

Ameliorative Effect of Nano and Organic Selenium on Some Productive and Hematological Values in Laying Hen during Force Molting

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Abstract

Due to its low toxicity and high bioavailability, nanotechnology has recently proposed a number of new effective forms of nutritional supplement ingredients. Nano-selenium is one of these due to its distinct characteristics of small particles and increased surface area of compounds, allowing for the possibility of biological interactions. 48 laying hens of a Ross breed, who were 47 weeks old, were employed in this investigation the trial ran for two months. Two different meals were provided to the birds: one was a conventional diet, and the other was a non-standard diet that included crushed yellow maize to facilitate molting. Six groups of birds were formed, each with regard to eight birds: A first group received a regular diet, while the second group was only received crushed yellow tops, the third group was given organic-Se (2 g/L) along with a standard diet, the fourth group was given nano-Se (0.5 ml/L) along with a standard stalk, the fifth group was given organic-Se with molting (2 g/L), and the sixth group was given nano-Se with molting (0.5ml/L). The results showed a rise in feed consumption, average egg weight, and production of eggs with an improvement in the feed conversion factor for egg production for the selenium groups, they also showed an elevate in the red and white blood cells, hemoglobin level, with packet cell volume, additionally, treatment with selenium increased glutathione while lowering malondialdehyde levels compared to the control and molting. We draw the conclusion from this study that organic and nano-selenium have beneficial effects in reducing the drawbacks of straw and bringing back the majority of the values to their normal state, which is close to control.

Keywords: nano-Se, laying hen, RBC, WBC, molting

Introduction

It is essential to have wholesome, premium animal products that are packed with micronutrients to support global human health (1). With over 30% of the world's total meat production, livestock outcome is the second most popular food. Selenium has been recognized as a crucial vitamin for improving the performance, well-being, and antioxidant system of chickens (2). Despite the fact that both organic and inorganic forms of selenium have positive effects on poultry productivity and are routinely used in animal feed, multiple studies reveal that the biological shape has a higher permeation in comparison to the inorganic form (3). Numerous hormones, including thyroid hormones, are metabolized by selenium, which also improves animal antioxidant capacity, controls immunity, productivity, and reproduction, and lessens the harmful effects of free radicals (5).

Molting is a common method in flocks of laying hens to boost egg production and quality during the subsequent laying cycle after temporarily halting egg production for a limited time (6). In many commercial farms, initiated shedding of laying chickens is a common practice in livestock husbandry. Molting is a wise management choice that encourages farmers to recycle laying hens by molting flocks rather than purchasing replacement pullets (7). The purpose of molting

is often to stop egg production during market changes by improving laying performance after the molt and extending the laying hens' productive lives (8,9).

Nanoparticles are defined as particles with dimensions ranging from one to one hundred nanometers, and because of their small size, they have characteristics that differ from their molecules when they are larger in size, such as a larger surface area and higher absorption capacity, which makes it easier for to use nanotechnology as feed sequel in animal diets, particularly avian diets (10). The Latin root of the term "nano" means "to dwarf" (11). Although many believe that nanotechnology is a relic of a bygone era, research into technologies that support nanotechnology has increased over the past ten years. Numerous companies have specialized in producing new types of nano-sized materials that are concerned with poultry and livestock production systems with the aim of improving animal production efficiency.

Employment materials and methods

Animals and the creation of memorable experiences

For this study, a total of 48 rose-type laying hens were used. The birds were raised and ground-bred for the duration of the two-month study in an open-type theatre. Two different types of meals were given to the birds: a conventional ration and a non-standard feed, represented by the use of crushed yellow maize for molting. N.R.C. (12) states that feed and water were freely available. Six groups of eight birds each were created, with the first group serving as the control and fed a standard diet, the second group molting with yellow corn that had only been crushed, the third group receiving organic selenium at a rate of 2 g/L with a standard diet, the fourth group receiving nano-selenium at an average of 0.5 ml/L with a standard stalk, the fifth group receiving organic selenium with molting at a rate of 2 g/L

Collection of blood samples

Following the completion of the trial, the hens were killed by cutting the jugular vein in order to collect blood samples using specialized tubes. To conduct blood tests, one portion of the blood samples was placed in test tubes containing anticoagulants; the other portion was placed in tubes without anticoagulants, allowed to coagulate at ambient temperature, and then throw away at a speed of 3000 rpm for 15 minutes to induce up to the time of the laboratory tests, serum specimens were kept at -20°C.

