



INVESTIGATING THE EFFECT OF NANO-METALS ON POULTRY HISTOLOGY: A REVIEW

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Abstract

Nanotechnology is a double-edged technology, given the benefits and uses of nano-metals that are common and many in the poultry industry, using them as antibacterials, antioxidants, and antifungals, and in improving immunity and fertility, as well as adding nano-mineral elements in feed and technology for transporting medicines and vaccines, however, many nano-metals may hurt the body cell and various body tissues (especially the liver, kidneys, and testicles), as high concentrations of it may cause necrosis and damage to the cells of the liver, kidneys, and testicles, which may harm the bird's health and thus its productivity, in addition, there may be a cumulative effect of the nano-metal in some tissues, which may be transmitted to the consumer, causing health damage, which may violate global health controls and conditions, and this is what called for nano green synthesis technology and organic chickens.

Keywords: Nano metals, poultry, histology, effect of nanotechnology.

Introduction

Nanotechnology provides a broad and diverse field of biological and therapeutic research and uses nanomaterials to address environmental problems. Nanotechnology is a promising and modern technology with enormous potential to revolutionize agriculture and livestock. Nano is a



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Latin word, and classified nanotechnology has been clarified. First introduced in 1959 by the famous physicist Richard Feynman (Gopi *et al.*, 2017; Al-Jebory *et al.*, 2023a), nanotechnology allows the dimensions of materials to be controlled on the nanoscale with particle sizes ranging from approximately 1 to 100 nanometers (Ognik *et al.*, 2016; Al-Jebory *et al.*, 2023b), which provides many properties as The process of converting these large molecules into small nano-sized molecules leads to changes in the physical and chemical nature of the matrix and these changes include change in solubility, absorption, transport mechanism, secretion and transport within the cellular level (Mohapatra *et al.*, 2014). The preparation of nanoparticles varies and depends on the purpose for which they are prepared. The stability of the substance and toxicity must also be considered, as there are physical, chemical, and biological methods (Agnihotri *et al.*, 2004; Al-Jebory *et al.*, 2023 c). The biological method is one of the simplest methods. The nanoparticles consumed and manufactured in this way are usually not dangerous, as they are considered an easy, effective, and environmentally friendly way (Mary, 2012) and also produce low-toxic nanomaterials (Narayanan and Sakthiv, 2010). Green Synthesis: The term green synthesis refers to the preparation of a nanomaterial by exploiting nanotechnology and plant biotechnology together, and it is one of the biological methods Plant compounds, such as sugar, amino acids, alkaloids, and polyphenols, bind with mineral elements, reducing their size to nanoscale, resulting in organic compounds that stabilize the mineral ions and make them more available to the organism (Parveen *et al.*, 2016). Though there are many toxicological studies on metal nanoparticles (N.P.s), it remains challenging to explain discrepancies observed between studies, mainly due to the lack of positive controls and disconnection between physicochemical properties of nanomaterials with their toxicities at feasible exposures in a specified test system (Al-Jebory *et al.*, 2023d).

Effects of nanoparticles in liver

Al-Saeedi, (2019) studied histological in the liver of treatments chickens at the age of 35 days of age of chickens, using treatments as control treatment, 300 mg vitamin E / kg feed, 1.0 mg nano selenium / kg feed , 2.0 mg nano selenium / kg feed, 3.0 mg nano selenium / kg feed, 1.5 mg nano selenium + 150 mg vitamin E / kg feed, respectively, and it was noted in the control treatment T1 that fatty and cellular degeneration occurred and blood congestion in the liver, where the changes in the liver tissue of the chickens of the control treatment T1 may be caused by heat stress to which the chickens of this treatment were exposed, as reported by Davis *et al.* (2000); Zulkifli *et al.* (2003) Turkyilmaz; (2008) that heat stress causes an increase in the H/L ratio, and they confirmed the possibility of using this ratio as evidence of the occurrence of environmental stress on chickens, as it appeared in the T2 treatment, cellular degeneration and blood congestion, while the T3 treatment showed the presence of cellular necrosis, congestion and degeneration. As for the T5 treatment, blood congestion, degeneration, and fibrosis appeared in the livers of the chickens. Gregory (1998) mentioned that when chickens are exposed to heat stress immediately, the chickens secrete the neuroprotective amines epinephrine and norepinephrine from the core of the adrenal gland, accompanied by an increase in breathing speed. Heart rate and blood pressure increase with increased blood flow, which leads to congestion of the liver, cell degeneration, and adipose tissue

