Histological Study of Jejunum in Broiler Chicks Fed in the Embryonic Period with Silver Nanoparticles and Exposed to Heat Stress

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Abstract

This study included two consecutive experiments, the first experiment was conducted in the hatchery of Al-Anwar company in Babylon province for the period from 30/7/2017 to 19/8/2017 in order to investigate the effect of injecting hatching eggs to broiler breeder with different levels of nano-silver, and then examining its subsequent effect on histology traits of the hatched chicks exposed to heat stress, as the experimental treatments were the treatment T1 without injection as a negative control, and the treatment T2 was an injection of 0.3 ml of Nacl solution as a positive control, and the treatments T3, T4, T5 and T6 were injections of hatching eggs with 12,14,16, and 18 ppm of a nano-silver solution, respectively. The hatched chicks from the above treatments were reared in the poultry farm of the Department of Animal Production at the College of Agriculture / Al-Qasim Green University for the period from 8/19/2017 to 9/22/2017, and were exposed to a periodic temperature. (28-32-28 m ◦) and the results showed A high significant (P≤0.01) of T6 was detected in the length of the villi in the jejunum period and the treatments were higher T3, T4, in width of villi while all the injection treatment significantly higher in the depth crypts compared with treatment control.

Keyword: broiler, nano-silver, injection, histology.

Introduction

Metallic silver nanoparticles (up to 100 nm) have a greater antibacterial impact than silver salts and are less likely to be destroyed by stomach acids. They also absorb slowly through the intestinal mucosa, minimizing any potential toxicity. The doses that stimulate physiological and productive effects in animals have also been demonstrated to be quite low (20 to 40 ppm), especially when compared to the 10 to 100-fold greater doses. Silver nanoparticles (nano-Ag) are a new type of feed additive for poultry that may also have medical uses. Animal health and metabolic function may be impacted by nano-ag (Albanese, 2012; Al-Khafaji and Al-Jebory, 2018&2019; Al-Gburi 2021; Al-Khafaji et al, 2022). Nano-antibacterial Ag's characteristics may have an impact on microbial populations without causing resistance and may boost anabolic activity, which could stimulate

animal growth and development, Nano-Ag would raise the pace of metabolism and increase the oxygen requirement, which would ultimately boost embryonic growth and development, - Fibroblast Growth Factor (FGF) gene expression is influenced by nano-ag, which increases the growth and development of blood vessels, muscles, and fibroblast cells and aids in angiogenesis; Vascular Endothelial Growth Factor (VEGF), which is essential for endothelial cell structure and proliferation and encourages angiogenesis; Paired box gene (Pax7) is a transcription factor that helps muscles produce satellite cells; Proliferating Cell Nuclear Antigen (PCNA) is a DNA repair and synthesis trimmer that is crucial for postnatal development, By promoting the creation of the heat shock protein (HSP), silver nanoparticles can boost immunity in cells without activating the pro-inflammatory pathway (Mahmoud, 2012), Additionally, several studies found that injecting silver nanoparticles into hatching eggs enhanced the traits of the chicks that emerged as well as the productive, physiological, and immunological performance of broiler chickens (Al-Khafaji and Al-Jebory, 2018&2019; Al-Gburi 2021; Al-Khafaji et al, 2022).

Materials and methods

Saline solution (Nacl) was used in the preparation of egg injection solutions, and nano-silver material was obtained from Nanosany Copration, with size (20 nm) and morphological shape (spherical). 480 broiler chicks were used and raised in 1 x 1.5 meter cages. The chicks were divided randomly into six treatments, and each treatment had four replications. Each replicate included 20 chicks. Nano-silver was used by injecting it into the eggs at concentrations (0, Nacl, 12, 14, 16, 18) ppm for the treatments (T1, T2, T3, T4, T5, T6), respectively, where the hatching eggs were injected at the age of 17.5 days of the embryos' age, and (0.25 ml / egg) was injected.

The chicks were exposed to a periodicity of (28-32-28 c)

Feed treatment:

The chicks were fed on the starter diet from the age of one day until the third week of the birds' life, after that they were replaced with the finisher diet until the end of the fifth week. Fodder and water were given freely (ad libitum), and the diet used is as shown in the table below (Table 1).

