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A comparative assessment of GPX-1 expression and histomorphometry evaluation in IBD vaccinated and supplemented broiler

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Abstract

This study was designed to investigate the bursa of Fabricius (BF) morphometric and effects of glutathione peroxidase enzyme (GPX-1) in infectious bursal disease vaccinated (IBDV) broiler supplemented with selenium-nanomaterials (Se-NPs). Ninety-six one-day-old of the Ross 308 broilers were assigned into E1, E2, E3, and E4 of 24 chicks each; those of E1 served as control, E2 vaccinated with an intermediate strain of IBD at 12 and 20 days old through the eye drop, E3 received a mix of Se-NPs and the vaccine, and E4 was supplemented with Se-NPs 0.3 mg/kg. At 19, 26, and 42 days of age, the diameters of the bursa of Fabricius were measured, and liver tissue was sampled to determine the reverse transcription polymerase chain reaction (RT-PCR) of the Glutathione peroxidase enzyme. Results indicated that group E4 (selenium-Nanoparticles group) significantly increased the bursal morphometric at 26 days old, and E3 increased the morphometric of the bursa Fabricius at 42 days old, as well as supplementation of Se-NPs significantly up-regulated RT-PCR of GPX-1 at 26 and 42 days old. The highest gene expression was in the selenium-nanoparticle group at 26 and 42 days old, in contrast to the other groups. Based on this finding, it can be concluded that nanomaterials improved the morphology of immune organs and enzyme peroxidase activity in vaccinated broilers with the Gumboro vaccine.

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Introduction

Infectious bursal disease (IBD) is a contagious disease that causes inflammation in the immunity organs, such as the spleen and bursa of Fabricius, and causes serious losses in the chicken industry, particularly in young birds (1,2). Due to the pathological and morphological changes in the bursa, this disease was later dubbed Infectious bursal disease (IBD); within 3-5 weeks of the rapid development of the bursa, the clinical form of the disease improves in infected birds, and the IBDV develops severely. The subclinical form occurs at less than three weeks of age, and the development of immunity in the acute and sub-clinical forms prevents adequate immune response to vaccinations and increases the possibility of secondary infection (3,4). Additionally, in terms of hygiene status, active and passive vaccination plays a major role in preventing bursal diseases (5). like mammals, the liver of birds is implicated in an array of homeostatic and metabolic functions and is deemed a biochemical factory accountable for most metabolism, synthesis, detoxification, and excretion processes; it plays a significant role in food digestion, metabolism production regulation, storage of lipids, and release of both proteins and carbohydrates (6). The liver of avian can be affected by diverse etiologic agents and conditions. Beyond the biological causes, some degenerative changes were detected, such as glycogenic, hydropic, fatty changes, and metabolic disorders. Such depositions are seen, for instance, urate, amyloidosis, and hemosiderosis (7-9), fatty liver hemorrhagic syndrome (10), circulatory disturbance (11), and neoplasms such as Cholangioma, hepatocellular adenoma, and cholangiocarcinoma were recorded as well (12,13).

Selenium is a nutrient that improves the productive performance in poultry production (14). It actively participates in diverse physiological functions, including antioxidant defense mechanisms, which are integrated into selenocysteine in combination with seleno-enzymes like glutathione peroxidase. In these regards, Se is added to the food of animals to improve their defense mechanism as antioxidants (15). Selenium's biological role is performed via glutathione peroxidase enzyme (GSH-Px). This enzyme, which, in its effective sites, implicates selenium together with superoxide dismutase, vitamin E, and catalase, participates in the cell membrane defense mechanism from damage of peroxidase (16). Nanomaterials are utilized in cellular, molecular biology, mineral nutrition biotechnology, and reproductive in both human and animal models (17-20). Nanomaterial is widely used in food and agricultural systems (21-23). The application of these materials in animal husbandry is very important (24,25). Amongst these materials, nano-Se has been attracted in the poultry industry due to its boosting bioavailability, catalytic effectiveness (26-28), capacity of absorption, and reducing toxic effects in comparison to the traditional sources of selenite. A few agents elaborate as antioxidant elements, the trace agents that are indirectly included in the demolition of the free radicals (17,18). A few elements, like selenium, zinc, and copper, are the basic deductive factors for the antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase (29,30). To counteract reactive oxygen species (ROS) effects, live organisms have developed a complex 3-line defense mechanism of antioxidants. The first line is the more efficient and includes antioxidant enzymes like catalase, glutathione, and superoxide dismutase (31-33).

The aim of this work is to detect the histopathological changes of vaccines and Se-NP on the bursa of Fabricius diameter and the molecular analysis of GPX-1 in the broiler liver.

Materials and methods

Ethical approval

The Ethical approval was agreed upon at the College of Veterinary Medicine-University of Mosul following the Animal Care Ethics Committee approval; the experimental protocol was used, numbered VET.2024-087, dated 15/11/2024.

Experimental design

Ninety-six one-day-old Ross 308 broilers were supplied with a source of heat, bedding, and ad libitum of food and water daily. According to their age, the birds were fed on pellets. They were divided randomly as follows: E1 was a control group and was supplemented with distilled water, E2 was vaccinated with 65PV-1 live intermediate strain of IBD at two-time points (12 and 20 days old) using the eye drop and the dose of vaccine used to follow the instructions of the manufacture and E3 were supplemented with a mix of Se-NPs and vaccinated with 65PV-1 live intermediate strain of IBD at two-time points (12 and 20 days old). E4 was supplemented with Se-NPs at 0.3mg/kg of body weight.

Birds were sacrificed at 19 26-day-olds, and on the last day of the experiment at 42 days, ten birds were sacrificed from each group: Liver and bursa of Fabricius were collected for conventional polymerase chain reaction (RT-PCR) and for micromorphological study, respectively. Gross morphology was done to detect any obvious macroscopic findings, particularly in the bursa of Fabricius. For histomorphometry, the collected tissue samples were preserved in 10% of formalin. Then, the samples were further treated and embedded in paraffin wax, sliced in 4-5 µm, and stained with routine stain (34). Follicles of the bursa, cortex, and medulla were measured under light microscope (35).