aggregation appeared, and blood congestion did not appear compared to the rest of the treatments, which indicates that exposure to heat stress was to a lesser extent compared to other treatments. Silver nanoparticles can destroy bacteria, viruses, and fungi so that they can be used as a drug in the treatment of some untreatable viral diseases in poultry (Al-Khafaji and AL-Jebory, 2019; Al-Khafaji *et al.*, 2022); Loghman *et al.*, (2012) noted the effects of nanosilver toxicity in broilers, where four treatments were used at (0) control levels, 4, 8 and 12 ppm of nanosilver added daily to drinking water, the first treatment (4 ppm) showed infrequent accumulations of nanosilver in liver cells, and hepatocyte hyperplasia was evident, as for the second and third treatments (8 and 12 ppm), dilatation of the central liver vein appeared, with severe fatty change, as appeared in the third treatment. (12 ppm) Increased connective tissue and focal change in liver cells, where the color of the nucleus of the cells was dark brown, detectable compared with other cells in the control treatment, where the number of these cells was deficient. However, in other treatments, it increased; there was an increase in the concentration of nanosilver. Chang *et al.* (2006) indicated that nanosilver enters the body through the skin, respiratory tract, and gastrointestinal tract, and the most important way is via the gastrointestinal tract in the form of colloidal nanosilver. Absorbed nanosilver binds to plasma proteins, can enter cells, and can be distributed to various body systems such as the liver, kidneys, heart, lymph nodes, brain, lung, stomach, and testes (Wijnhoven *et al.*, 2009). Nanosilver absorbed from the gastrointestinal tract enters the liver through the portal vein and can affect the liver. Since it enters the liver, it removes toxic substances from food absorbed by the gastrointestinal tract. Chemicals that can easily be excreted out of the body and high concentrations of ingested nanosilver particles may affect the liver and kidney excretion of nanosilver via urine was detected (Trop *et al.*, 2006). Recently, it has been reported that nanoparticles and nanomaterials generate free and oxidative radicals, and research results showed that nanosilver particles can damage various organs and tissues, such as liver cells (Trop *et al.*, 2006). Microscopic studies also confirmed that using nanosilver in high concentrations Mild causes damage to bacteria in the liver and the body. Also, high concentrations cause necrosis of liver cells, increase in phagocytic cells and increase connective tissue due to the toxicity of silver when used in high concentrations, High lead to decreased cellular activities due to glutathione depletion as well as damage to mitochondria and cell membranes and increased levels of liver enzymes. Kim *et al.* (2008) also found many cases of hepatic focal necrosis in high-dose treatments. Repeated oral doses of nanosilver for 28 days at a concentration of more than eight ppm led to increased glutathione and varicose veins and urethra. Al- kaabi, (2021) studied dosing selenium in broiler breeder cocks as (0, 1 mg organic selenium/kg feed, 1.5 mg organic selenium/kg feed, 2 mg organic selenium/kg feed, 0.5 mg nano selenium/kg feed, 0.75 mg nano selenium/kg feed, 1 mg nano selenium/kg feed) for treatments (T1, T2, T3, T4, T5, T6, T7) respectively and noted in liver It was noted that in the first treatment, T1, it showed a normal appearance of the liver, and the histological sections of the liver in treatment T2 were similar to those in the control group, as it showed a normal appearance of the central vein, hepatic cells, and sinusoids. However, in treatment T3, it was shown that there was slight congestion of the central vein with clear expansion and congestion of the sinusoids, which led to a slight compressive

