Clinicopathological Studies on the Impact of Nano Selenium Particles on the Growths and Some Biochemical Tests in *E-Coli* Experimentally Infected Broilers

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Abstract

This scientific investigation aimed to explore the impact of nano-selenium (nano-Se) supplementation on growth parameters and specific biochemical indicators in broiler chickens. The study proceeded to evaluate the impact of the limited nano-selenium supplementation (0.3 and 0.5 milliliter/l in water) and the *Escherichia coli* challenge on the experimental groups. The control group exhibited condensed body weight, body weight gain, and feed intake, accompanied by an augmented feed conversion rate (FCR). In contrast, the groups challenged with the pathogen and supplemented with nano-Se displayed improvement on weight gain, and FCR than the challenged non-treated group. These results indicated that nano-Se supplementation can effectively mitigate the detrimental impact of the pathogen on the growths of broiler chickens.

Leukogram analysis showed that *E. coli* infection increased total leukocyte count (TLC) and heterophil count on the 7th day, while on the 21st day, there was an increase in TLC and lymphocyte count. In contrast, the challenged groups supplemented with nano-Se showed reduced TLC and increased lymphocyte count on the 7th day and reduced TLC with increased heterophil count on the 21st day compared with the infected group. Regarding serum biochemical parameters, the infected non-treated group had elevated levels of liver enzymes. However, the challenged groups and supplemented with nano-Se showed decreased liver enzymes levels than the infected group. *Escherichia. coli* infection led to decreased total protein, albumin, and A/G ratio, along with increased globulin concentration. In addition, the challenged groups that received nano-selenium (nano-Se) treatment demonstrated elevated levels of the protein level, and albumin/globulin ratio, while the impact on globulin concentration varied among the groups. Furthermore, *E. coli* infection resulted in decreased serum glucose levels; imply that the nano-Se

supplementation in the challenged group enhanced the immune response compared with the infected group that did not receive any treatment. The challenged group also displayed hypercholesterolemia, which was lowered in the groups challenged and supplemented by nano-Se. Finally, *E. coli* infection increased uric acid levels, which were reduced in the challenged groups supplemented with nano-Se. In conclusion, nano-selenium supplementation had positive effects on growth parameters and various biochemical tests in chickens challenged with *E. coli.*

Keywords: *E. coli, nano-selenium, TLC, ALT, AST, Growth performance and FCR.*

Introduction

Selenium is one of the important elements that can help microbiota to complete its action within the gut ***(Yoon et al., 2007).*** Selenium positively affects feed utilization through participation in the metabolism of carbohydrates, lipids, and proteins ***(Stapleton, 2000; Attia et al., 2010 and Tufarelli et al., 2016).***

In the recent period, the poultry industry has seen several technologies, including nanotechnology, which are the study of materials at the nano-scale, where the size of particles is between 1-100 nm ***(Jiang et al., 2008 and Albanese et al., 2012).***

The major application of nanotechnology in poultry involves use of nano mineral elements, which can reduce any antagonistic behavior which is typically seen in traditional inorganic minerals in the gastrointestinal tract and to improve bioavailability ***(Gopi et al., 2017)*** and lower effective doses. Moreover, higher bioavailability and better utilization of trace minerals can have a desirable effect on metabolism while reducing mineral excretion into the environment ***(Surai et al., 2017)***. The utilization of nano-sized selenium (nano-Se) as a supplement shows great promise as an alternative to traditional inorganic sources. This is because nano-Se effectively masks the undesirable taste and odor of feed, possesses improved solubility, has a longer residence time, and exhibits enhanced bioavailability in the gastrointestinal tract of animals ***(Chen et al., 2006).*** Nano-Selenium has emerged as a potential dietary addition for broilers due to its low toxicity, high catalytic efficiency, and antibacterial properties ***(Wadhwani et al., 2016 and Skalickova et al., 2017).***

The poultry industry currently faces numerous challenges, with avian colibacillosis being a prominent concern. Avian colibacillosis, a contagious ailment, is primarily induced by the pathogenic bacterium *Escherichia coli*, and leading to mortality among birds. This ailment is responsible for

significant economic burden on the industry, as it can either act as a primary pathogen or contribute to various disease conditions as a secondary pathogen ***(Kabir, 2010).***

Material and methods

1-Chicks:

One hundred fifty-one-day old cobb broiler chicks, each weighting between 45 to 50 grams, were acquired from Ismailia/Misr Poultry Company, Ismailia city, Egypt. The chicks were housed in ground litters and divided into 6 groups at random, 25 birds per group. They were brought up for 35 days (5 wks.), during which they were permitted to eat and drink at any time. The formulation of the diet carried out to meet the nutritional requirements as recommended by the National Research Council ***(NRC, 1994)***.

At the 7^{th}, 14^{th} and 18^{th} day of age, the birds were vaccinated as described by ***Giambrone and Ronald (1986)***.

2- Nano-Selenium:

The Selenium nanoparticles (nano-se) were prepared according to ***(Ali et al.,***

2020). The Selenium powder was employed as a precursor for the nano-se and

Polyvinyl Alcohol (PVA) acted as a capping agent for the NPS to inhibit aggregation. At the same time, glucose serves as a reducing agent. Firstly, an

aqueous solution of Na_2SO_3 and selenium powder were mixed and refluxed under heating at 70 ºC for six h to create sodium seleno-sulfate solution. After that, the refluxed solution was filtered by filter paper to withdraw the unreacted materials. Then, one % PVA powder was added, followed by glucose powder (6%). The refluxing was continued again for another 6 hours. The solution color was changed from colorless to pale yellow, indicating the formation of the nano-se.

