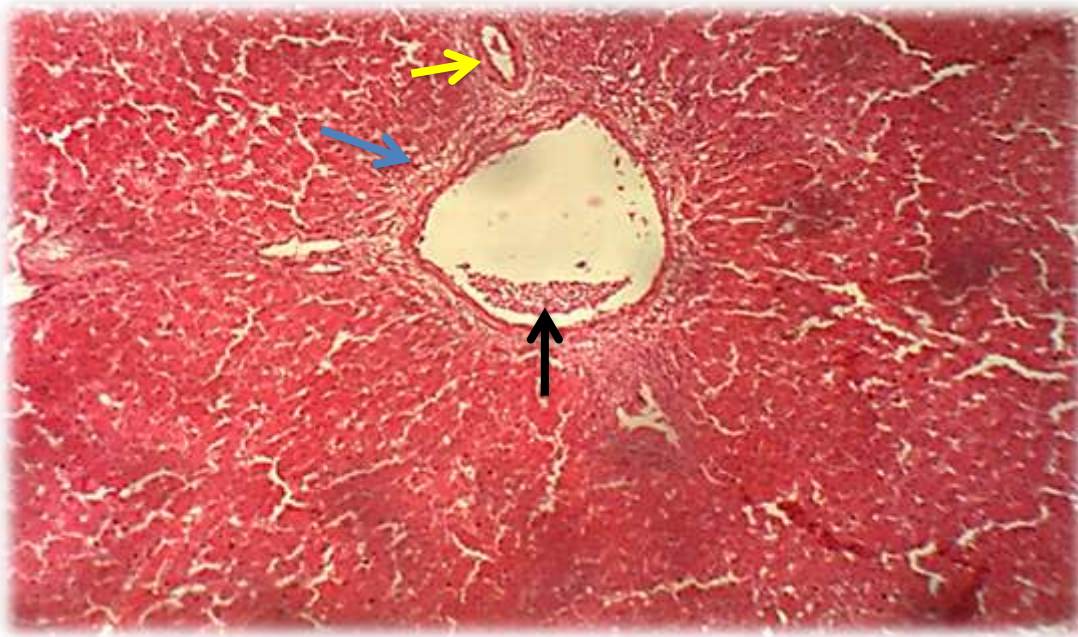
## Results and discussion:

### Histological characteristics of the liver at 35 days of age

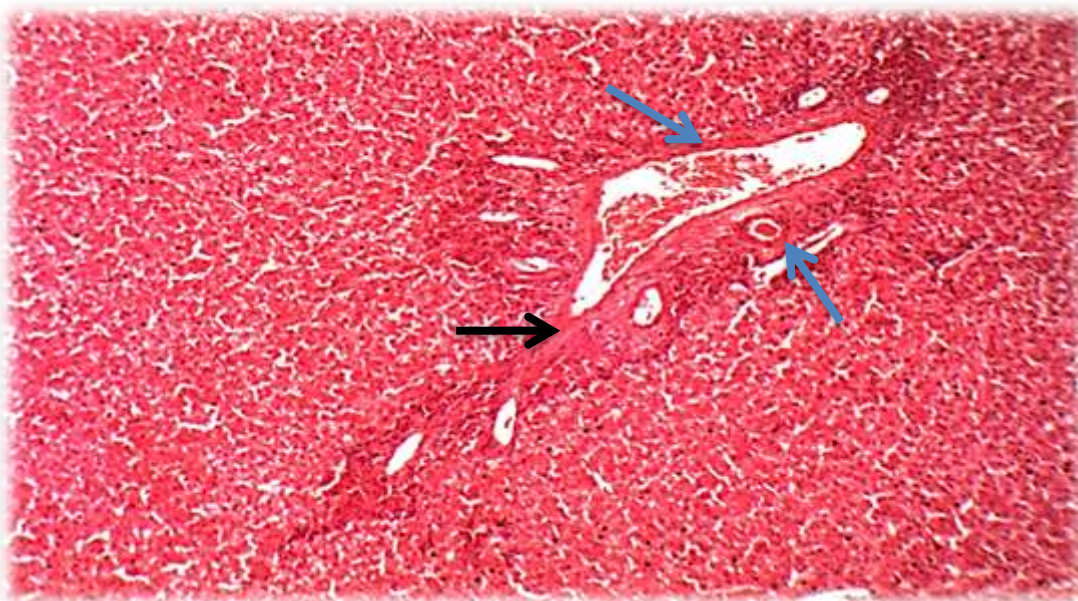
The histological sections of the liver of the treated birds age 35 days were studied (Pictures 1-11), and it was noticed in the T1 control treatment (Pictures 1 and 2) an accumulation of red blood cells and cirrhosis in the liver tissue, as well as the changes in the liver tissues of the picture (2) of the control treatment has shown congestion of the hepatic blood vessels with the exudation of white cells and the occurrence of cirrhosis in the liver around the area of the blood vessel. Treatment T2 (Picture 4, 3) has