atrophy of the hepatic cell cords compared to the control treatment. In the T4 treatment, slight congestion of the central vein was found, with the development of simple diffuse degeneration of the hepatic cells, compared to the control treatment; as for the nano-selenium treatments, the T5 treatment showed a normal appearance of the central vein, hepatic cells, and sinusoids, as in the control treatment. However, the T6 treatment showed congestion of the central veins with a clear expansion and congestion of the sinusoids, which led to slight compressive atrophy of the hepatic cell cords. In the T7 treatment, the sections showed the Liver histology of this group: Congestion of central and sinusoidal veins with moderately severe diffuse degeneration of hepatocyte cords, compared to the T1 treatment. The reason for the cytotoxic effect of increased selenium concentration in T7, T6, T4, and T3 treatments on cell proliferation may be that excessive selenium can lead to Cell cycle arrest, indicating that selenium deficiency may also restrict cell proliferation by mediating cell cycle expression (Shi *et al.*, 2017). The highest selenium concentrations in roosters were found in blood serum, liver, and chest muscles and increased with increasing dietary selenium levels. (0.03 to 1.3 mg/kg feed), and the magnitude of the increase was much greater when feeding nano-selenium than when feeding sodium selenite. Studies conducted on animals have shown that the liver is the main target organ for selenium toxicity as a result of excessive accumulation of absorbed selenium in the liver and the fact that the formation of reactive oxygen species generated by selenium (R.O.S.) are the main mechanisms of selenium toxicity (Mézès and Balogh, 2009), however, supplementation of 1.20 mg/kg selenium did not cause signs of toxicity suggesting that the optimal range of toxic dietary levels of nano-selenium is higher than that of sodium selenite. The likely reason for the higher tolerance to selenium in the form of nano-selenium is its higher rate of retention in the muscle, which may effectively reduce the selenite available to induce toxicity; his hypothesis was supported by a study using nano-selenium or sodium selenium intravenously; which showed that the percentages of nano-selenium in the whole body were much higher than those found in selenite (Hu *et al.*, 2012; Pelyhe and Mézès, 2013), it is worth noting that selenium deposition in testicular tissue will be different according to its chemical forms, duration of feeding, type of animal, age and physiological conditions. Testicular and liver degeneration may be caused by the high concentration of selenium deposited in the liver and testicle (Shi *et al.*, 2017), and also adequate selenium has a certain antioxidant effect, while excess selenium can lead to cell cycle arrest by increasing the level of R.O.S. (Shi *et al.*, 2017). Al-Jabawi (2019) study shows changes in the tissues of the liver of broilers exposed to heat stress at the age of 35 days (at concentrations 0.00, 0.1, 0.2, 0.3, 0.4 ppm/ L⁻¹) It was noted that heat stress generally leads to an increase in the amount of water consumed by the birds in the two hot periods of the day, and also leads to a decrease in the values of PCV, Hb, and R.B.C., and an increase in the L/H ratio. In the control treatment, we found the effect of heat stress in the control treatment T1, as it was observed that coagulative necrosis occurred in the visceral tissue of the liver, the reason for this may be due to an increase in body temperature above the normal rate, which increases the breakdown of the visceral tissue of liver cells (Guyton, 1996), as was observed, blood congestion and watery cellular degeneration occur, and the reason for this may be that birds exposed to heat stress secrete neuroamines such as adrenaline and noradrenaline from the adrenal