3- Experimental design

One hundred fifty-one-day old broiler chickens were subjected to an experimental design involving six distinct groups: Group 1 (G1): Control group; Group 2 (G2): Chicks received a 0.3 milliliter/L nano-selenium supplement in their water; Group 3 (G3): Chicks received a 0.5 milliliter /L nano-selenium supplement in their water; Group 4 (G4): Chicks were challenged with *Escherichia coli* at a concentration of 2×10^7 (I/N); Group 5 (G5): Chicks received a 0.3 milliliter /L nano-selenium supplement combined with an *Escherichia coli* challenge (I/N); Group 6 (G6): Chicks received a 0.5 milliliter /L nano-selenium supplement combined with an *Escherichia coli* challenge (I/N).

At 14 days of age, chicks in (G4, G5 and G6) were challenged with 2×10^7 (CFU) of *E. coli* via the intranasal route (I/N) using a 0.5 ml dosage ***(Peighambari et al., 2000)***.

4- Growth parameters:

The total body weight and weight gain, feed intake and "Feed

conversion ratio (FCR)" were established.

5- Blood samples:

The first sample: sterile tube containing the anticoagulant (EDTA) were used to aseptically collect a blood samples, which were specifically employed for the evaluation of leukogram studies.

The second sample: they were collected from wing vein and sera were then separated and preserved in -20º C until the biochemical tests were determined according to ***(Brady, 1968).***

6- Leukogram parameters: The total count of leucocytes and differential leucocytes count were determined following standard techniques described by ***Jain (1986)*** and ***Terry (1988)***.

7- Determination of biochemical tests:

The activity of alanine aminotransferase (ALT) and aspartic aminotransferase (AST) were measured. Additionally, uric acid, total cholesterol, glucose, total protein and albumin concentrations were determined. These parameters were analyzed following the instructions provided by the manufacturer (CUSABIO BIOTECH CO. Ltd., Houston, TX 77054, USA).

8- Statistical analysis:

The data obtained from the all groups were subjected to statistical analysis. The SPSS© (10) software was used to calculate mean values and standard errors according to ***Snedecor and Cochran, (1989***). Subsequently, Duncan multiple comparison tests were employed for post-hoc analysis to identify specific group differences.

Results

Table (1): *The impact of 2 concentrations of nano-selenium on the average live body weight (in grams) was assessed in in both control and E. coli experimentally-challenged chicks (n=25)*

Group	1 day	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.
G1	48.20 ±0.97^{a}	208.00 ±8.00^{a}	490.00 ±18.70^{a}	1150.00 ±31.60^{b}	1660.00 ±33.20^{b}	2170.00 ±40.60^{b}
G2	48.80 ±0.37^{a}	200.00 ±6.78^{a}	508.00 ±17.70^{a}	1180.00 ±25.50^{b}	1684.00 ±24.60^{ab}	2200.00 ±27.40^{a}
G3	48.80 ±0.58^{a}	208.00 ±6.63^{a}	506.00 ±16.30^{a}	1260.00 ±18.70^{a}	1746.00 ±12.90^{a}	2270.00 ±20.00^{a}
G4	48.20 ±0.97^{a}	208.00 ±6.32^{a}	490.00 ±18.70^{a}	822.00 ±10.20^{d}	1090.00 ±18.70^{e}	1424.00 ±14.40^{e}
G5	48.80 ±0.37^{a}	200.00 ±6.78^{a}	508.00 ±17.70^{a}	962.00 ±17.70^{c}	1322.00 ±13.60^{d}	1824.00 ±18.60^{d}
G6	48.80 ±0.87^{a}	208.00 ±6.63^{a}	512.00 ±18.50^{a}	980.00 ± 9.49^{c}	1398.00 ±21.30^{c}	1916.00 ±23.80^{c}

Groups with different letters are considered to have statistically significant differences within the same column.

Table (2): *The impact of 2 concentrations of nano-selenium on mean weight gain (g) was assessed in both control and E. coli experimentally-challenged chicks (n=25)*

Group	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.
G1	159.80 ±5.54[a]	282.00 ±18.50[a]	660.00 ±29.20[b]	510.0 ±18.70[a]	510.00 ±12.20[a]
G2	151.20 ±5.30[a]	308.00 ±14.60[a]	672.00 ±37.20[b]	504.0 ±12.90[a]	516.00 ±16.00[a]
G3	159.20 ±5.88[a]	298.00 ±15.70[a]	754.00 ±29.10[a]	486.00 ±18.60[a]	524.00 ±11.20[a]
G4	159.80 ±5.54[a]	282.00 ±18.30[a]	332.00 ±25.40[d]	268.00 ±22.20[c]	334.00 ±9.270[c]
G5	151.20 ±5.30[a]	308.00 ±14.60[a]	454.00 ±13.30[c]	360.00 ±26.10[b]	502.00 ±15.90[b]
G6	159.20 ±5.88[a]	304.0 ±18.20[a]	468.0 ±15.60[c]	418.00 ±30.60[b]	518.00 ±9.17[a]

Groups with different letters are considered to have statistically significant differences within the same column.