displayed the collection of inflammatory cells to form a microbial purulent lesion, as well as the occurrence of inflammation in the bile duct and a narrowing in the diameter and thickness of the walls. Pictures (6, 5) for the two treatments T4 and T3, respectively, did not show the presence of any pathological lesion in the tissue, where the hepatocytes appear normal and the sinusoids have equal alignment and coordination. As for treatment T5 in figure (8,7) as well as treatment T7 in figure (11), white blood cells were exuded outside the hepatic artery with an increase in the thickness of the artery walls and the presence of a blood clot in the hepatic vein that filled the diameter of the vein and closed it completely. It was also found that there was congestion in the hepatic vein with an expansion in diameter, as well as cirrhosis of the area surrounding the hepatic blood vessels and the presence of hemorrhagic areas in the liver parenchyma in the form of (10,9) for T6 treatment.

The reason for the occurrence of cases of congestion of the hepatic vein, the exudation of blood cells outside the vessels, or the congestion and narrowing of blood vessels may be that the liver in poultry birds is considered a filter for toxins inside the body, whether toxins that come from feed or the products of vital activities inside the body such as cases of free radical formation, and the fact is that the formation of Reactive oxygen species generated from nanoselenium are the main mechanisms of selenium toxicity [13]. As toxic substances (toxins) are associated with ornithine in the tissues of the liver and form a complex non-absorbable compound to be excreted outside the body and rid of toxins. The liver contains major immune cells permanently that play an important role in removing dead red blood cells, fighting bacterial infections, or eliminating toxins under normal physiological conditions [14] . The effective role of nano-selenium and astaxanthin as antioxidants also works to activate these cells that secrete many chemicals and cytokines, but an excess of selenium may lead to stopping the cycle of these cells by increasing the levels of free radicals and thus causing damage to the liver [15].