Table (1): The percentage of the ingredients used in the study and their chemical composition.

Feeding materials	starter%	finisher%
yellow corn	30	**40**
wheat	28.25	**24**
Soybeans (48% protein)	31.75	**24.8**
Proteins concentrate*	5	**5**
Sunflower oil	2.9	**4.4**
limestone	0.9	**0.6**
DCP) Calcium diphosphate)	0.7	**0.9**
Nacl	0.3	**0.1**
mixture of vitamins and minerals	0.2	**0.2**
Total	100	**100**

Calculated chemical analysis**

General protein (%)	23	20
Representative energy calculated (kilo calories / kg feed)	3027	**3195.3**
Lysine (%)	1.2	**1.1**
Methionine (%)	0.49	**0.46**
Cyctain(%)	0.36	**0.32**

Methionine + Cyctain (%)	0.85	**0.76**
Phosphorus is available (%)	0.45	**0.49**
C/P %	131.61	**159.77**

*procium processor: brokon-5 special w: chinese origin, each kg contains 40% raw protein, 3.5% fat, 1% fiber, 6% calcium, 3% phosphorus available, 3.25% lysine, 3.90% methionine + 2.2% sodium, 2,100 kg / kg of energy represented, 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 µg biotin, 1mg iron , 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antiphosphate .

**According to the chemical analysis of feeding according to (NRC, 1994).

studied traits

Preparing the histological slide

Four chicks from each replicator was slaughtered at the age of 7 days. The tissue samples were taken in the shortest possible period of time to ensure that the tissues of the bird's body are not damaged. A 2 cm cut was made from the second part of the jejunum small intestine. Intestinal contents were removed and all excised slides were washed with tap water and operated according to (Richard et al, 1973).

The tissue slides were examined using a compound microscope, Olympus BH2 of Japanese origin, and the tissue sections were photographed by a digital camera connected to a Lenovo computer. The jejunum sections of the small intestine were examined using an ocular micrometer with a power of 100x magnification for the purpose of recording measurements of the small intestine (villi length, villi width and crypt depth) in microns = 3-10 mm, and a stage micrometer was used for the purpose Calibration of the ocular lens, according to (Djolai et al, 1998). Villi length was measured from the apex of the villus to its attachment to the villus, and the crypt depth is defined as the depth or submersion distance between adjacent villi (Edens et al, 2009; Al-Shukri, 2011). and as for the width of the villus, it was measured according to what (Al-Shukri, 2011) mentioned As it was measured from the middle of the height of the villus or from the place of curvature of the villus that divides the villus, while (Awad et al, 2006) estimated the width of the villus from the base of the villus, so the width of the villus was measured according to (Al-Shukri, 2011), by taking the average of the two readings, noting that all measurements were made For (10) readings for each measurement and taking the average.

statistical analysis

The data were analyzed using a completely randomized design (CRD) to study the effect of the studied treatments on the different traits, and the significant differences between the means were compared using the (Duncan, 1955) multinomial test.

The program SAS, (2012) was used in the statistical analysis according to the following mathematical model:

$Yij = \mu + Ti + eij$

Since:

Yij: the observed j value of treatment i.

µ: the overall mean of the trait.

Ti: the effect of treatment i (as the study included the effect of 6 aforementioned treatments).