Table (3): *The impact of 2 concentrations of nano-selenium on feed intake (g) was assessed in both control and E. coli experimentally-challenged chicks(n=25).*

Group	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.
G1	123.04 ±2.86[a]	148.00 ±4.59[a]	626.00 ±4.30[a]	835.40 ±19.90[a]	873.80 ±18.80[ab]
G2	125.49 ±2.00[a]	148.20 ±4.83[a]	616.00 ±7.48[a]	833.80 ±21.6[a]	890.00 ±2.32[a]
G3	125.76 ±2.53[a]	152.00 ±4.64[a]	622.00 ±11.6[a]	740.00 ±12.20[b]	871.80 ±4.82[ab]
G4	123.04 ±1.94[a]	148.00 ±4.59[a]	416.40 ±9.22[c]	639.00 ±7.14[c]	741.60 ±11.90[c]
G5	125.49 ±2.21[a]	148.20 ±4.83[a]	525.40 ±9.55[b]	728.80 ±8.91[b]	840.40 ±2.58[ab]
G6	125.76 ±1.97[a]	152.00 ±4.64[a]	522.00 ±5.83[b]	729.80 ±17.5[b]	827.80 ±12.00[b]

Groups with different letters are considered to have statistically significant differences within the same column**.**

Table (4): *The impact of 2 concentrations of nano-selenium on feed conversion ratio was assessed in both control and E. coli experimentally-challenged chicks(n=25).*

Group	1st wk.	2nd wk.	3rd wk.	4th wk.	5th wk.
G1	0.77 ±0.02^{a}	0.52 ±0.03^{a}	0.95 ±0.05^{c}	1.64 ±0.06^{c}	1.71 ±0.03^{b}
G2	0.83 ±0.05^{a}	0.48 ±0.02^{a}	0.92 ±0.08^{c}	1.65 ±0.06^{c}	1.72 ±0.10^{b}
G3	0.79 ±0.03^{a}	0.51 ±0.03^{a}	0.82 ±0.08^{c}	1.52 ±0.07^{c}	1.66 ±0.02^{bc}
G4	0.76 ±0.02^{a}	0.52 ±0.03^{a}	1.25 ±0.06^{a}	2.38 ±0.23^{a}	2.22 ±0.06^{a}
G5	0.83 ±0.05^{a}	0.48 ±0.03^{a}	1.16 ±0.02^{b}	2.02 ±0.12^{b}	1.67 ±0.05^{bc}
G6	0.79 ±0.03^{a}	0.50 ±0.03^{a}	1.12 ±0.07^{b}	1.75 ±0.14^{bc}	1.60 ±0.03^{bc}

Groups with different letters are considered to have statistically significant differences within the same column.

Table (5): *the impact of 2 concentrations of nano-selenium on leukogram was assessed in both control and E. coli experimentally-challenged chicks(n=5) at 3 weeks of age.*

Group	TLC (×10^{3}/µL)	Heterophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
G1	32.67 ±0.76^{c}	36.31 ±0.56^{c}	55.70 ±0.76^{a}	4.66 ±0.42^{c}	3.33 ±0.21^{c}
G2	34.33 ±0.42^{c}	36.90 ±0.63^{c}	55.33 ±0.42^{a}	4.77 ±0.21^{c}	3.00 ±0.36^{c}
G3	33.00 ±0.63^{c}	37.40 ±1.59^{c}	55.00 ±0.76^{a}	4.60 ±0.36^{c}	3.00 ±0.21^{c}
G4	45.67 ±1.12^{a}	56.17 ±0.83^{a}	26.67 ±1.17^{c}	9.16 ±0.37^{a}	8.00 ±0.37^{a}
G5	39.67 ±0.92^{b}	49.33 ±0.97^{b}	39.67 ±0.21^{b}	6.00 ±0.36^{b}	5.00 ±0.36^{b}
G6	38.00 ±0.73^{b}	48.67 ±0.21^{b}	40.99 ±0.76 ^{b}	5.67 ±0.21^{b}	4.67 ±0.21^{b}

Groups with different letters are considered to have statistically significant differences within the same column.

Table (6): *The impact of 2 concentrations of nano-selenium on leukogram was assessed in both control and E. coli experimentally-challenged chicks(n=5) at 5 weeks of age.*

Group	TLC ($\times 10^3/\mu L$)	Heterophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
G1	56.67 ±0.92^{c}	45.00 ±0.97^{a}	49.00 ±0.63^{c}	3.00 ±0.37^{c}	3.00 ±0.36^{b}
G2	61.00 ±1.93^{b}	43.00 ±0.97^{a}	51.00 ±0.21^{b}	3.00 ±0.36^{c}	3.00 ±0.21^{b}
G3	62.67 ±3.31^{b}	42.33 ±0.21^{a}	51.67 ±0.21^{b}	2.67 ±0.21^{c}	3.33 ±0.21^{b}
G4	69.00 ±1.59^{a}	27.67 ±2.69^{b}	57.33 ±2.56^{a}	10.33 ±0.42^{a}	4.67 ±0.21^{a}
G5	62.33 ±2.64^{b}	39.33 ±0.42^{a}	52.00 ±0.56^{b}	4.67 ±0.21^{b}	4.00 ±0.36^{a}
G6	62.00 ±0.97^{b}	40.00 ±0.97^{a}	51.00 ±0.63^{b}	5.00 ±0.21^{b}	4.00 ±0.37^{a}

Groups with different letters are considered to have statistically significant differences within the same column.