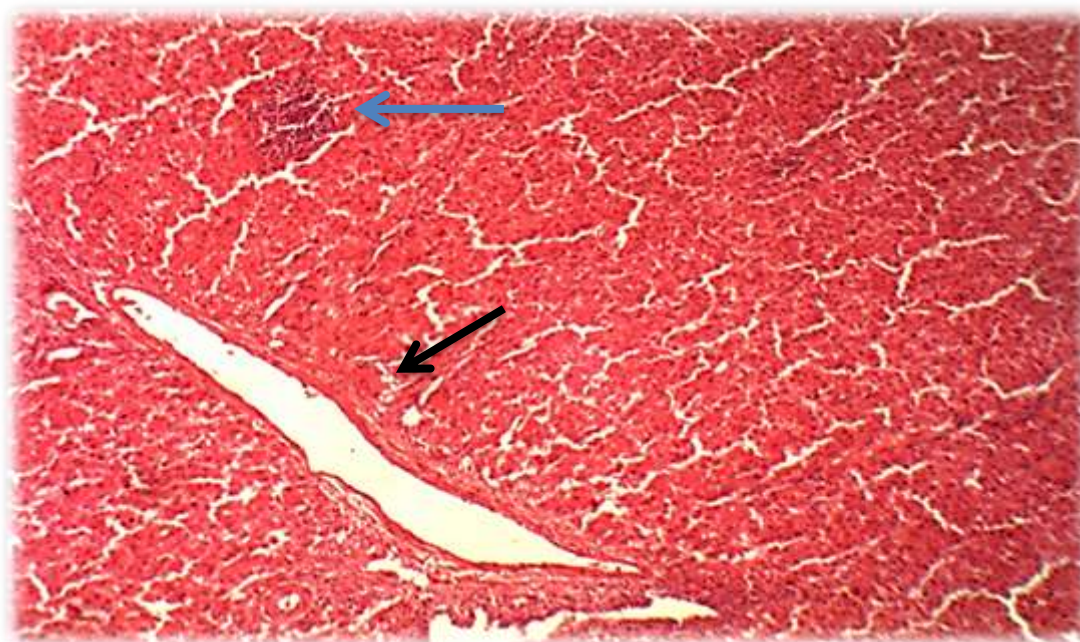


Picture (1) a section of the liver for the control of treatment without the any additions (T1) at the age of 35 days, showing the expansion of the hepatic vein with the accumulation of red blood cells inside the vein (indicator \_\_\_\_\_), as well as the observation of mesh tissue around the hepatic vein consisting of retinal fibers (indicator \_\_\_\_\_). In the artery, a decrease in the diameter of the artery can be observed (indicator \_\_\_\_\_) (Hematoxylin and Eosin stain X10).

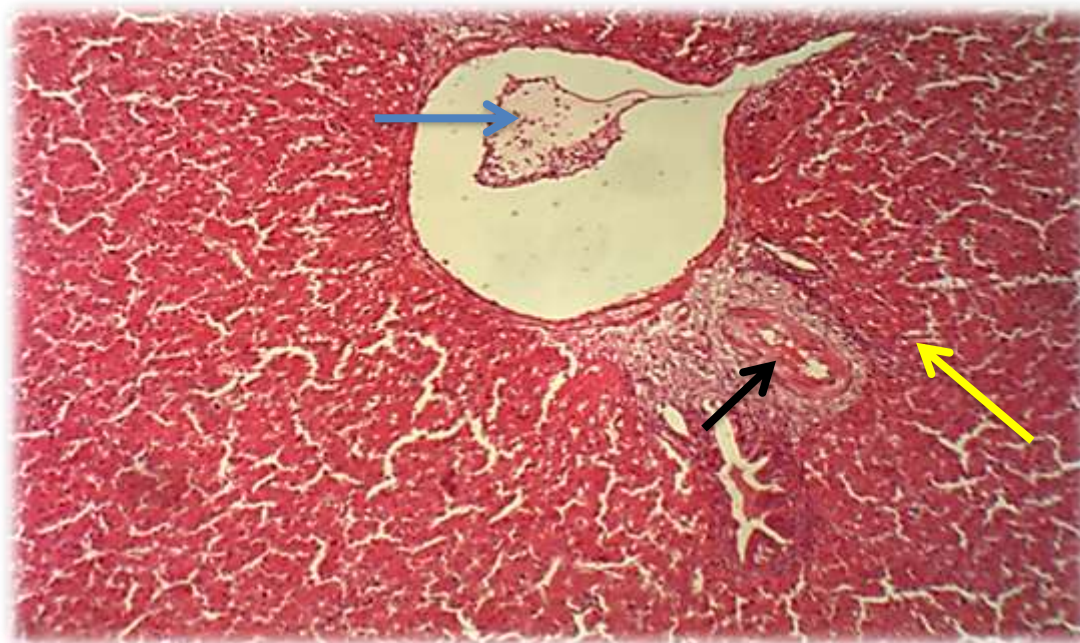


Picture (2) a section of the liver of the control treatment without additions (T1) at the age of 35 days, showing congestion in the hepatic blood vessels (indicator \_\_\_\_\_) and exudation of white blood cells and as well as occurrence of cirrhosis in the liver around the area of the blood vessel (indicator \_\_\_\_\_) Hematoxylin and Eosin stain X10.