Results and Discussion

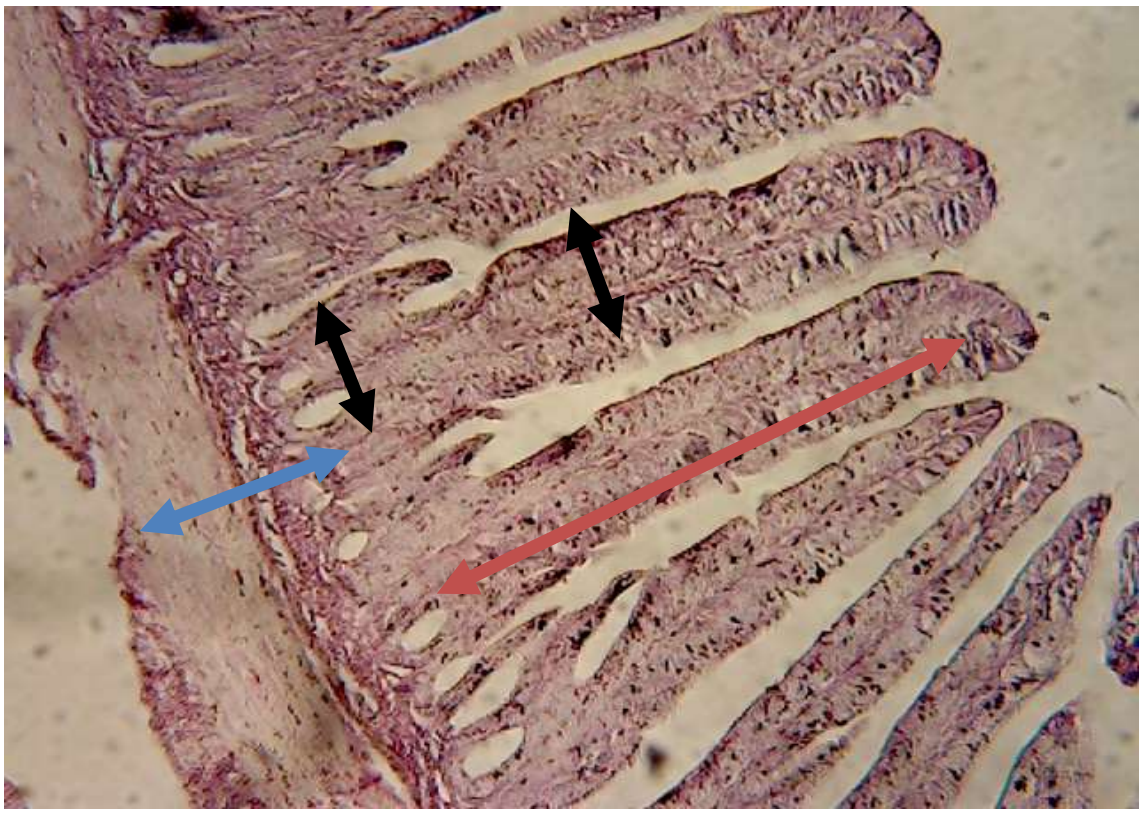
Table (2) shows the effect of injecting the hatching eggs with nano silver on the length and width of the villi and the depth of the crypts in the jejunum at the age of 7 days for broilers subjected to heat stress. It is noted that there is a significant superiority ($P \leq 0.01$) of the injection treatment with nano

silver T6 over the rest of the treatments in the trait The length of the villi followed by the T3 treatment, as well as the superiority of the T5 treatment over the T2, T4 treatments and the superiority of the T1 treatment over the T2 treatments and the superiority of the T4 treatment over the T2 treatment. Treatment T6 is superior to treatments T1, T2, T5, and no significant difference appeared between treatments T1, T2, T5 and with regard to the depth of crypts, we note a significant superiority (P≤0.01) for all treatments of nano-silver injection T3, T4, T5, T6 over treatments control T1, T2 and the table did not record a difference between the treatments T3, T4, T5, T6 as well as between the treatments T1, T2 (Sawosa, et al 2007) mentioned that nano-silver can affect the outer layer of the intestinal wall and thus a change in the length and width of the villi and the depth of the crypts also Katarzyna et al, (2016) found that feeding on nano-silver 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, and the reason may be due to the fact that nano-silver improved the intestinal environment and thus led to an increase in the length and width of the villi and the depth of the crypts. The histological sections of the jejunum of the small intestine of birds treated with nano-silver injection were studied, 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 of the injection treatments at the age of 7 days compared to the negative and positive control treatments (Pictures 1-8). And the cellular activity in the intestine sections 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 injection treatments with nano-silver at a concentration (12 ppm), and another section appears in the jejunum region for the injection treatment with a concentration (14 ppm), where it shows an increase in the width of the villi and their regularity, as well as the injection treatment with a concentration (16 ppm), the regularity of the villi forms appeared with their high lengths, and the appearance of cell proliferation in the crypts area that does not vibrate, while the injection treatment with a concentration (18 ppm) showed the elongation of the villi 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 may be due to the superiority of the injection treatments with nano-silver over the treatments. The control indicated that the injection of nano-silver in the amniotic fluid of the fetus and its ingestion orally by the fetus in the last third of incubation (19 days), 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 of the crypts (Al-Khafaji, 2012).