Table (7): *The impact of 2 concentrations of nano-selenium some on serum biochemical parameters in both control and E. coli experimentally-challenged chicks(n=5) at 3 weeks of age.*

Group	ALT (U/L)	AST (U/L)	T.protein (g/dl)	Alb. (g/dl)	Glob. (g/dl)	A/G. ratio	Gluc. (mg/dl)	Cholest. (mg/dl)	Uric A (mg/dl)
G1	19.26 ±1.78^{c}	144.65 ±4.19^{d}	2.42 ±0.04^{b}	1.29 ±0.002^{a}	1.13 ±0.03^{a}	1.14 ±0.04^{a}	338.60 ±12.2^{a}	156.80 ±8.83^{cd}	6.13 ±0.02^{c}
G2	20.785 ±0.79^{bc}	162.2 ±5.20^{cd}	2.52 ±0.043^{a}	1.35 ±0.029^{a}	1.17 ±0.014^{a}	1.15 ±0.013^{a}	321.60 ±3.23^{ab}	164.85 ±4.42^{bc}	6.10 ±0.01^{c}
G3	22.75 ±1.75^{bc}	172.2 ±11.3^{c}	2.55 ±0.02^{a}	1.37 ±0.01^{a}	1.18 ±0.01^{a}	1.16 ±0.02^{a}	337.80 ±11.3^{a}	145.75 ±6.32^{d}	6.12 ±0.09^{c}
G4	37.03 ±1.03^{a}	304.85 ±5.63^{a}	1.89 ±0.003^{d}	0.81 ±0.04^{d}	1.08 ±0.05^{a}	0.75 ±0.003^{c}	297.25 ±1.41^{c}	194.40 ±0.34^{a}	7.61 ±0.01^{a}
G5	24.29 ±0.77^{b}	210.00 ±5.77^{b}	2.12 ±0.008^{c}	1.03 ±0.017^{c}	1.09 ± 0.05^{a}	0.94 ±0.07^{b}	310.50 ±2.08^{bc}	173.85 ±0.95^{b}	7.15 ±0.08^{b}
G6	24.56 ±0.76^{b}	210.50 ±4.33^{b}	2.22 ±0.014^{c}	1.14 ±0.02^{b}	1.08 ±0.009^{a}	1.05 ±0.01^{b}	301.40 ±7.10^{bc}	174.6 ±2.14^{b}	7.26 ±0.04^{b}

Groups with different letters are considered to have statistically significant differences within the same column.

Table (8): *The impact of 2 concentrations of nano-selenium on some serum biochemical parameters in both control and and E. coli experimentally-challenged chicks (n=5) at 5 weeks of age.*

Group	ALT (U/L)	AST (U/L)	T.protein (g/dl)	Alb. (g/dl)	Glob. (g/dl)	A/G. ratio	Gluc. (mg/dl)	Cholest. (mg/dl)	Uric A (mg/dl)
G1	4.71 ±0.84^{c}	136.35 ±7.71^{c}	2.75 ±0.02^{b}	1.58 ±0.02^{b}	1.17^{c} ±0.01	1.35 ±0.002^{a}	283.9 ±12.7^{a}	146.60 ±6.35^{b}	6.52 ±0.09^{b}
G2	6.27 ±1.54^{bc}	117.75 ±5.05^{c}	2.87 ±0.04^{a}	1.60 ±0.03^{b}	1.27 ±0.01^{a}	1.25 ±0.01^{c}	288.55 ±5.92^{a}	147.60 ±10.5^{b}	6.51 ±0.02^{b}
G3	6.70 ±1.52^{bc}	120.35 ±1.47^{c}	2.91 ±0.02^{a}	1.66 ±0.02^{a}	1.25 ±0.01^{ab}	1.32 ±0.01^{b}	284.6 ±30.4^{a}	147.10 ±12.0^{b}	6.52 ±0.04^{b}
G4	13.81 ±0.618^{a}	258.4 ±11.1^{a}	2.40 ±0.02^{d}	1.18 ±0.01^{e}	1.22 ±0.01^{b}	0.96 ±0.002^{f}	241.7 ±16.3^{b}	168.55 ±5.46^{a}	7.32 ±0.14^{a}
G5	8.52 ±0.02^{b}	208.25 ±2.86^{b}	2.59 ±0.03^{c}	1.34 ±0.01^{d}	1.25 ±0.02^{ab}	1.07 ±0.01^{e}	289.5 ±15.3^{a}	143.30 ±8.26^{b}	6.62 ±0.26^{b}
G6	8.20 ±0.18^{b}	215.45 ±4.36^{b}	2.74 ±0.03^{b}	1.46 ±0.01^{c}	1.28 ±0.01^{a}	1.14 ±0.01^{d}	285.9 ±14.0^{a}	145.20 ±5.14^{b}	6.66 ±0.02^{b}

Groups with different letters are considered to have statistically significant Groups with different letters are considered to have statistically significant differences within the same column