Collection and processing of liver tissue

Representative liver tissue samples, approximately 1-2 cm in size, were collected and immediately fixed in 10% neutral buffered formalin for proper preservation. The fixed tissues were then dehydrated through a graded series of alcohol concentrations and cleared using xylene. Subsequently, the samples were embedded in paraffin wax. Paraffin-embedded liver sections were cut into thin slices of 4-5 µm thickness using a rotary microtome. These sections were then mounted on glass slides and stained routinely with hematoxylin and eosin to assess the histological architecture (34).

RNA extraction from liver tissue

RNA was extracted from frozen liver tissue 50-100 mg following the protocol provided by Addbio (Korea). The sample was placed in a 1.5 ml microcentrifuge tube, to which 400 µl of lysis buffer, 4 µl of β-mercaptoethanol, and 20 µl of proteinase K solution (20 mg/ml) were added. Homogenization was performed using appropriate equipment, followed by vortexing to ensure complete mixing. The homogenized sample was incubated at 26ºC for 10 minutes and then centrifuged at 13,000 rpm for 3 minutes. The supernatant was carefully transferred to a spin column (white ring) seated in a 2.0 ml collection tube and centrifuged at 13,000 rpm for 30 seconds. The flow-through was retained.

To the collected flow-through, 400 µl of binding buffer was added and mixed by pulse vortexing for 10 seconds. The mixture was then centrifuged at 13,000 rpm for 1 minute. Approximately 500-600 µl of the resulting supernatant was transferred to a fresh microcentrifuge tube. An equal volume of binding buffer and 200 µl of absolute ethanol were added to this tube and mixed thoroughly. Subsequently, 600 µl of this lysate was loaded onto a second spin column (green ring) and placed in a 2.0 ml collection tube, ensuring the rim was kept dry. The sample was centrifuged for 10 seconds at

13,000 rpm. This step was repeated with the remaining lysate to ensure full processing.

Following binding, the column was washed with 500 µl of Washing Solution 1 and centrifuged for 10 seconds. After discarding the flow-through, the spin column was reassembled with a fresh collection tube and left at room temperature 20-30ºC) for 15 minutes. A second wash with 500 µl of Washing Solution 1 followed by centrifugation at 13,000 rpm for 1 minute was performed. Next, 700 µl of Washing Solution 2 was added and centrifuged under the same conditions. To eliminate residual ethanol, an additional centrifugation was carried out for 1 minute. Finally, the spin column was transferred to a clean 1.5 ml tube, and 50 µl of elution solution was applied. After allowing the solution to sit for at least one minute, RNA was eluted by centrifugation at 13,000 rpm for 1 minute and stored at -20ºC.

Conventional PCR for GPX1 gene detection

Complementary DNA (cDNA) synthesis was conducted using the AddScript cDNA synthesis kit (Addbio, Korea), which includes all necessary reagents for first-strand synthesis. The 20 µl reaction mixture contained 10 µl of the AddScript cDNA Master Mix, 5 µl of total RNA, 1 µl of oligo dT20 primers (10× random hexamer), and 4 µl of nuclease-free water. The reverse transcription was performed using the MiniAmp Plus™ Thermocycler (USA) under the following thermal profile: priming at 25ºC for 10 minutes, reverse transcription at 50ºC for 60 minutes, and enzyme inactivation at 80ºC for 5 minutes. The resulting cDNA was then subjected to PCR amplification using AddStart Taq DNA Polymerase (Addbio, Korea). Specific primers targeting the Glutathione Peroxidase 1 (GPX1) gene were used: forward primer 5′-CGGCTTCAAACCCAACTTCA-3′ and reverse primer 5′-AAGTTCCAGGAGACGTCGTT-3′, all synthesized by Macrogen (Korea). The PCR mixture (20 µl) consisted of 10 µl of the polymerase mix, 0.5 µl of each primer, 5 µl of cDNA template, and 4 µl of nuclease-free water. PCR was carried out for 40 cycles under these conditions: initial denaturation at 95ºC for 10 minutes, denaturation at 95ºC for 10 seconds, annealing at 61ºC for 10 seconds, and extension at 72ºC for 30 seconds, followed by a final extension at 72ºC for 7 minutes. Amplicons were analyzed via electrophoresis on a 1.5% agarose gel stained with gel-safe dye using equipment from Cleaver Scientific (UK). The target product size for GPX1 was 183 base pairs.

Gene expression analysis of GPX1 using qRT-PCR

Quantification of GPX1 gene expression in liver tissue was performed using the One RT-PCR SYBR Master Kit from Addbio (Korea), designed for both reverse transcription and real-time PCR in a single-step reaction. Primer sequences used for GPX1 were identical to those used in conventional PCR and were also synthesized by Macrogen (Korea). The 20 µl reaction mix included 10 µl of the SYBR Master Mix, 0.5 µl of each primer, 2 µl of RNA template, and 7 µl of nuclease-free water. According to the manufacturer's protocol, the reaction began with reverse transcription at 50ºC for 20 minutes, followed by denaturation at 95ºC for 10 minutes to both denature RNA hybrids and inactivate the reverse transcriptase. The real-time PCR was then conducted for 40 cycles using the following settings: denaturation at 95ºC for 15 seconds and annealing/extension at 61ºC for 60 seconds. Amplification and fluorescence detection were performed on the StepOnePlus™ Real-Time PCR system (USA).

Primers design

On the NCBI gene bank, we designed two sets of primers with 20 nucleotides for each primer we use, in which the GPX1 F: CGGCTTCAAACCCAACTTCA, GPX1 R: AAGTTCCAGGAGACGTCGTT (the primers were set at the flank of exon 4 of the GPX-1 gene). The total length of the product is 183 nucleotides, as demonstrated in the gel image. These two primers were specifically chosen because they are part of the mRNA sequence for gene expression. Upon rechecking across the entire genome, no misalignments were observed in any position, except for GPX1 in *Gallus gallus*. The gel also confirms the absence of any false or mismatched bands.

Statistical test

Analysis of the research data was done using the Windows software package SPSS (20.0 version; SPSS, USA, IL, Chicago). The significant means values were at $P<0.05$. Variations amongst the groups were analyzed by one-way ANOVA and Duncan's tests (36).

Results

Bursa of Fabricius diameter measurement

The results of the Bursa of Fabricius diameter at 19-day-old chicks (Table 1), the non-significant difference between the follicle length and width, cortex thickness, and Length of the medulla are shown between all four groups, and the microscopic morphology images showed non-significant variation changes at 19 days old (Figure 1).