Table (2): Effect of injecting hatching eggs with silver nanoparticles on jejunum villi tissue at 7-day-old broilers exposed to heat stress

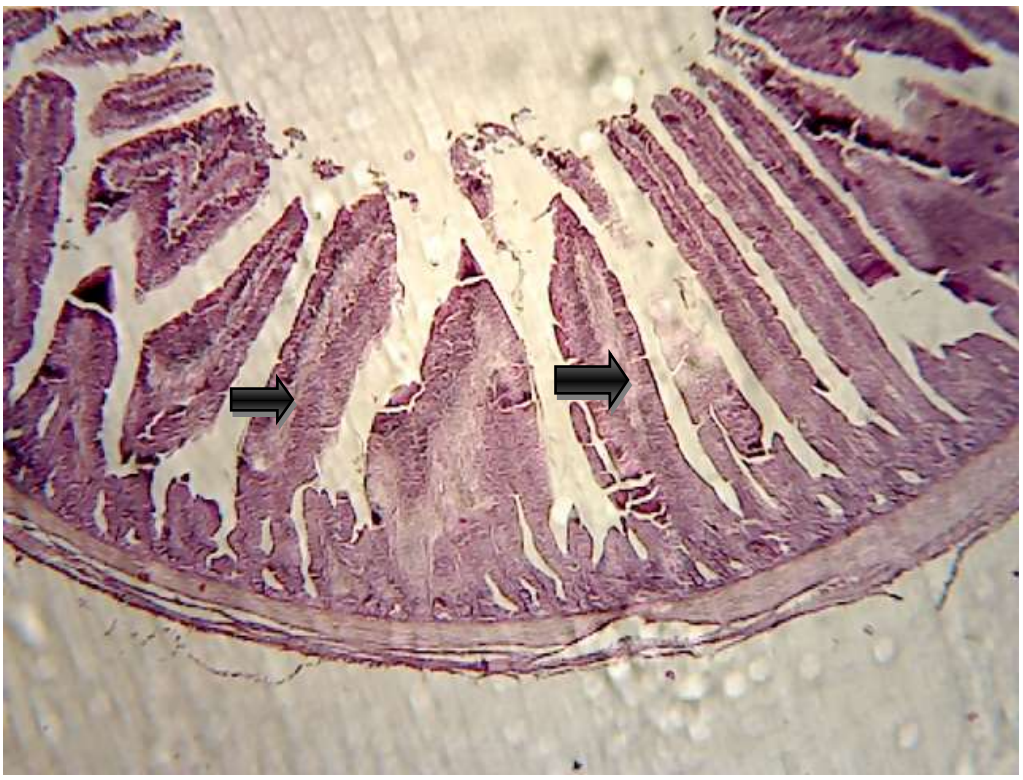
Treatments	stander error± Means		
	Length	Width	crypts depth
T1	cd 0.23 ± 7.53	c 0.02 ± 1.18	b 0.12 ± 2.40
T2	e 0.05 ± 6.14	c 0.04 ± 1.09	b 0.05 ± 2.53
T3	ab 0.09 ± 8.20	a 0.03 ± 1.76	a 0.09 ± 3.04
T4	d 0.15 ± 7.24	a 0.05 ± 1.79	a 0.07 ± 2.98
T5	bc 0.11 ± 7.91	c 0.04 ± 1.09	a 0.04 ± 3.14
T6	a 0.16 ± 8.48	b 0.01 ± 1.58	a 0.05 ± 3.11
Significant	**	**	**

Means with different letters indicate a significant difference in probability level 0.01, N. S: Not significant. *(P≤0.01). The treatment T1, T2, T3, T4, T5, T6 are (0, Nacl, 12, 14, 16, 18), respectively.