Discussion

Based on the results of growth performance parameters (Tables 1, 2, 3 & 4), the group challenged by *E. coli* (G4) exhibited a substantial decline in body weight, body weight gain, and feed intake contrasted to control. Additionally, there was a major increase in the feed conversion ratio (FCR). This decrease in growth can potentially be attributed to factors such as the production of toxins, the utilization of essential nutrients by the host, or the suppression of microbes responsible for synthesizing vitamins and other growth factors necessary for the host's development. The outcomes of this investigation align with the findings reported by ***Russell (2003)*** and ***Ask et al. (2006)*** which indicated the deleterious effects of colibacillosis on growth performance and general well-being. The key problem identified was growth retardation, which was accompanied by a decrease in appetite and consequently reduction in feed intake. In contrast, the nano-se infected groups (G5 and G6) exhibited an increase in “the total body weight and gain”, and a notable lower in feed conversion ratio than the infected group. Due to the participation of selenium in the expression of selenoprotein P and selenoenzymes, which are essential for the manufacture of hormones of thyroid gland and selenium transfer ***(Zhan et al., 2014 and Belal et al., 2021).*** Therefore, these results indicate that the enhanced growth performance may be attributed to increased levels of thyroid hormones, which regulate the body's energy metabolism, as well as improved protein digestibility ***(Saleh, 2014).*** Nano-se supplemented groups (G2 and G3)

revealed a major raise in body heaviness with non-significant difference in body weight gain, feed eating and feed conversion ratio in contrast to control at 5th week and, throughout the experimental period, no detrimental impact on the growth of the chickens was determined due to nano-se supplementation. These consequences came in parallel with ***Cai et al. (2012)*** and ***Mahmoud et al. (2016)*** investigated that, the non-changes in weight grow, feed eating and FCR in broilers fed diets supplemented by 0.3 mg nano-se per kg diet as contrasted to control. In contrast to ***Selim et al., (2015)*** showed that, the body weight gain and FCR improved than control in broilers supplemented with nano-selenim (0.30 ppm) in broiler feeds or in water.

Avian leukocytes serve as the primary defense mechanism against invading microorganisms ***(Powell, 1987)***. In gallinaceous birds, heterophils, the predominant granulated leukocytes, play a vital role in the acute inflammatory response. They possess highly phagocytic capabilities and exhibit a wide range of antimicrobial activity ***(Barry, 1998)***. Lymphocytes, on the other hand, are the predominant leukocytes found in the peripheral blood of healthiest chickens. They play a significant role in both humoral and cell-mediated immunity, making lymphocytosis indicative of immunogenic stimulation ***(Thrall, 2004)***.

As shown in (Tables, 5&6), the leukogram results of the present study demonstrated that the non-supplemented group challenge with *E. coli* (G4) exhibited a significant leukocytosis and heterophilia during the third week of the experiment. By the fifth week, there was a noteworthy increase WBC, and differential leukocytic count (lymphocytes, and monocytes) in *E. coli* challenged group. These findings align with previous studies conducted by ***Hanan (2002)***, ***Fatma (2005)***, and ***Kilany et al. (2018)***, who similarly observed leukocytosis and heterophilia in experimentally infected broilers after one week of infection. However, after two weeks of infection, leukocytosis, lymphocytosis, and monocytosis were observed. These results are also aligned with the research conducted by ***Sabah et al. (2009)***, who reported a significant increase in total leukocyte count, heterophils, monocytes, and eosinophils at the 2nd and 9th day of infection, along with a significant increase in lymphocyte count at 15 days' post-infection. ***El-Tahawy et al. (2022)*** also reported a higher rate of total leukocyte count and heterophils, along with a dimension in lymphocytes in broilers challenged with *E. coli* than non-challenged group. These findings are supported by ***Fraser et al. (1991)***, who suggested that leukocytosis, lymphocytosis, and monocytosis are associated with infection, and ***Barry (1998)***, who demonstrated that

leukocytosis accompanied with heterophilia is a response to *E. coli* airsaculitis in chickens. The increased presence of heterophils appears to be an inflammatory response to *E. coli* infection ***(Nakamura et al., 1990)***. In contrast, nano-se challenged groups (G5 and G6) observed an improvement concerning to leukogram in contrasting with the challenged group (G4) which demonstrated a major lower rate in TLC, heterophil, monocyte with a higher level in lymphocyte at 3rd week of the experiment and revealed a higher level in TLC, lymphocyte and monocyte counts with a diminish in heterophil count at 5th week of the experiment. These findings could be owing to nano-se improves the immune response ***(Surai, 2006 and Mohapatra et al., 2014)***. Furthermore, Nano-se treated groups (G2 and G3) showed was a significant leukocytosis and lymphocytosis as compared with control (G1) at 5th week of age. Nearly, same finding was obtained by ***Fuxiang et al. (2008)*** and ***Selim et al. (2015)*** who noted that the addition of nano-selenium to broiler feeds and water resulted in a higher level of lymphocytes contrasted to non-challenged group. As well, ***Abdulkrem and Tareq (2021)*** who showed that, the number of WBC and lymphocyte in nano-se treated group were significantly higher than control. The observed effect may be attributed to the potential of nano-selenium to enhance cellular immunity, as suggested by ***(Mohapatra et al., 2014)***. In difference to ***Rizk et al. (2017)*** who found an important reduction in lymphocyte count for Sinai hens when supplemented with nano-se in the diet, ***Ibrahim et al. (2020)*** found that the supplementation of nano-selenium in broilers did not result in any changes in the leucocytic cell (WBC). Liver enzymes (ALT & AST) activities serve as reliable markers for assessing liver function and overall health. Increased enzyme activity indicates liver damage and degeneration of hepatocytes, leading to the release of these enzymes into the bloodstream. This finding is consistent with the research findings reported by ***(Kubena et al., 1995)***. The ALT is particularly sensitive in detecting acute liver damage, and its elevation is uncommon in non-hepatic diseases ***(Soumendra et al., 2010)***. Compared to AST, ALT is more specific to liver parenchymal cells ***(Nkosi et al., 2005)***. As shown in (tables 7& 8), In the serum biochemical study, the *E. coli* challenged group (G4) observed a higher level of AST and ALT as contrasted to the control at both 3rd and 5th week. ***Madian et al. (2008)***; ***Sharma et al. (2015)*** and ***kilany et al. (2018)*** illustrated that the increasing the value of AST and ALT with *E. coli* challenged broilers. The rise of these levels is attributed to hepatocellular harm triggered by an *E. coli* infection ***(Campbell and Coles, 1986)***. Also,