A significant increase in the length of follicle 690.67±142.84 and medulla length 484.67±149.54 was observed in the E4 (Se-NPs) group at 27 days of the experiment, and non-significant variation was observed between E2 (Gumboro vaccinated) and E3 (Mix) groups as well as the follicle Width and cortex thickness did not show any significant between all groups at the same period (Table 2), bursa of Fabricius morphometric revealed a significant increase in the follicle and medulla length in E4 (Se-NPs) group (Figure 2).

A significant increase in the length of the Follicle and medulla was observed in E3 (Mix) group at 42 days old, 546.33±47.06, and 430±45.03 and non-significant difference

in the width of follicle and cortex thickness was observed between all groups and non-accounting difference in the medulla length between the E4 (Se-NPs) and E2 (Gumboro vaccinated) group 304.67±46.2 and 296±34.51 (Table 3). Morphometric revealed a significant increase in the follicle and medulla length in the fourth group (Figure 3).

Table 1: Bursa of Fabricius measurement at 19 days of age

Groups	Follicle length	Follicle width	Cortex thickness	Medulla length
E1 (control)	449±64.65a	235.67±51.47 a	81±17.58a	314.33±95.76a
E2 (Gumboro vaccinated)	324±73.14a	231±107.62 a	51.33±11.72a	201.33±74.19a
E3 (Mix)	442.33±116.38a	274.67±12.42 a	77.33±27.54a	347.67±100.83a
E4 (Se-NPs)	317.67±76.5a	245.33±55.22 a	67±12.77a	274±5.57a

The same letters within each column (vertically) represent no significant difference at a P value < 0.05.

Table 2: Bursa of Fabricius measurement at 27 days of age

Groups	Follicle length	Follicle width	Cortex thickness	Medulla length
E1 (control)	531.67±89.95ab	350.67±41.65a	76±28.16a	349±49.57ab
E2(Gumboro vaccinated)	338.67±109.56b	244±54.01a	60±5.57a	306.33±51.6b
E3 (Mix)	371±116.99b	348±130.29a	73±16.37a	290.67±36.67b
E4 (Se-NPs)	690.67±142.84a	340±15.72a	94.33±17.9a	484.67±149.54a

Different letters within each column (vertically) represent significant differences at P value < 0.05.

Table 3: Bursa of Fabricius measurement at 42 days of age

Groups	Follicle length	Follicle width	Cortex thickness	Medulla length
E1 (control)	506±39.04ab	321±13.89a	81.67±20.74a	355±70.87ab
E2 (Gumboro vaccinated)	427.67±32.19c	271±112.09a	59±8.54a	296±34.51b
E3 (Mix)	546.33±47.06a	328.67±57.1a	70.33±5.13a	430±45.03a
E4 (Se-NPs)	546.33±47.06a	328.67±57.1a	70.33±5.13a	430±45.03a

Different letters within each column (vertically) represent significant differences at P value < 0.05.

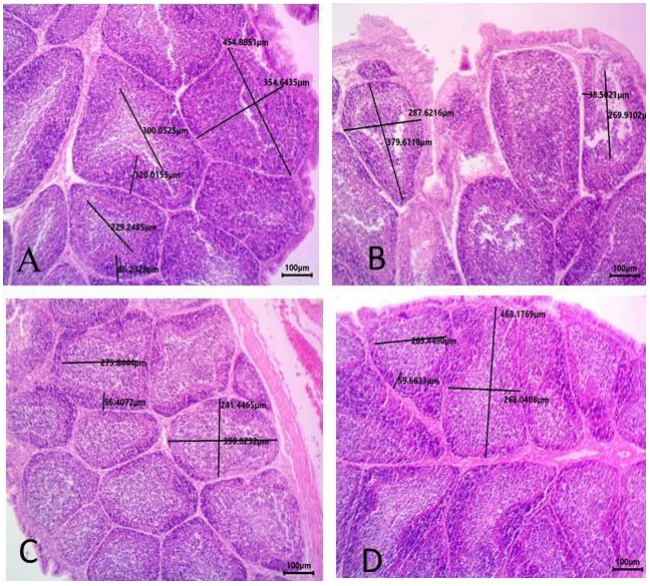

Figure 1: Bursa of Fabricius photomicrographs at 19 days old, A: E1 (Control), B: E2 (Gumboro vaccinated), C: E4 (Se-NPs), D: E3 (Mix), H&E,100nμ.

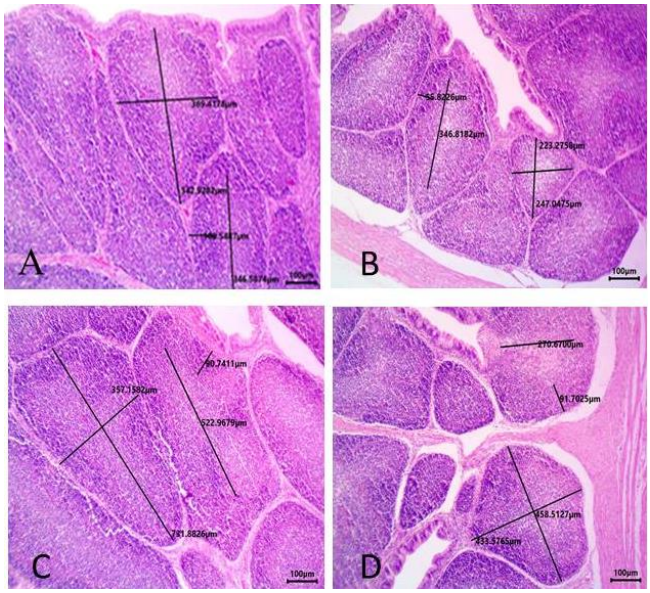

Figure 2: Bursa of Fabricius photomicrographs at 26 days old, A: E1 (Control), B: E2 (Gumboro vaccinated), C: E4 (Se-NPs), D: E3 (Mix), H&E,100nμ.