Examined characteristics

Hemoglobin concertation (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concertation (MCHC) (13) in addition to red blood cell count (RBC), white blood cell count (WBC), leukocyte differential count (DLC), stress index (heterophils/lymphocytes), based on Campbell (14) were imposed using blood samples that included anticoagulants, malondialdehyde and glutathione levels in serum samples were calculated.

An analysis of the data

The results were initially analyzed using one-way variance analysis (ANOVA), and Duncan's multiple variety trial was used to assess the importance of variability among firms on the opportunity level (P0.05) using the SPSS system to analysis the information. (15).

Results

According to the findings in Table (1), the total feed consumption rate, egg weight rate, egg production, and egg production percentage all significantly decreased at the probability level ($p \leq 0.05$), while the feed conversion factor for egg production significantly increased (negative effect) comparing the lashing to the control group. In comparison to the control, the organic and nano-selenium groups at a rate 2g/L and 0.5ml/L significantly increased the total feed consumption rate, average egg weight, egg production, and the percentage of egg production, the nano-selenium group in these above traits outperformed the organic selenium group, and both groups showed a significant decrease (improvement) in the feed conversion factor for egg production in comparison to the control. The results of the organic and nano-selenium groups with molting indicated a significant increase in the total feed consumption rate, the average egg weight, egg production and the proportion of egg production compared with the molting group alone, while the nano-selenium group with molting outperformed the organic selenium group with molting in these above characteristics, the feed conversion factor for egg production significantly decreased (leverage) in both groups when compared to the molting group alone. These findings show that the feed conversion factor for egg production in the nano-selenium group with molting has reverted to normal levels, coming close to the control group.

Table (1) Impact of nano and organic selenium some productive values in laying hens during forced molting (for eight weeks).

Parameters Groups	Average feed intake (g/hen)	average egg weight (g)	Egg production/hen	H.D%	F.C.R (g feed/g egg)
Control (stander ration)	734.39 ± 1.94 c	51.05 ± 0.21 e	4.70 ± 0.05 c	67.18 ± 0.75 c	3.06 ± 0.03 c
Molting (yellow corn)	289.66 ± 32.63 e	47.41 ± 0.43 f	0.28 ± 0.11 e	4.01 ± 1.71 f	7.31 ± 3.15 a
Organic selenium (2g/L)	784.57 ± 3.57 b	61.59 ± 0.32 b	4.95 ± 0.04 b	70.75 ± 0.57 b	2.59 ± 0.01 e
Nano- selenium (0.5ml/L)	799.74 ± 5.47 a	65.85 ± 0.10 a	5.07 ± 0.05 a	72.54 ± 0.75 a	2.39 ± 0.02 d

Organic selenium+ molting (2g/L)	687.74 ± 11.50 d	52.99 ± 0.16 d	3.52 ± 0.03 d	50.44 ± 0.55 e	3.67 ± 0.08 b
Nano-selenium + molting (0.5ml/L)	689.21 ± 16.23 d	55.94 ± 0.11 c	3.98 ± 0.03 d	56.91 ± 0.58 d	3.11 ± 0.05 c

- The aforementioned numbers stand for the mean ± standard error.
- There are significant variations between the clusters at the rate of probability ($p \leq 0.05$), as indicated by the little English letters within the same column.
- H.D% = Hen Day
- F.C. R= Food conversation ratio (g feed/g egg)

As per the findings in Table (2), the numbers of erythrocyte, leukocyte, hemoglobin concentration, and hematocrit were all substantially lower ($p \leq 0.05$) in molting than those of the control group. In compared with the control group, the organic and nano-selenium groups with concentrations of 2g/L and 0.5ml/L, respectively, substantially increased the rate of erythrocyte, leukocyte, Hb concentration, hematocrit. In comparison to the molting group alone, the organic and nano-selenium groups with molting displayed a considerable increase in the quantity of erythrocyte, Hb concentration, and packet cell volume. The digit of red and white blood cells was higher in the group of nano selenium with molting than in the group of organic selenium with molting. The aforementioned data show that hemoglobin concentration, packet cell volume, and the number of red and white blood cells have all returned to normal levels as in the control group in the nano selenium group with molting, and the same is true in the organic selenium group with molting in the numbers of RBC.