Picture (3) a section of the liver for T2 treatment, adding nano-selenium at a concentration of 0.3 mg/kg of feed at the age of 35 days, showing the accumulation of inflammatory cells in the liver tissue to form a micro-purulent lesion (indicator \_\_\_\_\_). Also, an elongation of the hepatic vein is noted with an accumulation of fibrous tissue around the hepatic vein (indicator \_\_\_\_\_) hematoxylin and eosin stain X10



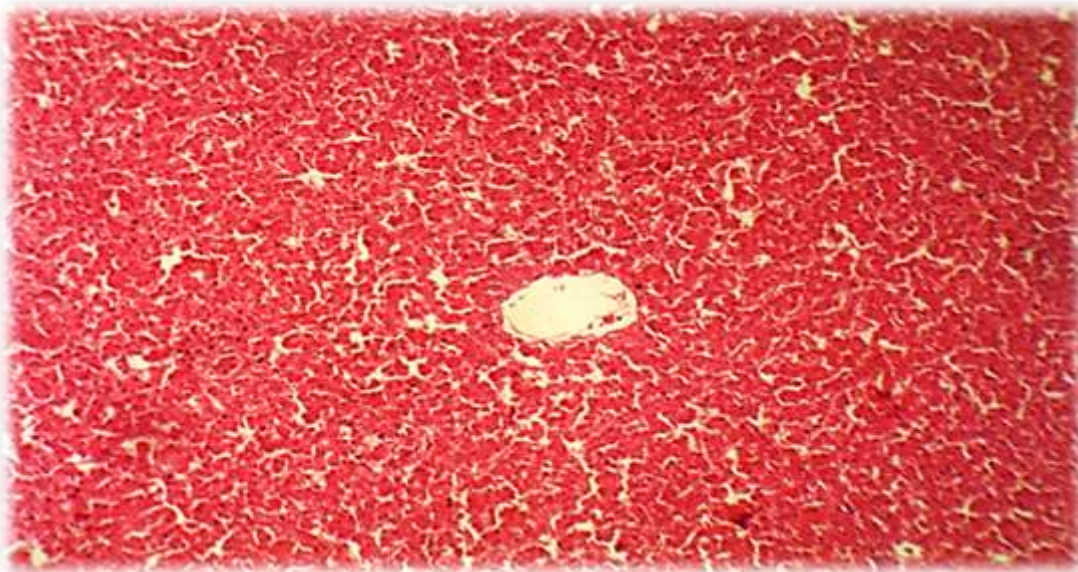
Picture (4) a section of the liver for T2 treatment, adding nano-selenium at a concentration of 0.3 mg / kg feed at the age of 35 days, showing an expansion in the diameter of the hepatic vein with the dissolution of the remnants of red blood cells collected inside the vein (indicator \_\_\_\_\_). A narrowing in the diameter of the hepatic artery is noted with increasing the thickness of the arterial wall (indicator \_\_\_\_\_). It is also noted the emergence of fibrous tissue around the hepatic artery (indicator \_\_\_\_\_). Additionally, the hepatic



bile duct is noted to have a narrowing in diameter of the bile duct thickens) as a result of bile duct and an increase in the thickness of its wall (the wall inflammation.