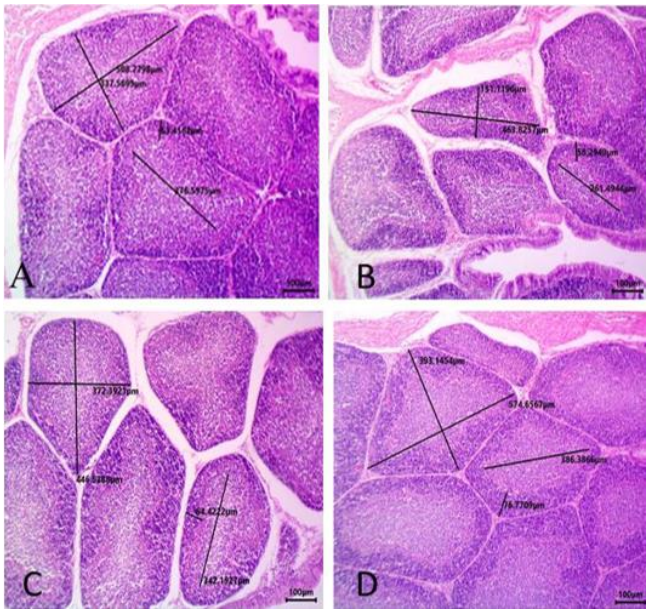

Figure 3: Bursa of Fabricius photomicrographs at 42 days old, A: E1 (Control), B: E2 (Gumboro vaccinated), C: E4 (Se-NPs), D: E3 (Mix), H&E, 100nμ.

Follicle length

The E4 (SE-NPs) group showed a lower percent value in the length of the follicle at 19 days old -29.25 compared to the control group; at 26 and 42 days old, the E2 (Gumboro vaccinated) group showed a lower percentage value in the follicle length, -36.3, and -15.48 respectively (Figure 4).

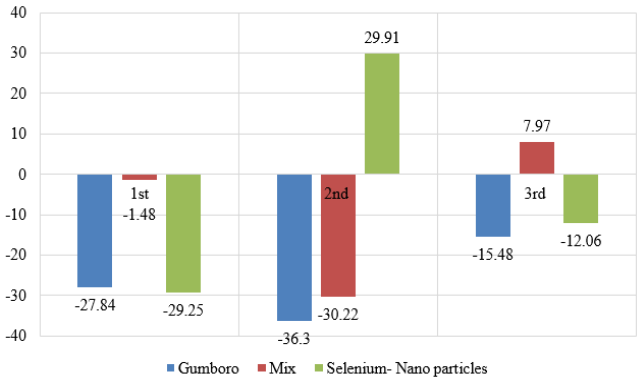

Figure 4: Percentage change compared to the control follicle length.

Follicle width

The follicle width percentage For the E3 (Mix) group showed a higher value in the follicle width in contrast to the control group at 19 days old at 16.55. However, the E2 (Gumboro vaccinated) group showed a higher change value at 26 and 42 days old, -30.42 and -15.58, respectively (Figure 5).

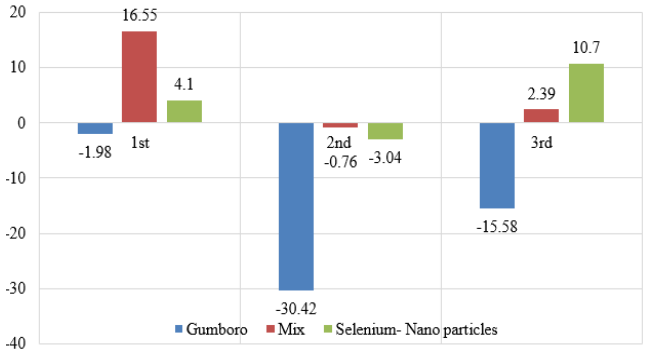

Figure 5: Percentage change compared to the control follicle width.

Cortex thickness

Figure 6 revealed a higher percentage change in the cortex thickness in the second group (E2) (Gumboro vaccinated), in contrast to the control group at 19 days old, which was -36.63 at 26 days old, the Se-NPs treated group (E4) showed a higher percentage value of 24.12, while the vaccinated group (E2) at 42 days old showed a lower cortex thickness percentage comparing to the control group -27.76.

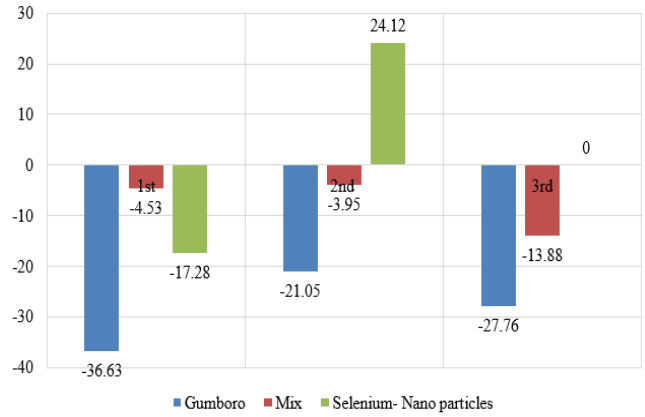

Figure 6: Percentage change compared to the control Cortex thickness.

Medulla length

As depicted in figure 7, the medulla length percentage change in the Se-NPs group (E4) was higher at 19 and 26 days old, at 29.25 and 38.87, respectively. The result showed a higher percentage of the medulla length in the mixed group (E3) at 42 days old, which was 21.13.

Detection of Se-NPs and Gumboro vaccine effects by RT-PCR

Out of 24 chicken liver samples tested with RT-PCR, figure 8 represents the product size we have done as per step to detect the gene expression of GPX-1; each number represents a different sample.

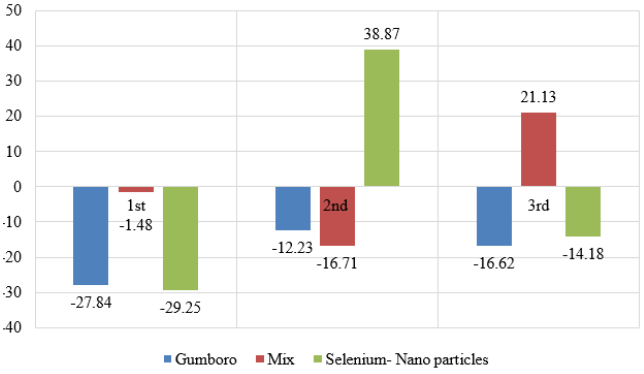

Figure 7: Percentage change compared to the control medulla length.