nano-se challenged groups (G5 and G6) denoted a reduction in the levels of ALT and AST values as contrasted to the challenged non-supplemented group at both 3rd and 5th week. These consequences agreed with ***Ali et al. (2020)*** study which reported that, ALT and AST were significantly reduced in nano-se supplemented infected group with *E. coli* as contrasted to control. Based on the information provided, it appears that the inclusion of nano-selenium in broilers' feeds and water has shown potential hepatoprotective effects. This effect can be attributed to selenium's involvement in the manufacture of selenoproteins and enzymes, particularly glutathione peroxidase, which are part of the antioxidant protection system in the body. Nano-selenium is believed to inhibit the formation of free radicals, which are known to contribute to inflammatory processes and liver damage. By reducing the formation of free radicals, nano-selenium helps maintain liver health and minimize potential harm ***(Lesnichaya et al., 2021)***. Regarding the supplementation of nano-selenium (G2 and G3), no changes observed in the AST and ALT values in contrast to non-challenged chicks at the 5th week. This indicates the safe usage of nano-se on the liver. According to ***Bityutskyy et al. (2019***), adding nano-se to the diet did not have a deleterious effect on the liver. These findings are consistent with the studies conducted by ***Selim et al. (2015)*** and ***Jamima et al. (2020)***, who reported no variations in the AST and ALT values of broilers given nano-se. Conversely, ***Azab et al. (2019)*** and ***Ibrahim et al. (2022)*** description the liver enzyme (ALT and AST) levels were lowered in birds given nano-se as contrasted to non-challenged chicks.

(Tables 9 and 10), the results of serum protein indicated a major lower in proteinogram concentration, along with a higher value of globulin concentration, in *E. coli* challenged chickens (G4) as contrast to non-challenged chicks at the 5th week. These findings align with the research conducted by ***Zaki et al. (2012)***, which reported a reduction in the protein concentration in *E. coli* challenged broiler chickens. ***Kumari et al. (2014)*** and ***Sharma et al. (2015)*** also found a higher level of globulin concentration, along with a reduction in the protein concentration, in *E. coli* challenged broiler chickens. In contrast, ***Ogunbanwo et al. (2004)*** observed an elevation in the protein concentration in *E. coli* challenged birds. According to ***Blood et al. (1994)***, Hypoproteinemia can be caused by three main factors: kidney disease, leading to the drop of proteins; liver disease, which hinders plasma protein synthesis; or heart disease. Decreased albumin levels may result from reduced feed intake, anorexia, and hepatic damage ***(Deshmukh, 2006)***. Albumin acts as a reliable marker for liver

dysfunction, diminished uptake, or protein depletion ***(Sacher and McPherson, 2000)***. *E. coli* infection caused an increase in globulins, associated with liver lesions ***(Sharma et al., 2015)***. In contrast, the challenged groups supplemented with nano-se (G5 and G6) demonstrated a major elevation in protein level and albumin concentrations as contrasted to the challenged group at 5th week. Also, G5 presented a non-considerable higher in globulin level and G6 showed a considerable higher in globulin as contrast to the challenged group (G4) at 5th week. The rise in protein levels noted in the nano-selenium-supplemented groups (G2 and G3) may be ascribed to the capacity of selenium to bolster plasma lipoproteins, as indicated by earlier studies ***(Iizuka et al., 2001)***. Moreover, at the third week of the experiment, the nano-selenium-supplemented groups exhibited a major rise in protein levels, while non-changes were demonstrated in albumin, globulin, and the albumin/globulin (A/G) ratio as contrasted to non-challenged group (G1). The products aligned with ***Selim et al. (2015)*** who investigated that, nano-se was not significantly affected the albumin, globulin and A/G ratio. ***Jamima et al. (2020)*** who found that, the Protein level was significantly higher in nano-se supplemented birds contrasted to non- challenged group. Meanwhile, A/G ratio was not significantly differed than control. The increased levels of globulin and lowered A/G ratio are indicative of immunity status of the animal ***(Bunglavanetal.,2014)***. These results similar with ***Mohapatra et al. (2014)***; ***Ismail et al. (2016)*** and ***Abdulkrem and Tareq (2021)*** who observed that, the protein level and globulin conc. in nano-se supplemented group were significantly higher as contrasted to non-challenged group. While, albumin was not significantly differed contrast to non-challenged group.