Picture (5) a section of the liver for the T3 treatment, adding nanoselenium at a concentration of 0.5 mg / kg of feed at the age of 35 days, showing the absence of a pathological lesion in the tissue, where the hepatocytes appear normal and the sinusoids have an equal arrangement and alignment. Hematoxylin and eosin stain X10.

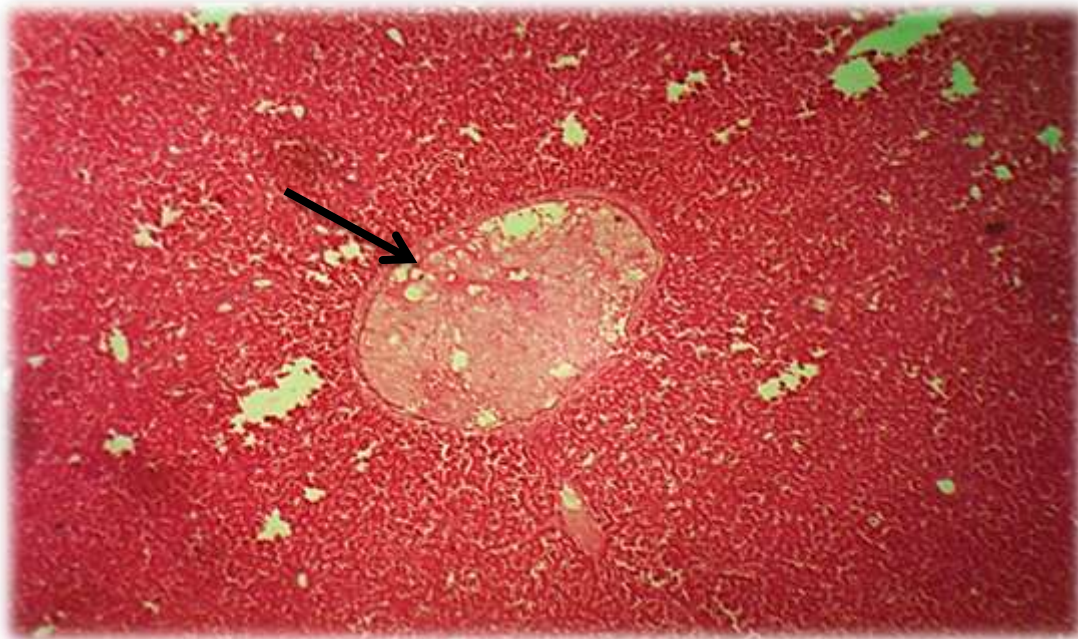


Picture (6) a section of the liver for T4 treatment, adding astaxanthin at a concentration of 60 mg / kg of feed at the age of 35 days, showing the absence of a pathological lesion in the tissue, where the hepatocytes appear normally and the sinusoids are of equal alignment and arrangement. Hematoxylin and eosin stain X10.