medulla, which is accompanied by an increase in respiratory rate, as well as an increase in heart rate and blood pressure and an increase in blood flow, which leads to liver congestion (Gregory , 1998), it is also observed that there is aqueous cellular degeneration and coagulative necrosis in treatment T2, while in treatment T3 it is observed that there is congestion of the blood vessel around the inflammatory cells and coagulative necrosis with the presence of inflammatory cells, the reason for the congestion of the blood vessel may be due to the birds being exposed to a little heat stress, which increases the speed of blood flow to the tissues, which causes congestion of some blood vessels (Ewing *et al.*, 1999), as for treatment T4, it appears that blood congestion and the deposition of hemosiderin occur, and the reason for the deposition of this dye may be due to the decomposition of red blood cells, an effect of heat stress was also found in the liver tissue for treatment T5, which shows the occurrence of deposition of fibrils, blood congestion, and deposition of hemosiderin, it turns out that Heat stress affected the liver tissue of all treatments, but the addition of nano-zinc reduced its effect on the birds of these treatments, as Zulkifili *et al.*, (2003); Turkyilmaz, (2008) mentioned; stated that heat stress causes an increase in the L/H ratio by emphasizing the use of this ratio as evidence of stress on birds at high altitude, the reason may be due to the role of nano-zinc in reducing the effects of heat stress through its role as an antioxidant or through its role in enhancing the body's immunity. Nano-zinc is also involved in synthesizing the S.O.D. enzyme (Superoxide dismutase - MnSOD). The hypothalamic-pituitary-pituitary axis releases the hormone corticosterone from the adrenal gland, which appears to be involved in reducing the damage resulting from exposure to heat stress (Ramiah *et al.*, 2019), as the corticosterone hormone activates, MnSOD enzyme, which works to convert peroxide or superoxide O_2^- into hydrogen peroxide H_2O_2 (Starkov, 2008), which indicates the role of the S.O.D. enzyme in eliminating free radicals in two steps that include recycling the copper element from Cu^{2+} to Cu^+ , and then to Cu^{2+} (Clara *et al.*, 2013).

In general, adding nano zinc enhanced broilers' antioxidant activity, as evidenced by increased Cu/Zn S.O.D. activity and reduced M.D.A. (malondialdehyde) accumulation in both blood and liver (Vural *et al.*, 2010). Zinc is also essential for regular growth, maintenance of body tissues, and tissue repair, and it is damaged and necessary for forming bones and feathers (Martin, 2016). Exposure of broilers to high temperatures results in the formation of reactive oxygen species at the cellular level, which hinders enzymatic activities and can cause a disease condition (Mishra, 2019). Research has indicated that nano-zinc has great potential as an antibacterial agent, as nano-zinc prevents the growth of bacterial strains. Such as *E. coli* and *Aureus*, where nano-zinc interacts with the cell walls of bacteria, and then Zn^{2+} ions enter through the cell membrane, destroying the bacterial cell (Brayner *et al.*, 2006; Li *et al.*, 2011). In addition, the presence of nano-zinc causes the production of peroxide hydrogen (H_2O_2) through S.O.D. enzyme activity, as hydrogen peroxide has an effective role in eliminating germs, which leads to reducing the use of antibiotics in feed and improving the intestinal epithelium and its performance, which affects the absorption and digestion of nutrients in chickens (Deplancke and Gaskins, 2001; Younas *et al.*, 2023; Zaki, and Al-jebory, 2021) Epithelial cells, goblet cells that secrete mucus, and intraepithelial lymphocytes provide a barrier to the entry of harmful germs through the intestinal tubule into the