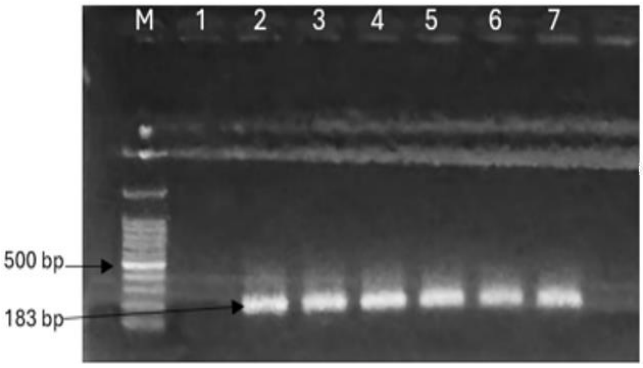

Figure 8: Gel electrophoresis of Glutathione Peroxidase 1 product with a molecular weight of 183 bp revealed that well No. 1 was negative, while wells 2-7 were positive for GPX1, and well M was for ladder 100bp.

The expression levels of GPx1 in liver tissue were assessed using real-time RT-PCR at three different time points: 19, 26, and 42 days of age. The amplification curves in figures 9, 10, and 11 show the relative fluorescence intensity (ΔRn) across PCR cycles for eight samples representing four experimental groups: control (samples 1 and 2), Gumboro-vaccinated (samples 3 and 4), mixed group (Gumboro vaccine + Se-NPs; samples 5 and 6), and Se-NPs only (samples 7 and 8). On day 19 (Figure 9), early amplification was observed in the Se-NPs group (E4) compared to the control group (E1), indicating an upregulation of GPx1 expression. By day 26 (Figure 10), this trend became more pronounced, with the mixed group (E3) and Se-NPs group (E4) showing higher fluorescence signals and earlier Ct values than both the control (E1) and vaccinated-only (E2) groups. At day 42 (Figure 11), the amplification curves of the Se-NPs and mixed groups (E3 and E4) remained elevated and shifted leftward, reflecting sustained higher GPx1 expression, whereas the control and Gumboro-vaccinated groups (E1 and E2) showed comparatively delayed amplification.

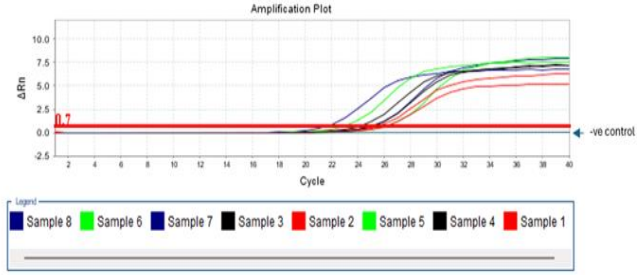

Figure 9: Real-time PCR amplification curves of tested liver samples for the Glutathione Peroxidase-1 at 19 days old using One Step RT- PCR SYBR® Green Master Mix Kit. ΔRn is the magnitude of the normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification.

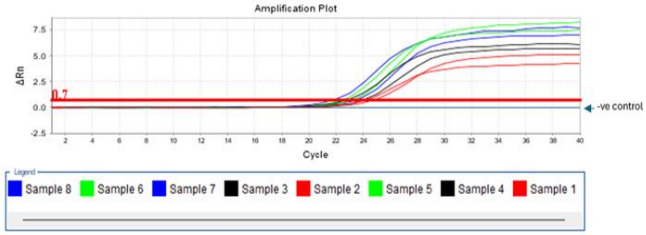

Figure 10: Real-time PCR amplification curves of tested liver samples for the Glutathione Peroxidase-1 at 26 days old using One Step RT- PCR SYBR® Green Master Mix Kit. ΔRn is the magnitude of the normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification.

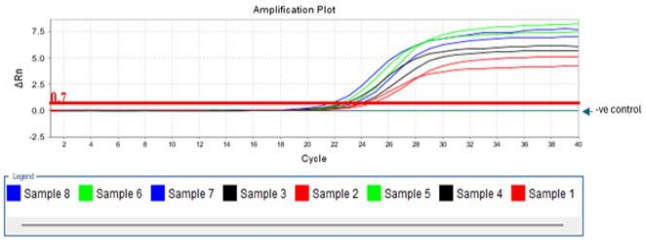

Figure 11: Real-time PCR amplification curves of tested liver samples for the Glutathione Peroxidase-1 at 42 days old using One Step RT- PCR SYBR® Green Master Mix Kit. ΔRn is the magnitude of the normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification.

As shown in table 4, the gene expression of GPX-1 for all sample collection periods showed the heights expressed 20.25 in the control group (E1) at 19 days old in contrast to the E2, E3, and E4 groups. At 26 and 42 days old, the last group (E4) showed the heights expressed 23.35 and 22.55 for the gene in contrast to the other groups.

Table 4: Cycle Threshold (CT) for GPX-1 gene expression in different liver samples for multiple sampling procedures

Sample	CT means 19 days old		CT means 26 days old		CT means at 42 days old	
Sample 1	20.4	20.25	26.1	25.80	24.1	24.45
Sample 2	20.1		25.5		24.8	
Sample 3	21.3	20.85	25.1	24.60	23.9	23.35
Sample 4	20.4		24.1		22.8	
Sample 5	21.0	20.70	22.9	24.50	23.1	22.70
Sample 6	20.4		26.1		22.3	
Sample 7	25.2	25.55	25.1	23.35	23.3	22.55
Sample 8	25.9		21.6		21.8	

CT: Cycle Threshold; samples 1-2: (E1: Control); samples 3-4: (E2: Gumboro vaccinated)); samples 5-6: (E3: Mix); samples 7-8: (E4: Se-NPs).

Discussion

The infectious bursal disease is one of the serious economic and epidemiological problems on a large production scale. The disease causes direct and indirect losses resulting from immunity suppression. The above excuses the systematic use of prophylaxis in poultry flocks against the disease (37). At all sampling periods, no significant difference was observed in the follicle length and width, cortex thickness, and medulla length in the vaccinated group (E2) in contrast to the other groups. This may be attributed to the (live attenuated vaccine) exhibiting poor immunity response even with the negative titer of ELISA at the first 3 weeks due to the neutralization remaining of the antibody vaccine activity (23,38), bird age, dose of the intermediate vaccine, experimental condition, periods of it and species of chickens (39,40).