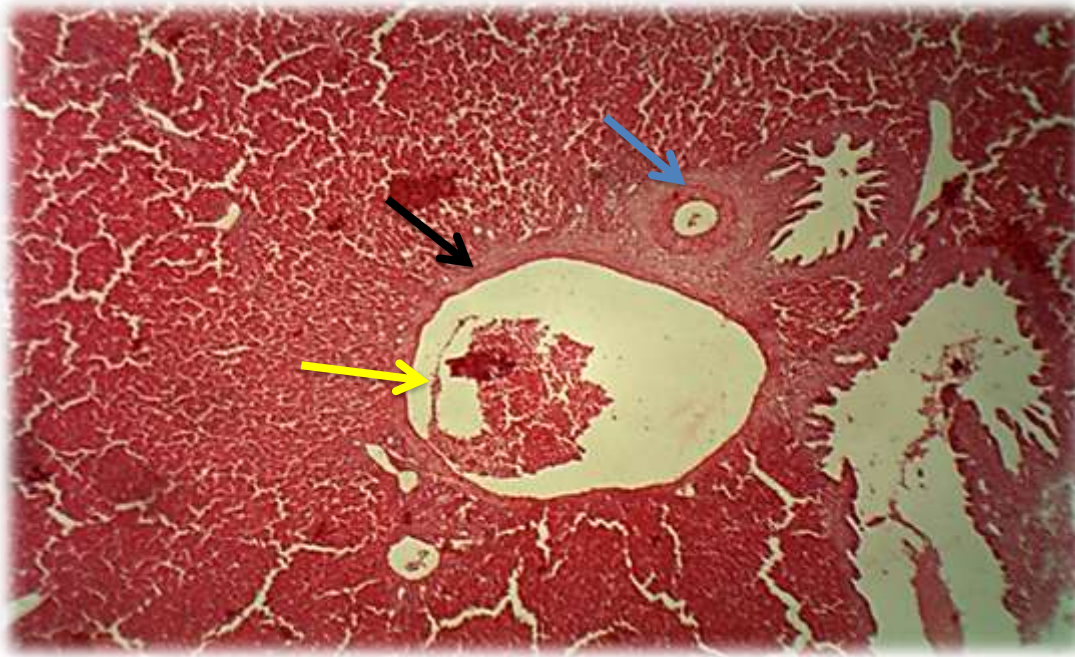


Picture (7) a section of the liver for T5 treatment, (indicator \_\_\_\_\_) with an increase in the thickness of the walls of the hepatic artery (artery thickening, adding astaxanthin at a concentration of 70 mg / kg of feed at the age of 35 days, showing the exudation of white blood cells outside the hepatic artery indicator \_\_\_\_\_), hematoxylin and eosin stain x10.

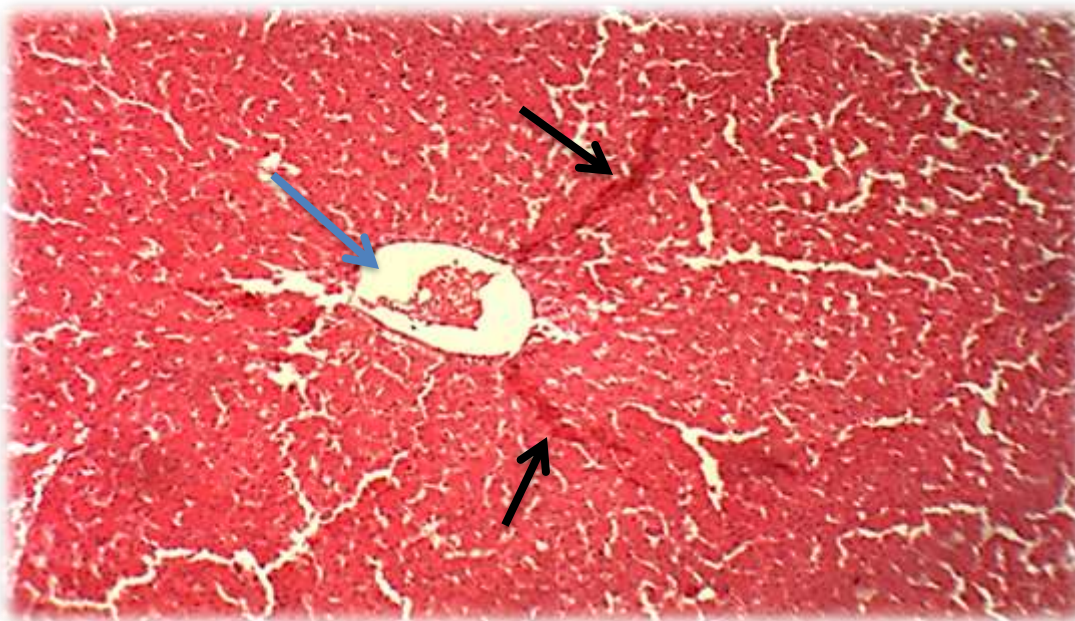


Picture (8) a section of the liver for T5 treatment, the diameter of the vein and closes it completely (indicator \_\_\_\_\_), hematoxylin and eosin stain X10. adding astaxanthin at a concentration of 70 mg / kg of feed at the age of 35 days, showing the presence of a blood clot in the hepatic vein and this clot fills