(Tables 9 and 10), Tissue receives glucose through two primary pathways: absorption of dietary glucose in the intestines and the synthesis of glucose by the liver from its building blocks ***(Kaneko et al., 1997)***. In the present study, *E. coli* challenged non supplemented group (G4) showed a dimension in serum glucose level contrasted to non-challenged group at 3rd and 5th weeks of age Anorexia could be to blame ***(Hazelwood and Lorenz, 1959)***. This finding agreed with the results previously reported by ***Coles (1986)***, who reported that hypoglycemia caused by anorexia, decreased intestinal glucose absorption also reduced blood flow and oxygen levels cause alterations in tissue metabolism. Also, this result was similarly to ***Kilany et al. (2018)*** and ***Farouk et al. (2021)*** which demonstrated a dimension in serum glucose level in the *E. coli* challenged chicken. While challenged and supplemented

groups (G5 and G6) demonstrated a non-significant elevation in glucose level at 3rd week in contrast to the challenge non-supplemented group (G4) and a major rise at 5th week, these results may be due to improvement in feed conversion ratio and feed eating, and the intestinal absorption improvement. Nano-selenium exhibits new transfer and uptake properties, according to ***(Liao et al., 2010)***, resulting in higher assimilation efficiencies. The enhanced performance of nanoparticles can be pointed to their small particle volume, improved mucosal permeability, large surface area, enhanced intestinal amalgamation and increased tissue declaration, as highlighted by ***(Mohapatra et al., 2014)***. In contrast, the nano-se treated groups (G2 and G3) exhibited no significant change in serum glucose levels at both the 3rd and 5th weeks contrasted to the non-challenged group (G1). These results agreed with ***Ismail et al. (2016)*** who illustrated, there was non-changes in serum glucose levels in nano-se supplemented group contrast to the non-challenge groups. While, the result differed from ***Mohapatra et al. (2014)*** who showed that, the serum glucose level was increased quadratically with increase nano-se concentration in the diet of layers.

The only type of dietary cholesterol that may be absorbed is the non-esterified variety, which is found in both free and esterified forms. Non-esterified cholesterol is taken up by the body and then carried through the lymphatic system before eventually entering the bloodstream ***(Kaneko et al., 1997)***. (Tables 9 and 10), the present study revealed that the *E. coli* challenged non-supplemented group (G4) exhibited a substantial rise in cholesterol levels than the control group at the 3rd and 5th weeks. This finding is consistent with the observations made by ***Farouk et al. (2021)*** who reported a rise in cholesterol levels in *E. coli* challenged chicks. The elevation in cholesterol levels could be attributed to liver disease ***(Kaneko et al., 1997)***. As opposed to, the challenged and supplemented groups (G5 and G6) exhibited a dimension in cholesterol conc. as contrast to the challenged group (G4). This can be aspect to the crucial role of selenium in modulating the impacts of thyroid hormone on metabolism of fat ***(Masukawa et al., 1983)***. Selenium is involved in the formation of the active center of glutathione peroxidase (GSH-Px), which acts as an antioxidant and may contribute to the decrease in cholesterol levels (***Radwan et al., 2015 and Abdou et al., 2019)***. This finding is supported by ***Brown and Jessup (1999),*** who showed that an increased dietary antioxidant content led to a decrease in cholesterol concentration. The reduction in cholesterol levels may also be attributed to increased lipolysis associated with selenium intake.

It has been demonstrated that selenium stimulates the PPAR- -γ (sterol regulatory element-stimulated receptor-gamma), which lowers the levels of SREBP-2 (sterol regulatory element-binding protein-2). This, in turn, can contribute to decreased cholesterol synthesis, as reported by ***(Klopotek et al., 2006)***. In the nano-se treated groups (G2 and G3), there were non-significant changes in cholesterol rates contrasted to the non-challenged group (G1) at the 3rd and 5th weeks. These findings align with the observations of ***Abdulkrem and Tareq (2021)***, who reported no significant difference in total cholesterol levels between broilers treated with nano-se and the control group. However, these results contradict the conclusions of ***Abdel-Moneim et al. (2022)***, who demonstrated a major diminish in cholesterol rates in broilers receiving nano-se in their diet contrast to the non-challenged group.

The uric acid content in birds serves as an indicator of protein utilization and nitrogen excretion, as described by ***(Wright, 1995)***. Uric acid, being the primary product of amino acid and purine breakdown in birds, exhibits an inverse correlation with protein degradation and reflects the equilibrium between protein consumption, utilization, degradation, and the excretion of protein metabolites by the kidneys. Values of serum uric acid are commonly utilized to evaluate kidney function, with hyperuricemia (raised serum uric acid values) frequently combined with kidney disease ***(Kolmstetter and Ramsay, 2000)***. (Tables 9 and 10), regarding the uric acid results, the *E. coli* challenged chickens (G4) exhibited a major rise in uric acid levels at the 3rd and 5th weeks contrasted to the non-challenged group. These conclusions are constant with the reports of ***Hanan (2002)***, ***kilany et al. (2018)***, and ***El-Tahawy et al. (2022)***, who observed elevated serum uric acid levels in chickens challenged with *E. coli.* The escalation of this phenomenon can be ascribed to the declination of plasma proteins. The rise in blood urea levels may be aspect to the impact of microbes and their toxins on renal function ***(Obrig et al., 1987)***. On the other hand, the challenged groups and supplemented with nano-se (G5 and G6) demonstrated a major lower in uric acid levels contrast to the challenged group. These results indicate an improvement in the health of the chicks. This improvement may be attributed to the renal protective effect of nano-se, which is attributed to its antioxidant properties. In contrast, the nano-se treated groups (G2 and G3) demonstrated no changes in the level of uric acid contrasted to the non-challenged group at both the 3rd and 5th weeks, suggesting that nano-se had no dangerous effects on the kidneys. This finding is matching the observation of ***Abdel-Moneim et al. (2022)*** who reported no significant