capillary network (Deplancke and Gaskins, 2001). The intestinal structure is affected by zinc levels by increasing the height of the villi, and zinc also has a major role in developing the immune system and helps improve cellular and humoral immune responses (Moghaddam and Jahanian, 2009). Zhao *et al.* (2014) studied feeding broilers with 60 mg/kg zinc oxide and 20, 60, and 100 mg/kg nanozinc. It was noted that concentrations of 20 and 60 mg/kg nano-zinc improved live body weight, feed conversion coefficient, and decreased body weight. The body is treated with 100 mg of nanozinc. Ibrahim *et al.* (2017) found that adding 50 mg/kg of inorganic zinc, nano-zinc oxide, organic zinc (zinc-methionine), and a mixture of organic zinc (zinc-methionine) with nano-zinc oxide, respectively, to the diet of broilers improved significantly. The live body weight and food conversion factor favored the nano-zinc treatment. The two zinc treatments also showed an increase in the relative weight of abdominal fat and the concentration of zinc in the body. Ramiah *et al.* (2019) indicated that adding 100 mg of nano zinc oxide/kg in heat stress conditions to broilers reduced feed consumption and improved the feed conversion factor compared to concentrations of 0, 60 mg zinc oxide/kg, 40, 60, and 100. mg nano zinc oxide/kg. Zhao *et al.* (2014) studied feeding broilers 60 mg/kg zinc oxide and 20, 60, and 100 mg/kg nano-zinc. An increase in S.O.D. enzyme activity was observed in 60 and 100 mg/kg nano-zinc treatments, while catalase activity in blood serum was lower. It was higher in the 20- and 60-mg nano-zinc treatments, but its activity in the liver was not affected by the different zinc levels, and the M.D.A. level decreased in all nano-zinc treatments. Ibrahim *et al.* (2017) studied the addition of 50 mg/kg of inorganic zinc, nano-zinc oxide, organic zinc (zinc-methionine), and a mixture of organic zinc (zinc-methionine) with nano-zinc oxide, respectively, to the diet of broilers, where results were shown. The study showed a significant increase in the zinc addition treatments in the concentration of iron and copper in the liver and zinc in the bone marrow. The average total cholesterol in the blood, triglycerides, very low-density lipoprotein, and M.D.A. decreased. At the same time, S.O.D. enzyme activity increased in the zinc addition treatments, and the treatments of nano-zinc mixture with organic zinc increased. Nano-zinc reduced insulin-like growth factor-1 gene expression compared to inorganic zinc. Ramiah *et al.* (2019) indicated that the concentration of 40 and 60 mg of nano-zinc/kg decreased the concentration of M.D.A. in the thigh muscles and the concentration of corticosterone in the blood in conditions of heat stress compared to the concentration of 0.100 mg nano-zinc/kg. According to Ognik *et al.* (2019), dietary additions of Cr can range from 3 to 6 mg/kg, depending on whether Cr-organic or Cr-nano particle form is utilized, decreased visceral fat, boosted the body's defenses against oxidative stress, raised blood sugar levels, hindered growth, interfered with liver function, and led to pathological alterations in the liver and pancreas, therefore, in the case of both organic forms and nanoparticles, future studies attempting to demonstrate the favorable effects of chromium on broiler chicks should utilize lower levels of this element than 3 mg/kg feed. Abdel-Rahman *et al.*, (2022) study the effects of nano-iron alone or in combination with methionine were investigated using 0, 4 mg Fe/kg, and 4 mg Fe/kg plus 4 g kg of methionine, the findings revealed that the birds in the control group had significantly higher final weights, and that Fe compared to Fe⁺ meth partially relieved the adverse effects of heat stress on growth, Fe⁺ meth demonstrated a significantly increased free iron (Fe) level and transferrin

saturation index, while Fe^+ meth significantly reduced total iron-binding capacity and transferrin level when compared to 0 and Fe, in addition to a poor lipid profile, the Fe^+ meth group also displayed hepatic impairment and an inflammatory response when compared to the O and Fe groups, additionally, their hepatic tissues had elevated levels of ferritin and thiobarbituric acid reactive as well as a significant decrease in reduced glutathione levels, these findings suggest that the high absorption of nano-iron at the level of 4 mg kg^{-1} in this study is harmful, and more research is required to determine the appropriate supplemental level. Zhang *et al.* (2022) examined how surface charge and particle size affected the in vitro mutagenic response and the in vivo embryonic toxicity of recently synthesized silver nanoclusters at exposures relevant to humans or the environment. The new findings were compared with titanium dioxide nanoparticles (TiO_2 NPs, as a positive control), one of the most widely used nanoscale particles. The hypothesis was that the interaction between the test system and the physicochemical properties of nanomaterials is crucial in determining their toxicities at concentrations relevant to human or environmental exposures, the mutagenicity of the AgNCs (approximately 2 nm) and the two sizes of TiO_2 NPs (i.e., small: 5–15 nm, big: 30–50 nm) were evaluated using the Salmonella reverse mutation assay (Ames test), when it came to TiO_2 NPs, the size effect was obstructed by the agglomeration of TiO_2 NPs in media and the generation of oxidative stress from the N.P.s; the embryonic toxicity and the liver oxidative stress were assessed using a chicken embryo model at three doses (0.03, 0.33, and $3.3 \mu\text{g/g}$ egg), the smallest size of AgNCs demonstrated the highest mutagenic activity with the Salmonella strain TA100 in the absence and presence of the S9 mixture, both sizes of TiO_2 NPs had negative effects on the development of chicken embryos; the non-monotonic response was used to assess the developmental toxicity of the tested N.P.s; the data on AgNCs differed from earlier findings on AgNPs; the results on chicken embryos showed some size dependency of nanomaterials, but they were more strongly correlated with lipid peroxidation (malondialdehyde) in the fetal livers of the chickens; and there was a distinct degree of agglomeration of TiO_2 NPs and AgNCs in the Ames and chicken embryo assay media.