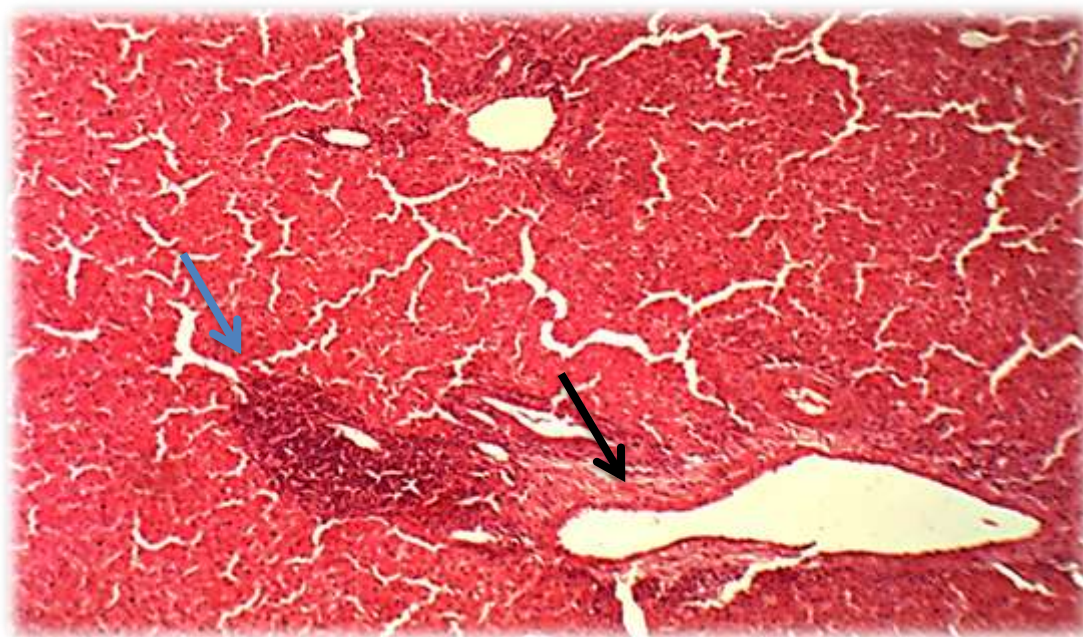


Picture (9) a section of the liver for T6 treatment, adding 0.3 mg of nano-selenium mixture + astaxanthin 60 mg/kg of feed at the age of 35 days, showing congestion in the hepatic vein with the expansion of the diameter of the vein (indicator \_\_\_\_), fibrosis of the area around the hepatic blood vessels, which is fibrous tissue (indicator \_\_\_\_), thickening of the hepatic artery wall (hepatic artery wall thickening, index \_\_\_\_), hematoxylin and eosin stain X10.



Picture (10) a section of the liver for T6 treatment, with the presence of hemorrhagic areas in the liver parenchyma (indicator \_\_\_\_), adding 0.3 mg of nano-selenium mixture + astaxanthin 60 mg/kg of feed at the age of 35 days. Hepatic vein congestion (indicator \_\_\_\_ ) is noted eosin stain X10.





Picture (11) a section of the liver for T7 treatment adding a mixture of nano-selenium 0.5 mg + astaxanthin 70 mg / kg feed at the age of 35 days, showing the exudation of inflammatory cells (white blood cells) outside the blood vessel and their infiltration into the hepatic tissue parenchyma (indicator \_\_\_\_\_) with elongation of hepatic vein and narrowing (index \_\_\_\_\_), hematoxylin and eosin X10 stain.

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