Pictures (1) showing the method of measuring the length of the villi (__) and the width of the villi (___) according to Al Shukri (2011) and the depth of the crypts (__)

Picture (2) A section of the jejunum of the negative control treatment without T1 injection, at the age of seven days, and it shows contrast in the length and width of the villi (hematoxylin and eosin stain) 200X

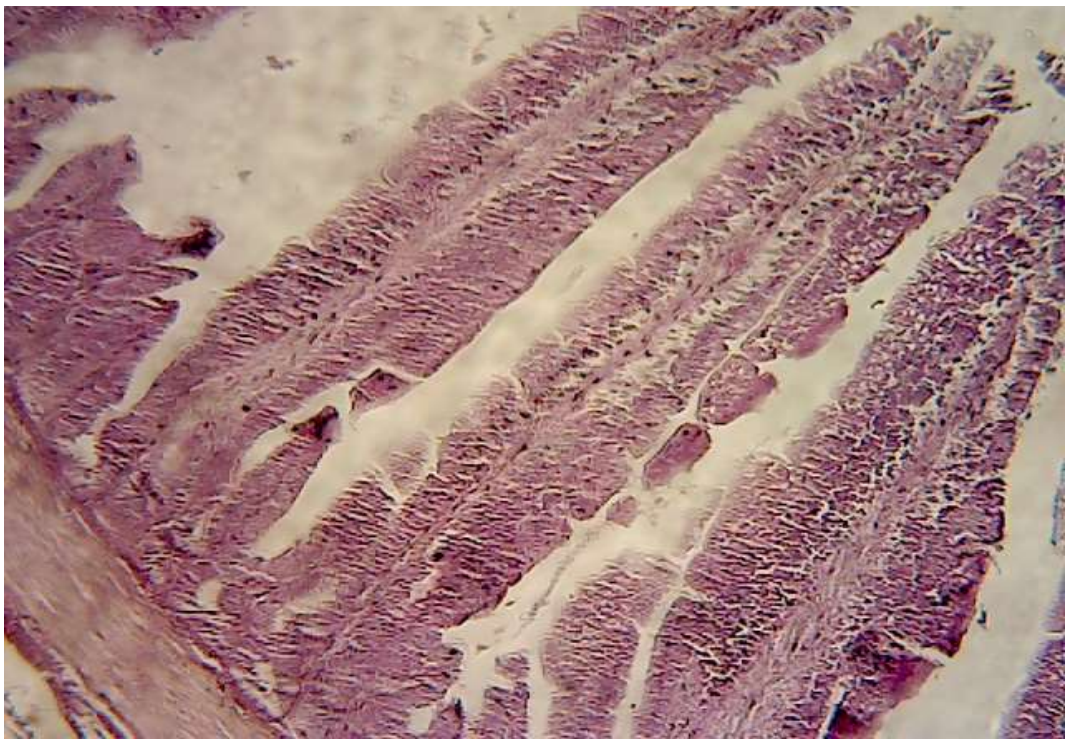


Picture (3) A section of the jejunum of the negative control treatment without T1 injection at the age of seven days, and it shows a variation in the length and widthof the villi (hematoxylin and eosin stain) 40X (indicator)

Picture (4) A section of the jejunum of the T2 positive control treated, injected with saline solution Nacl, at the age of seven days, and it is noted that irregularity and variation in the length and width of the villi (hematoxylin and eosin stain) 200X