Effect of nanoparticles on intestinal

Sawosz *et al.* (2007) mentioned that nanosilver could affect the outer layer of the intestinal wall and, thus, cause a change in the length and width of the villi and the depth of the crypts. Katarzyna *et al.* (2016) found that feeding on nanosilver at a concentration of 5 mg/kg feed It led to an increase of 11% in the average length and width of the villi and 7% in the depth of the crypts. The reason may be because nanosilver improved the intestinal environment and thus led to an increase in the length and width of the villi and the depth of the crypts. Al-Jebory, (2018) studied the histological in the jejunum of the small intestine of chickens treated with different nanosilver injections under heat stress in breeding house, and the results showed a significant increase in the length and width of the villi and the regularity of their shapes, as well as an increase in the depth of the crypts for the nanosilver injection treatments at a concentration of (14, 16, 18 ppm), at the age of 7 days compared to the negative and positive control treatments, and the cellular activity in the intestinal segments showed an increase through the multiplication of intestinal cells in the

crypts region, as well as in the surface of the villi, especially in the treatment of nanosilver injections at a concentration of (12 ppm) as well another section appears in the jejunum after the injection treatment with a concentration of (14 ppm), where it shows an increase in the width and regularity of the villi as well as the injection treatment with a concentration of (16 ppm), injection with a concentration of (18 ppm), the villi elongated with their regularity and an increase in cellular activity in the crypts area, as for the negative and positive control treatments, the variation in the length and width of the villi and the irregularity of their shapes appear, and the reason may be due to the superiority of the nanosilver injection treatments on control indicated that the injection of silver nanoparticles into the amniotic fluid of the embryos and its oral ingestion by the embryos in the last third of incubation (19 days) (Al-Jebory *et al.*, 2021 a & b), which causes activity in the gastrointestinal tract in general and the intestinal region, and in the jejunum in particular, which led to an increase in the length and width of the villi and an increase in the depth Crypts (Al-Khafaji, 2012; Ali Al-Gburi *et al.*, 2021; Al-Saeedi *et al.*, 2021).