Morphometric results revealed a significant increase in the follicle and medulla length in the groups supplemented with Se-NPs (E3 and E4) material when compared to the other groups; nano-material enhances immunity (41) and improves bursa lymphatic follicular area, size of the follicle, lymphocyte cell concentration inside bursa of Fabricia and the number of producing antibodies cell (42). Furthermore, the Se-NPs stimulate the follicles to produce several lymphocyte cells that help improve the bird's immune response (43). As an important trace element, selenium is essential for the antioxidant system synthesis (GPX-1), which blocks oxidative injury. The current study delineated the protective effects of Se-NPs in birds vaccinated with IBV. Liver enzyme expression helps to understand the functionality of the liver. The low enzyme level indicates the healthy function of the liver. Se-NPs increase the production of Glutathione peroxides (GPX1), which improves the anti-inflammatory responses, immunity statutes, repair of the DNA, and reinforces the resistance to diseases (44). The nanomaterial can reduce oxidative stress by eliminating the excessive production of free radicals and reducing stress indicators in animals, including birds. Selenium stimulates the immune system, improves bird productivity, and protects body cells and membranes from oxidative damage (45). The GPX-1 gene controls the production of enzymes, including glutathione peroxidase. Which protects body cells from damage free radicals (46). Sunde and Hadley (45) reported that selenium feed additives have significantly increased liver enzymes (GPX-1 and GPX-4) in male turkeys. In the present work, we found that the level of GPX-1 increased in the Se-NPs (E4) group. We can assume that Se-NPs promote anti-oxidant activity, which ultimately prevents hepatocytes from peroxidation of lipids and reduces the level of MDA (47,48). The protective role might be mediated by a specific type of selenoprotein, such as GPX. These proteins reduce the oxidative stress. Therefore, the current result concludes that the hepatocyte protective effects against vaccine stress may be related to selenium modulation up-regulation of GPX-1 of broiler chickens. No significant changes in the expression of GPX-1 in the liver tissue of the vaccinated bird suggest that our experimental results' slight differences may be due to the vaccination stress factor, which leads to decreased growth performance, antioxidant levels, and ROS amplified levels (49), oxidative stress impact specific liver enzymes, particularly ALT, AST and GSK that regulate the synthesis of glycogen (50,51), additionally to the doses and type of vaccine, point time and experimental condition (52,53).

Conclusion

The current study describes Se-NPs as nutritional material supplemented in chicken vaccinated with infectious bursal disease. We found that birds' administration of Se-NPs at 0.3 mg/kg had a good effect on the exhibition of the bursa of Fabricius morphometric immune response and increased GPX-1 RT-PCR expression in the liver tissue of these birds. However, the intermediate plus strain vaccine did not have a positive effect on the bursal morphometric and gene expression of GPX-1.

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Interest Collision

The authors proclaim no interest in conflicts.