Table (2) Impact of nano and organic selenium on blood picture in laying hens during forced molting

Parameters Groups	RBCs 1x10⁶/mm³	WBC 1 x10³/mm³	Hb mg/100ml	PCV%
Control (Stander ration)	3.18 ±0.13 cd	4.64 ±0.13 c	6.92 ±0.15 b	30.28 ±0.42 b
Molting (Yellow corn)	2.50 ±0.07 e	3.54 ±0.29 d	5.18 ±0.14 e	27.27 ±0.30 d
Organic selenium (2g/L)	3.94 ±0.05 b	5.45 ±0.23 b	7.38 ±0.15 b	32.13 ±0.38 a
Nano-selenium (0.5ml/L)	4.41 ±0.08 a	6.48 ±0.36 a	8.96 ±0.20 a	33.05 ±0.35 a

Organic selenium+ molting (2g/L)	2.97 ±0.04 d	3.79 ±0.12 d	6.31 ±0.22 cd	28.91 ±0.20 c
Nano-selenium + molting (0.5ml/L)	3.29 ±0.10 c	4.04 ±0.06 c	6.62 ±0.11 bc	29.87 ±0.27 bc

- The aforementioned numbers stand for the mean ± standard error.
- There are significant variations between the clusters at the rate of probability ($p \leq 0.05$), as indicated by the little English letters within the same column.

According to Table (3) findings, the mean corpuscular volume substantially elevates in the molting group compared to the control group, while the MCH and MCHC considerably lowered. When compared to the control group, the results demonstrated a substantial decrease in MCH and MCV for the organic and nano selenium groups at concentrations of 2g/L and 0.5ml/L, respectively. Significantly higher mean corpuscular hemoglobin concentration after exposure to nano selenium compared to control, in comparison to the organic selenium group, the nano selenium group similarly demonstrated a large increase in MCH and MCHC with a considerable decrease in mean corpuscular volume. In comparison to the molting group alone, the results of the organic and nano-selenium groups with molting showed a substantial decrease in the MCV and a significant rise in the MCH and MCHC. In comparison to the nano selenium group with molting, the organic selenium group did better in terms of MCV and MCH, as well as with a considerable drop in the mean corpuscular hemoglobin concentration. The aforementioned findings also demonstrated that the mean corpuscular volume may be restored to normal levels, which are close to those of the control group, in the organic selenium group with molting.

Table (3) Impact of nano and organic selenium in MCV, MCH and MCHC in laying hens during forced molting

Parameters	MCV Micron	MCH Picogram	MCHC%
Control (Stander ration)	95.75 ±3.75 c	21.89 ±0.91 a	22.88 ±0.57 b
Molting (Yellow corn)	109.00 ±4.75 a	18.88 ±1.91 c	19.42 ±0.38 d
Organic selenium (2g/L)	81.49 ±1.88 e	18.71 ±0.55 c	22.99 ±0.60 b
Nano-selenium (0.5ml/L)	74.89 ±1.09 f	20.30 ±0.38 b	27.11 ±0.39 a
Organic selenium+ molting (2g/L)	97.37 ±1.76 b	21.31 ±0.79 a	21.84 ±0.88 c
Nano-selenium + molting (0.5ml/L)	90.87 ±2.26 d	20.19 ±0.67 b	22.12 ±0.36 b

- The aforementioned numbers stand for the mean ± standard error.

- There are significant variations between the clusters at the rate of probability ($p \leq 0.05$), as indicated by the little English letters within the same column.