Relationship of nano-elements with apoptosis in cocks' testes cells

Fertility is the first and most important condition for raising poultry; the number of fertilized eggs produced for hatching determines the final profitability of the chickens; infertility is also a major economic loss in the poultry industry (Heydari *et al.*, 2015). Although both males and females contribute to reducing fertility, Low fertility is thought to be a major problem in males because the ratio of males to females in the herd is meager (Ommati *et al.*, 2013). The deterioration of fertilization capacity is attributed to many factors, such as age, weight, and low semen quality (Khan, 2011). The testicle has two important functions: spermatogenesis and steroidogenesis, spermatogenesis occurs in the germinal epithelium of the seminiferous tubules, and steroid formation is done by interstitial cells (Leydeck cells) that are controlled by gonadotropin (Mathur and D'Cruz, 2011). The testicle is the basis for the production and reproduction of poultry. Which means that if the testicles are damaged for some reason, the quality of sperm will be affected, which ultimately leads to a defect or deformity in the sperm, which will negatively affect the success rate of mating (Shao *et al.*, 2018; Siva Kumar and Neeraja, 2019), on the other hand, oxidative stress is the main factor for infertility and a defect in the reproductive efficiency of the bird, in addition to other factors such as exposure to anti-endocrine agents, physiological stress, temperature, pH imbalance, etc. (Bandi *et al.*, 2018; Rehman *et al.*, 2018), testicular cell regulation Sertoli cells and sperm, are highly regulated in number and viability throughout reproduction, as testicular retraction was thought to be due to necrotic processes resulting from the failure of hormones to maintain the integrity of the germinal epithelium (Nazarabadi *et al.*, 2019), however, Recent studies in mammals have mostly shown that testicular cell death is not random but initiates highly organized cellular suicide mechanisms instigated by a series of intracellular protein interactions (Thurston and Korn, 2000; Kadhim *et al.*, 2021; Ajafar *et al.*, 2023), and physiological apoptosis is believed to occur continuously to control the size of the cell population. germ cells (Nazarabadi *et al.*, 2019), and there is much evidence that Sertoli cells are involved in the control of apoptosis and germ cell function via different pathways in which they are involved (Lee *et al.*,

1997), and the number of Sertoli cells and their functional state determine testicular size and progression. Normal sperm formation plays a crucial role in the reproductive function of males (Chen *et al.*, 2015). As for plasma membrane fats, they have an important role in enhancing the fertilizing ability of sperm because the membranes of chicken sperm cells contain a much higher concentration of polyunsaturated fatty acids. than mammalian sperm cells and are therefore more susceptible to lipid peroxidation in the presence of reactive oxygen species (R.O.S.) (Heydari *et al.*, 2015). During the reproductive period of roosters, the testis undergoes numerous cellular changes related to proliferation and degradation, and proliferation may be regulated by endocrine mechanisms. Paracrine and autocrine, which are driven by hormones, cytokines, and transcriptional regulators, may begin to deteriorate due to certain conditions, such as decreased gonadotropin, testosterone levels, or oxidative stress, which are conditions that increase the regulation of Sertoli cells, causing apoptosis of germ cells, regulation of relevant proteins may help protect the cells from, programmed cell death, here I have described the factors responsible for the production of reactive oxygen species in the reproductive organs, compared to abnormal sperm formation, they can cause attacks by free radicals that threaten the various organs of the body by blocking the arteries and increasing oxidative stress, thus causing serious damage to the tissues. High production of harmful oxidative action factors leads to in the testicle, the natural regulatory mechanisms (high cell division, cell competition for the rate of oxygen, and low oxygen pressure). These conditions lead to the destabilization of the vessels in the testicle and excessive production of cholesterol and fatty acids. Therefore, the harmful effect of oxidative stress causes the inability to activate the antioxidant system, which leads to It leads to infertility in males as well as females, and apoptosis is the main pathway of apoptosis under oxidative stress conditions responsible for the production of reactive oxygen species (R.O.S.) and apoptosis in rooster testis (Nazarabadi *et al.*, 2019). Al- kaabi, (2021) studied dosing selenium in broiler breeder cocks as (0, 1 mg organic selenium/kg feed, 1.5 mg organic selenium/kg feed, 2 mg organic selenium/kg feed, 0.5 mg nano selenium/kg feed, 0.75 mg nano selenium/kg feed, 1 mg nano selenium/kg feed) for treatments (T1, T2, T3, T4, T5, T6, T7) respectively and noted in testes an improvement was found in the thickness of the interstitial tissue of the seminiferous tubules, the diameter of the seminiferous tubules, the height of the germinal epithelium, the diameter of the blood vessels, and the number of interstitial cells. The reason for the improvement in the histological characteristics of treatments T5, T3, and T2 was attributed to the role of nano-selenium and organic selenium, which works to increase the size of the testicles, and the seminiferous tubules and the increase in the characteristics of the seminiferous tubule and daily sperm production by increasing the secretion of testosterone and thus increasing the efficiency of Sertoli cells and Leydeck cells and the diameter of the lumen (Okpe and Udoumoh, 2016). It was also found that changes in selenium levels (deficiency or increase) can lead to Increased apoptosis in spermatogenic cells and thus reduced fertility of roosters (Qazi *et al.*, 2019), and improvement in seminiferous tubule tissue characteristics and daily sperm production resulted from enhanced testosterone and Sertoli cell efficiency (Safaa *et al.*, 2019). Twenty-four adult male rabbits were used in the study, according to (Taha and Ismail, 2023), and they were split into four groups: the first group received an