Reference

1. Ateş MB, Özdemir Ö, Çelik Z, Salik R, Dağar O, Bulut A, et al. Histopathological evaluation of the effects of live Infectious bursal disease vaccine originated from WF2512 strain on bursa Fabricius in the broilers. J Adv VetBio Sci Tech. 2022;7(2):250-8. DOI: 10.31797/vetbio.1069954
2. Triyatjaya Y, Hermawan FA, Zega DN, Pratiwi U, Khairani S. Diagnosis of infectious bronchitis in a local chicken. Arshi Vet Lett. 2024;8(1):15-6. DOI: 10.29244/avl.8.1.15-16
3. Müller H, Mundt E, Eterradossi N, Islam MR. Current status of vaccines against infectious bursal disease. Avian Pathol. 2012;41(2):133-9. DOI: 10.1080/03079457.2012.661403
4. Niu X, Ding Y, Chen S, Gooneratne R, Ju X. Effect of immune stress on growth performance and immune functions of livestock: Mechanisms and prevention. Animals. 2022;12(7):909. DOI: 10.3390/ani12070909
5. Mohammed AR, Al-Mahmood SS. Effects of ProbChick® on the immunological response after new castle virus using LaSota stain vaccination in broiler. Iraqi J Vet Sci. 2023;37(1):17-32. DOI: 10.33899/ijvs.2022.133248.2193
6. Zaefarian F, Abdollahi MR, Cowieson A, Ravindran V. Avian liver: The forgotten organ. Animals. 2019;9(2):63. DOI: 10.3390/ani9020063
7. Lopes MC, Freitas Neto OC, Amaral CI, Lacerda MS, Fonseca CS, Martins NR, Ecco R. Hepatic changes in *Gallus gallus domesticus* in Brazil. Pesq Vet Bras. 2022 Jul 11;42:e07078. DOI: 10.1590/1678-5150-pvb-7078
8. Carnaccini S, Shivaprasad HL, Cutler G, Bland M, Meng XJ, Kenney SP, Bickford AA, Cooper G, Charlton B, Sentíes-Cué CG. Characterization of seven outbreaks of hemorrhagic hepatopathy syndrome in commercial pullets following the administration of a Salmonella Enteritidis bacterin in California. Avian Dis. 2016;60(1):33-42. DOI: 10.1637/11297-100915-reg.1
9. Crespo R, Shivaprasad HL. Developmental, metabolic, and other noninfectious disorders. In: Swayne DE, editor. Diseases of Poultry. John USA: Wiley & Sons, Inc; 2013. 1233-70 p. DOI: 10.1002/9781119421481.ch30
10. Trott KA, Giannitti F, Rimoldi G, Hill A, Woods L, Barr B, Anderson M, Mete A. Fatty liver hemorrhagic syndrome in the backyard chicken: a retrospective histopathologic case series. Vet Pathol. 2014;51(4):787-95. DOI: 10.1177/0300985813503569
11. Az Zahra S, Harlina E, Subangkit M, Wiranti RW, Priosoeryanto BP. Liver histopathology of broiler chicken (*Gallus domesticus*): A veterinary forensic model 48 h postmortem. Arshi Vet Lett. 2023;7(4):79-80. DOI: 10.29244/avl.7.4.79-80
12. He W, Tian X, Zhou Q, Lv J, Li Y, Jin C, Liu H, Yang H, Xu Y, He F, Liu T. Selenium nanoparticles ameliorate lumbar disc degeneration by restoring GPX1-mediated redox homeostasis and mitochondrial function of nucleus pulposus cells. J Nanobiotechnol. 2024;22(1):634. DOI: 10.1186/s12951-024-02890-x
13. Elnaggar A, Ghazalah A, ElSayed AH, Abdelalem A. Impact of selenium sources on productive and physiological performance of broilers. Egypt Poult Sci J. 2020;40(3):577-97. DOI: 10.21608/epsj.2020.112468
14. Basharat S, Tahir SK, Majeed KA, Yousaf MS, Hussain KK, Rashid MA. Effects of iron oxide nanoparticle supplementation on the growth performance, serum metabolites, meat quality, and jejunal basal morphology in broilers. Animals. 2023;14(1):99. DOI: 10.3390/ani14010099
15. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: Molecular pathways and physiological roles. Physiol Rev. 2014;94(3):739-77. DOI: 10.1152/physrev.00039.2013
16. Rahawi AM, Al-Taee SK, Ali FF, Altaey OY, Abdullah DA. Protective role of biosynthetic silver nanoparticles in broilers with aflatoxicosis through histopathological study of the spleen. Iraqi J Vet Sci. 2024;38(3):565-72. DOI: 10.33899/ijvs.2024.146024.3414
17. Jabori EE, Ismail HK, Khaleel LW, Flaih AN. Costus-loaded silver nanoparticles mitigated AMPK and related pathways in the albino rat atherosclerosis model. Iraqi J Vet Sci. 2024;38(1):77-87. DOI: 10.33899/ijvs.2023.140352.3039
18. Bader OA. Study the efficacy of silver nanoparticles in enhancing tracheal anastomosis healing in rabbit model. Iraqi J Vet Sci. 2024;38(4):941-8. DOI: 10.33899/ijvs.2024.152022.3788
19. Al-Shammari MS, Al-Saaidi JA. Influence of magnetic iron oxide nanoparticles in reproductive efficiency of adult male rats. Iraqi J Vet Sci. 2023;37(2):507-13. DOI: 10.33899/ijvs.2022.133978.2326
20. Taha AN, Ismail HK. The impact of nano zinc oxide particles on the histology of the male reproductive system of adult male rabbits. Iraqi J Vet Sci. 2023;37(1):105-13. DOI: 10.33899/ijvs.2022.133632.2270
21. Taha AN, Ismail HK. The amelioration of vitamin E on histological changes of rabbit's brain treated with zinc oxide nanoparticles. Iraqi J Vet Sci. 2023;37(1):95-104. DOI: 10.33899/ijvs.2022.133599.2265
22. Holovská K, Holovská K, Boldižárová K, Čekonová S, Lenártová V, Levkut M. Antioxidant enzyme activities in liver tissue of chickens fed diets supplemented with various forms and amounts of selenium. J Anim Feed Sci. 2003;12(1):143-52. DOI: 10.22358/jafs/67691/2003
23. Zhou Q, Chen W, Gu C, Liu H, Hu X, Deng L. Selenium-modified bone cement promotes osteoporotic bone defect repair in ovariectomized rats by restoring GPx1-mediated mitochondrial antioxidant functions. Regen Biomater. 2023;10. DOI: 10.1093/rb/rbad011
24. Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defense systems and oxidative stress in poultry biology. An update. Antioxidants. 2019;8(7):235. DOI: 10.3390/antiox8070235
25. Al-Fahady MQ, Hameed HM. Bioceutical role of nano and organic selenium on certain reproductive value of laying hen during force molting. Iraqi J Vet Sci. 2023;37(2):325-31. DOI: 10.33899/ijvs.2022.134401.2364
26. Jasim JY, Al-Taee SK. Evaluation of the role of green synthesis silver nanoparticles as adsorbents and protective agents for broilers tissue treated with aflatoxin. Iraqi J Vet Sci. 2023;37(3):675-81. DOI: 10.33899/ijvs.2023.136771.2614
27. Jabori EA, Ismail HK, Khaleel LW, Al-Hadidy AA. Hematological, biochemical, and histological alteration induced by nano silver material on male rats. Iraqi J Vet Sci. 2023;37(3):707-17. DOI: 10.33899/ijvs.2023.137239.2660
28. Oke OE, Akosile OA, Oni AI, Opowoye IO, Ishola CA, Adebiyi JO. Oxidative stress in poultry production. Poult Sci. 2024;103(9):104003. DOI: 10.1016/j.psj.2024.104003
29. El-Gogary MR, El-Khateeb AY, Megahed AM. Effect of physiological and chemical nano garlic supplementation on broiler chickens. Plant Arch. 2019;19(1):695-705. [available at]
30. Ammen MH, Wahhab MA, Muhammad SS, Salih SA. Impact of mixed dietary vitamin E-Selenium powder on reproductive hormones' concentration of males and females in Japanese quail bird (*Coturnix coturnix japonica*) Mesopotamia J Agric. 2023;51(3):99-108. DOI: 10.33899/magrj.2023.139953.1233
31. Huang Z, Aaron HR, Hoffmann PR. The role of selenium in inflammation and immunity: From molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2012;16:705-43. DOI: 10.1089/ars.2011.4145
32. Li Y, Wang K, Li C. Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. Antioxidants. 2024;13(11):1375. DOI: 10.3390/antiox13111375
33. Alkhashb A, Alhaji T, Thalij K. Effectiveness of Chitosan and Ag-Nanoparticle Films on the Quality of Chicken Meat. Mesopotamia J Agric. 2024;52(2):14-26. DOI: 10.33899/mja.2024.145729.0011337
34. Al-Sabaawy HB, Rahawi AM, Al-Mahmood SS. Standard techniques for formalin-fixed paraffin-embedded tissue: A pathologist's perspective. Iraqi J Vet Sci. 2021;35(I-III):127-35. DOI: 10.33899/ijvs.2021.131918.2023