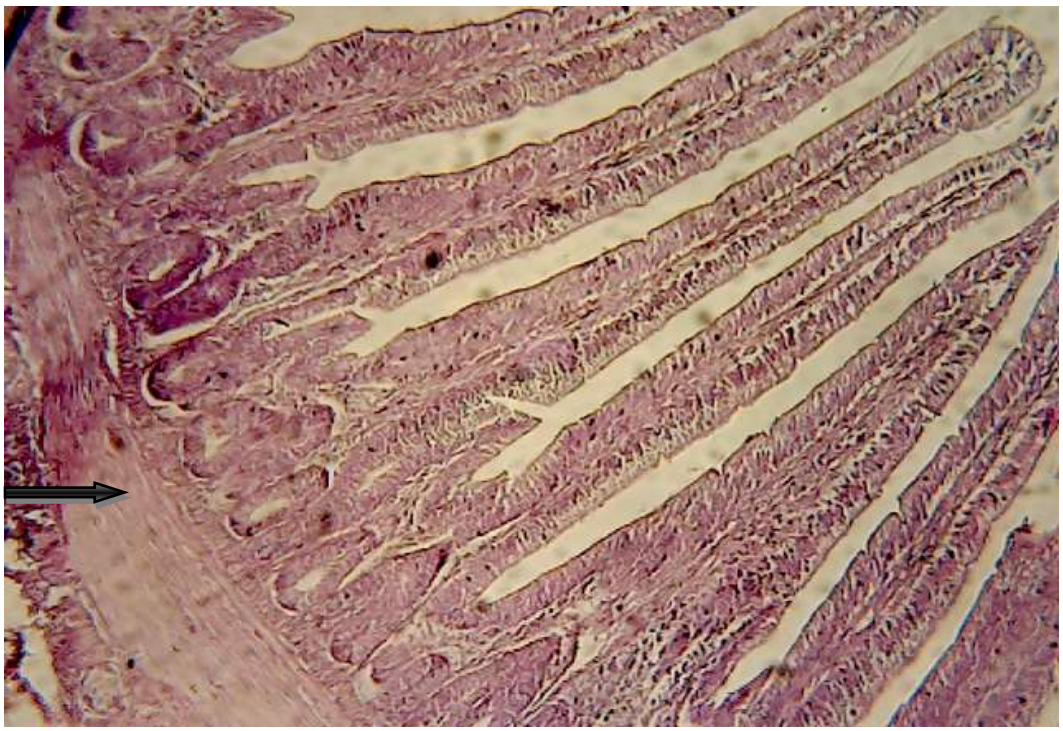
Picture (5) A section of the jejunum of the T2 positive control treated, injected with saline solution Nacl, at the age of seven days, and it is noted that irregularity and variation in the length and width of the villi (hematoxylin and eosin stain) 40X

Picture (6) A section of the jejunum of the T3 treatment (12 ppm) at the age of seven days, and a significant increase in the length and width of the villi (__) is noted, as well as in the depth of the crypts with an increase in cellular activity in the crypts area (__) (hematoxylin stain and eosin) 200X



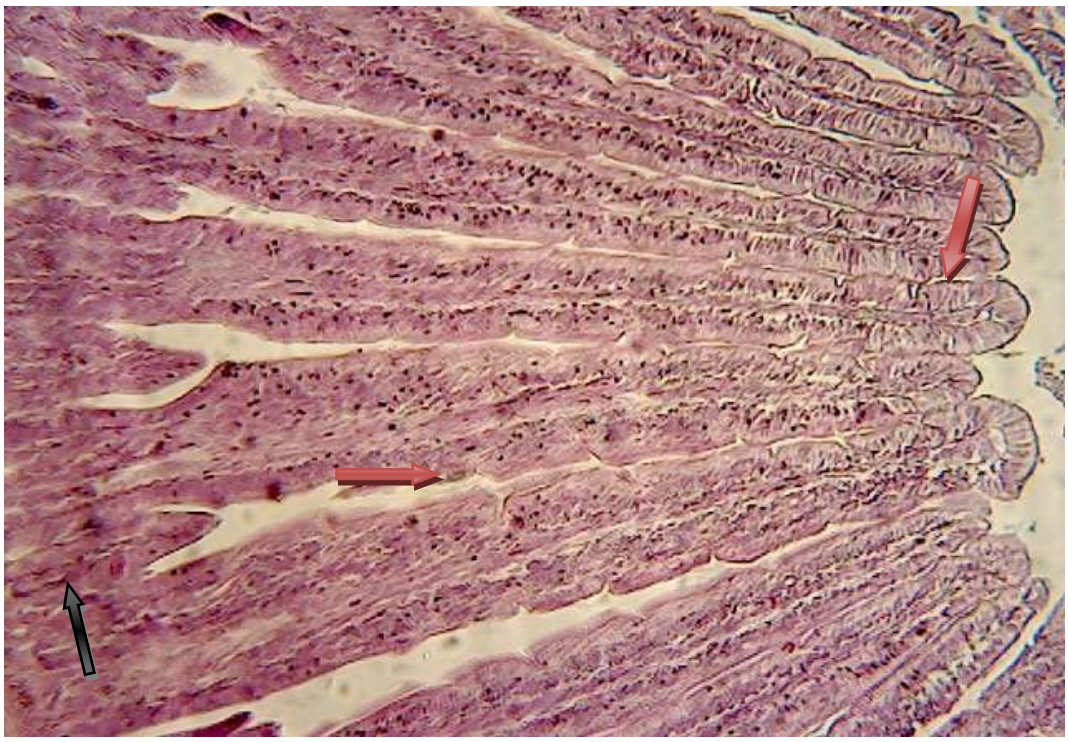
Picture (7) A section of the jejunum of the T4 treatment (14 ppm) at the age of seven days, and it shows a significant increase in the length, width and regularity of the villi compared to the control treatments T1 and T2 (hematoxylin and eosin stain) 200X