intraperitoneal distal water injection as the control; the second group received an injection twice a week for three weeks at a dose of 600 mg/kg body weight of nano ZnO particles I.P.; the third group received an injection of 600 mg/kg body weight of nano ZnO particles I.P. and 100 mg/kg body weight of vitamin E orally; and the fourth group was given an oral dose of 100 mg/kg body weight of vitamin E, the testis and epididymis undergo discernible alterations as a result of the nano ZnO particle therapy, according to the histology data, the testis's tunica albuginea thickens as a result of these modifications, giant cell development, spermatogenesis arrest, degradation and necrosis of the epithelial cells lining the epididymis canals, and alterations to the germ cells lining the seminiferous tubules, sperm-free canals are visible, the animals treated with nano ZnO particles in addition to vitamin E showed improved histological changes when compared to the control group, while the vitamin E-treated animals alone displayed normal testicular and epididymal architecture. Furthermore, compared to other groups, the animals treated with micro ZnO particles had a lower concentration of testosterone.

Muscle and other tissue

Aljumaily and Aljumaily, (2021) find when adding nano-selenium at a concentration of 0.25 and 0.35 mg/kg + with V. E at a concentration of 300 mg/kg, in physical properties of the meat, there were significant differences in favor of the nano-selenium treatments, where recorded the highest concentration of selenium in the breast meat tissue, while recorded a significant decrease in the pH of the meat compared with the control treatment, this reduces the microbial activity and prolongs the storage and preservation period of meat, and in the chemical properties, the results indicated the presence of significant differences in the percentage of protein, moisture, fat, and ash, and therefore the results showed that the use of nano-selenium and vitamin E led to the improvement of most of the biochemical and immunological properties of blood, in addition to improve the physical and chemical properties of broiler carcasses. (Czyż *et al.*, 2023) noted an application of preparation based on nanosilver caused an increase in Ag content in chickens' liver, lung, and abdomen skin, Cu content decreased in all experimental groups, except for abdomen skin, while Zn content increased in most cases compared to the control, no apparent decrease of the increasing tendency was found for Se, it did not demonstrate an antagonism concerning the content of Cu, Se and Zn in examined samples. (Bień *et al.*, 2023) indicate that the birds were fed with a standard commercial diet containing inorganic Se in the form of inorganic Se at the level of 0.3 mg/kg diet and an experimental diet with an increased level of Se (0.5 mg/kg diet), using other forms of Se (nano-Se) versus sodium selenate significantly influences a higher collagen content and does not impair physicochemical properties in the breast muscle or the growth performance of the chickens, in addition, using other forms of selenium at an increased dose versus sodium selenate affected the elongation of sarcomeres in the pectoral muscle while reducing mitochondrial damage in hepatocytes and improving oxidative indices, the use of nano-Se at a dose of 0.5 mg/kg feed has high bioavailability and low toxicity without negatively affecting the growth performance while improving breast muscle quality parameters and the health status of the chickens, (Eletmany *et al.*, 2022-2024).

Conclusion

Although most of the research that analyzed poultry meat indicated that the level of minerals in it was average, some also found high levels of nano-minerals in the liver, chest muscles, thighs, kidneys, and testicles, which may pose a threat to the health of birds and consumers and therefore requires more research to be conducted in order to find out. Toxic levels of it, as well as conducting chemical and histological analyses of meat after storage to study the extent of deterioration occurring in the tissues.

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