35. Ayman U, Alam M, Das S. Age-related development and histomorphological observations of the bursa of Fabricius in Sonali chicken. J Adv Biotechnol Exp Ther. 2020;3(1):20. DOI: 10.5455/jabet.2020.d103
36. Running data analysis using SPSS. In: Statistical Methods in Psychiatry Research and SPSS. USA: CRC Press; 2014. 274-315 p. DOI: 10.1201/b17560-27
37. Tulu D. Epidemiology, status and economic importance of infectious bursal disease in poultry production, Ethiopia. Epidemiol Int J. 2019;3(3). DOI: 10.23880/eij-16000129
38. Al-Zebary MS, Isihak FA, Al-Jameel WH. Immune and immune-histochemical response for bivalent attenuated vaccines against Newcastle and infectious bronchitis diseases in broilers. Iraqi J Vet Sci. 2025;39(2):207-15. DOI: 10.33899/ijvs.2025.157260.4111
39. Sedeik ME, El-Shall NA, Awad AM, Abd El-Hack ME, Alowaimer AN, Swelum AA. Comparative evaluation of HVT-IBD vector, immune complex, and live IBD vaccines against vvIBDV in commercial broiler chickens with high maternally derived antibodies. Animals. 2019;9(3):72. [available at]
40. Roh JH, Kang M, Wei B, Yoon RH, Seo HS, Bahng JY, Kwon JT, Cha SY, Jang HK. Efficacy of HVT-IBD vector vaccine compared to attenuated live vaccine using in ovo vaccination against a Korean very virulent IBDV in commercial broiler chickens. Poult Sci. 2016;95(5):1020-4. DOI:10.3382/ps/pew042
41. Korzeniowska M, Madej JP, Stefaniak T, Kopec W. Influence of selenium on the morphology of immune system organs in healthy broilers. Acta Vet. 2019;69(4):379-90. DOI: 10.2478/acve-2019-0032
42. Abd El-Haliem HS, Attia FA, Saber HS, Hermes IH. Impacts of zinc oxide nano-particle supplementation in broiler diets on growth performance, some carcass characteristics, and immune organs. Egypt J Nutr Feeds. 2020;23(1):3-122. DOI: 10.21608/ejnf.2020.95825
43. Al-Haideri DH, Al-Timmemi HA. Efficacy of chitosan nanoparticles and mesenchymal stem cells in rabbit models for sciatic nerve regeneration. Iraqi J Vet Sci. 2024;38(2):369-77. DOI: 10.33899/ijvs.2023.142572.3186
44. Abdelsalam M, Al-Homidan I, Ebeid T, Abou-Emera O, Mostafa M, Abd El-Razik M, Shehab-El-Deen M, Abdel Ghani S, Fathi M. Effect of silver nanoparticle administration on productive performance, blood parameters, antioxidative status, and silver residues in growing rabbits under hot climate. Animals. 2019;9(10):845. DOI: 10.3390/ani9100845
45. Hefnawy AEG, Tórtora Pérez JL. The importance of selenium and the effects of its deficiency in animal health. Small Rumin. Res .2010;89(2-3):185-92. DOI. :10.1016/j.smallrumres.2009.12.042
46. Sunde RA, Hadley KB. Phospholipid hydroperoxide glutathione peroxidase (Gpx4) is highly regulated in male turkey poults and can be used to determine dietary selenium requirements. Exp Biol Med. 2010;235:23. DOI: 10.1258/ebm.2009.009262
47. Han D, Hanawa N, Saberi B, Kaplowitz N. Mechanisms of liver injury. III. Role of glutathione redox status in liver injury. Am J Physiol Gastrointest Liver Physiol. 2006;291(1):G1-7. DOI: 10.1152/ajpgi.00001.2006
48. Zhang L, Zhou J, Obianwuna UE, Long C, Qiu K, Zhang H, Qi X, Wu S. Optimizing selenium-enriched yeast supplementation in laying hens: Enhancing egg quality, selenium concentration in eggs, antioxidant defense, and liver health. Poult Sci. 2025;104(1):104584. DOI: 10.1016/j.psj.2024.104584
49. Abbas AO, Alaqil AA, Mehaisen GMK, El Sabry MI. Effect of organic selenium-enriched yeast on relieving the deterioration of layer performance, immune function, and physiological indicators induced by heat stress. Front Vet Sci. 2022;9. DOI: 10.3389/fvets.2022.880790
50. Maty HN, Hameed HM, Hassan AA. Potency of nano-zinc oxide on caspase-3 of male quail exposed to lipopolysaccharide. Iraqi J Vet Sci. 2024;38(1):163-71. DOI: 10.33899/ijvs.2023.141585.3121
51. Li Y, Wang K, Li C. Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. Antioxidants. 2024;13(11):1375. DOI: 10.3390/antiox13111375
52. Hafez HM, Attia YA. Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. Front Vet Sci. 2020;7. DOI: 10.3389/fvets.2020.00516
53. Wang X, Liu X, Liu S, Qu J, Ye M, Wang J. Effects of anti-stress agents on the growth performance and immune function in broiler chickens with vaccination-induced stress. Avian Pathol. 2022;52(1):12-24. DOI: 10.1080/03079457.2022.2114874

التقييم المقارن لتعبير إنزيم الكلوتاثيون بيروكسديز والتقييم الهيستومورفومتري في دجاج التسمين الملقح والمكمل

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الخلاصة

تم تصميم هذا البحث للتحقق في مورفولوجية جراب فابريشيا وتفاعل البلمرة المتسلسل لأنزيم الكلوتاثيون بيروكسديز في الدجاج الملقح بلقاح الكمبورو والمعامل بمادة السلينيوم النانوية، تم تقسيم ستة وتسعون دجاجة نوع روس الى أربعة مجاميع متمثلة بالمجموعة الأولى والثانية والثالثة والرابعة. المجموعة الأولى عوملت كمجموعة سيطرة، المجموعة الثانية تم تطعيمها بلقاح الكمبورو في اليوم الثاني عشر والتاسع عشر من عمر الدجاج بواسطة قطرة العين. وعوملت أفراخ المجموعة الثالثة بمادة السلينيوم النانوية بالإضافة الى تطعيمها باللقاح. أما أفراخ المجموعة الرابعة فعوملت بمادة السلينيوم النانوية فقط. في اليوم التاسع عشر والسابع والعشرون وفي اليوم الثاني والأربعون من عمر الأفراخ، تم اخذ عينات جراب فابريشيا للقياسات المورفولوجية واخذ عينات من أنسجة الكبد لتفاعل البلمرة المتسلسل. بينت النتائج الارتفاع المعنوي في قياسات جراب فابريشيا في المجموعة المعاملة بالسلينيوم النانوية عند اليوم ٢٦ من عمر الافراخ وفي المجموعة المعاملة بالمزيج عند اليوم ٤٢ من عمر الافراخ واعلى تعبير جيني في المجموعة المعاملة بالمادة النانوية وفي اليوم ٢٦ و ٤٢ من عمر الأفراخ بالمقارنة ببقية المجاميع. نستنتج من نتائج الدراسة بان جزئيات السلينيوم النانوية قد حسنت من مورفولوجية الأعضاء المناعية وتفاعل إنزيم الكلوتاثيون بيروكسديز في الدجاج اللاحم والملقح بلقاح الكمبورو.