Figure (8) A section of the jejunum of the T5 treatment (16 ppm) at the age of seven days, and it is noted that the shapes of the villi are regular with a high length (indicator) and the appearance of cell proliferation in the crypts of Leberken (hematoxylin and eosin stain) 40X



Picture (9) A section of the jejunum of the T5 treatment (16 ppm) at the age of seven days, and it is noted that the shapes of the villi are regular with a high length and the appearance of cell proliferation in the Leberken crypts (indicator) (hematoxylin and eosin stain) 200X

Picture (10) a section of the jejunum of the T6 treatment (18 ppm) at the age of seven days, and it is evident that the villi are elongated with their regularity and an increase in cellular activity in the crypts (indicator) (hematoxylin and eosin stain) 40X



Picture (11) a section of the jejunum of the T6 treatment (18 ppm) at the age of seven days, and it is evident that the villi are elongated with their regularity (indicator-) and an increase in cellular activity in the crypts area (indicator--) (hematoxylin and eosin stain) 200X

Conclusion

Increasing the amount of minerals, especially the uncommon ones, helps the body's cells and tissues carry out the majority of their metabolic functions while also enhancing the immune system's performance and the bird's metabolic rate. Therefore, despite being raised in settings of heat stress, we observe an improvement in the experimental chicks' histological properties. The use of nanoparticles is a promising technology in many industries, including the poultry sector. In particular, nano-silver plays a significant role in improving physiological traits and serving as a vital support for the immune system's functionality by being able to eliminate harmful germs.

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دراسة نسيجية لمقطع في الصائم لفروج اللحم المغذى جنينياً على جسيمات الفضة النانوية والمعرض لإجهاد حراري

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الملخص:

تضمنت هذه الدراسة تجربتين متتاليتين ،أجريت التجربة الأولى في مفقس شركة الانوار في محافظة بابل للمدة من 30/ 7 /2017 لغاية 19 /2017/8 وذلك لبحث تأثير حقن بيض التفقيس لأمهات فروج اللحم بمستويات مختلفة من نانو الفضة ومن ثم بحث تأثيره اللاحق على الصفات الفسلجية للأفراخ الفاقسة والمعرضة لإجهاد حراري أذ كانت معاملات التجربة هي المعاملة T1 من دون حقن سيطرة سالبة و معاملة T2 حقن بـ 0.3 مل من محلول Nacl سيطرة موجبة ، و المعاملات T5,T4,T3 و T6 حقن بيض التفقيس بـ 12،14،16،18 جزء بالمليون من محلول نانو الفضة على التوالي، ربيت الافراخ الفاقسة من المعاملات اعلاه في حقل الطيور الداجنة التابع لقسم الإنتاج الحيواني في كلية الزراعة /جامعة القاسم الخضراء للمدة من 19 /8 /2017 لغاية 9/22 /2017، وعرضت الى درجة حرارة دورية (28–32–28 م°) واظهرت النتائج تبين وجود ارتفاع عالي المعنوية ($P\leq0.01$) للمعاملة T6 في طول الزغابات في الصائم و تفوقت المعاملات T4,T3 في عرض الزغابات بينما تفوقت جميع معاملات الحقن T6,T5,T4,T3 في عمق الخبايا مقارنة بمعاملتي السيطرة.

الكلمات المفتاحية: فروج اللحم، نانو الفضة، حقن البيض، الاجهاد الحراري.