differences in serum uric acid concentrations in Ross broilers fed nano-se (0.1 and 0.2 mg/kg) contrasted to control. However, this finding contradicts the conclusions of ***Azab et al. (2019)***, demonstrated a lowering in level of uric acid in Cobb broilers fed 0.15 ppm nano-se contrasted to control.

Conclusion

In conclusion, nano-selenium supplementation had positive effects on the growth performance and various biochemical parameters in chickens infected with *E. coli*. These results imply that nano-Se supplementation may serve as a beneficial strategy to improve chicken health and mitigate the negative effects of *E. coli* infection. Further research is warranted to elucidate the underlying mechanisms and optimize the dosage and duration of nano-Se supplementation for optimal results.

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دراسة باثولوجية إكلينيكية على تأثير استخدام النانوسيلينيوم (على النمو و القياسات البيوكيميائية فى بدارى التسمين فى الحالات الطبيعية والمصابة تجريبيا بالميكروب القولونى

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الملخص العربي

إستهدف هذا العمل دراسة تأثير استخدام النانوسيلينيوم على النمو و القياسات البيوكيميائية في بدارى التسمين في الحالات الطبيعية والمصابة تجريبيا بالميكروب القولوني

اجريت الدراسة على عدد مائة و خمسون من دجاج التسمين عمر يوم وقسموا إلى ست مجموعات علاجية كل مجموعة إشتملت على 25 كتكوت.

أظهرت النتائج وجود زيادة معنوية في اوزان الطيور في المجموعات المعالجة بالنانو سيلينيوم و نقص واضح فى نمو المجموعة المصابة بميكروب الإيشريشيا القولوني مع إرتفاع معنوي في معدل التحويل الغذائي مع ظهور أعراض مرضية تمثلت فى إنكماش و قلة الحركة وصعوبة التنفس مع وجود افرازات من العين والأنف والإسهال وانخفاض معدل استهلاك العلف..

بينما أظهرت المجموعات المصابة والمعالجة بالنانوسيلينيوم إرتفاعا معنويا في معدل النمو وإنخفاضا معنويا في معدل التحويل الغذائي بالمقارنة بالمجموعة المصابة.

القياسات البيوكيميائية:

1- أوضحت النتائج عدم وجود تغير معنوي في مستوي إنزيمات الكبد في المجموعات المعالجة بالنانوسيلينيوم , في حين لوحظت زيادة معنوية في مستوي إنزيمات الكبد (ألانين أمينوترانسفريز و أسبرتيت أمينو ترانسفريز) فى الطيور المصابة تجريبيا بالمقارنة بالمجموعة الضابطة بينما أظهرت المجموعات المصابة والمعالجة بالنانوسيلينيوم انخفاضا معنويا بالمقارنة بالمجموعة المصابة الغير معالجة.

2- لوحظ وجود إرتفاع معنوى في البروتينات الكلية و نسبة الجلوبيولين في المجموعات المعالجة بالنانوسيلينيوم بالمقارنة بالمجموعة الضابطة بينما عانت المجموعة المصابة من إنخفاض معنوي في البروتينات الكلية و نسبة الألبيومين مع زيادة معنوية في نسبة الجلوبيولين بالمقارنة بالمجموعة الضابطة , هذا و قد أظهرت المجموعات المصابة و المعالجة بالنانوسيلينيوم زيادة معنوية في البروتينات الكلية و نسبة الألبيومين بالمقارنة بالمجموعة المصابة الغير معالجة.

3- المجموعات المعالجة فقط بالنانوسيلينيوم لم يحدث فيها تغيرمعنوي في مستوى السكر بينما لوحظ إنخفاض معنوي فى مستوى السكر في المجموعة المصابة بالمقارنة بالمجموعة الضابطة أما المجموعات المصابة و المعالجة بالنانوسيلينيوم أظهرت زيادة معنوية بالمقارنة بالمجموعة المصابة.

4- المجموعات المعالجة فقط بالنانوسيلينيوم لم تظهر تغير معنوي فى مستوى الكولستيرول بينما لوحظ إرتفاع معنوي فى مستوى الكولستيرول في المجموعة المصابة بالمقارنة بالمجموعة الضابطة بينما أظهرت المجموعات المصابة و المعالجة بالنانوسيلينيوم إنخفاض معنوي بالمقارنة بالمجموعة المصابة الغير معالجة.

5- كما لم تظهر المجموعات المعالجة بالنانوسيلينيوم فقط أي تغير معنوي في مستوى حمض البوليك بينما أظهرت المجموعة المصابة وجود زيادة بالمقارنة بالمجموعة الضابطة بينما أظهرت المجموعات المصابة و المعالجة بالنانوسيلينيوم انخفاض معنوي بالمقارنة بالمجموعة المصابة الغير معالجة.