As per the results in Table (4), there was a significant increase at the eventuality level ($p \leq 0.05$) in the percentage of heterophiles, the percentage of monocytes, and the stress index in the molting group compared to the control group, with a statistical decline in the percentage of lymphocytes. The organic and nano-selenium groups at a rate 2g/L and 0.5ml/L respectively showed a dramatic increase in the proportion of monocyte in comparison with control, and the organic selenium group indicate a dramatic raise in the proportion of lymphocytes with a statistical decline in the rate of heterophile and stress index in comparison with control, whereas the selenium nano group showed a dramatic reduction in lymphocyte proportion as compared with control, and the nano selenium group outperformed the organic selenium group in the proportion of heterophile and stress index which in turn showed a significant increase in lymphocyte proportion compared to the selenium nano group. When compared to the molting group alone, treatment with organic selenium with molting cause an eventuality decline in the proportion of heterophile and stress index with a significant increase in the lymphocytes, whereas treatment with nano selenium with molting resulted in a dramatic raise in the proportion of heterophile with a significant decrease in the lymphocytes. The results also revealed an excess in the rate of heterophiles and the stress index, as well as an eventually decline in the percentage of lymphocytes for the nano selenium molting group compared to the organic selenium molting group. The results show that the organic and nano-selenium groups with molting returned the proportion of monocytes and stress index to normal levels, hassling to the control.

Table (4) Impact of nano and organic selenium in differential leukocyte count in laying hens during forced molting

Parameters Groups	Lymphocyte%	Heterophils%	Monocyte%	Basophils%	Eosinophils%	H/L
Control (stander ration)	72.81 ± 3.11 c	14.82 ± 1.63 d	10.24 ± 0.60 b	0.94 ± 0.39 a	1.12 ± 0.32 a	0.20 ± 0.03 b
Molting (yellow corn)	70.78 ± 1.73 e	18.19 ± 2.34 b	12.04 ± 0.91 a	0.99 ± 0.40 a	1.14 ± 0.39 a	0.22 ± 0.04 a
Organic selenium (2g/L)	74.69 ± 3.17 b	12.71 ± 1.13 f	11.21 ± 0.87 a	0.89 ± 0.36 a	1.17 ± 0.41 a	0.17 ± 0.02 c
Nano- selenium (0.5ml/L)	70.48 ± 1.62 e	15.18 ± 1.92 c	12.64 ± 0.98 a	0.97 ± 0.40 a	1.10 ± 0.29 a	0.21 ± 0.03 b
Organic selenium+	76.19 ± 4.06 a	13.25 ± 1.19 e	10.54 ± 0.72 b	0.87 ± 0.31 a	1.15 ± 0.41 a	0.17 ± 0.02 c

molting (2g/L)						
Nano-selenium + molting (0.5ml/L)	71.89 ±2.87 d	18.61 ±2.83 a	10.52 ±0.69 b	0.93 ±0.37 a	1.21 ±0.42 a	0.25 ±0.06 a

- The aforementioned numbers stand for the mean ± standard error.
- There are significant variations between the clusters at the rate of probability ($p \leq 0.05$), as indicated by the little English letters within the same column.

The statistical analysis results shown in Figures (1,2) at the level of probability ($p \leq 0.05$) indicated a significant reduction and an elevate in glutathione and malondialdehyde value in the molting group compared to the control group. The organic and nano selenium groups at a rate 2g/L and 0.5ml/L respectively showed a statistical raise in glutathione levels with a significant dropping in malondialdehyde value when compared to the control group, while the nano selenium group outperformed the organic selenium group and showed a significant increase in glutathione levels with a decrease in malondialdehyde levels. The results also showed a dramatic raise in glutathione rate with an eventually reduction in malondialdehyde value in the organic and nano selenium groups with molting compared to molting alone, and the nano selenium group with molting caused a significant decrease in malondialdehyde levels compared to the organic selenium group with molting. When compared to the control group, the data show that nano selenium with molting improves the state of antioxidants by increasing glutathione levels and decreasing malondialdehyde levels to normal levels.

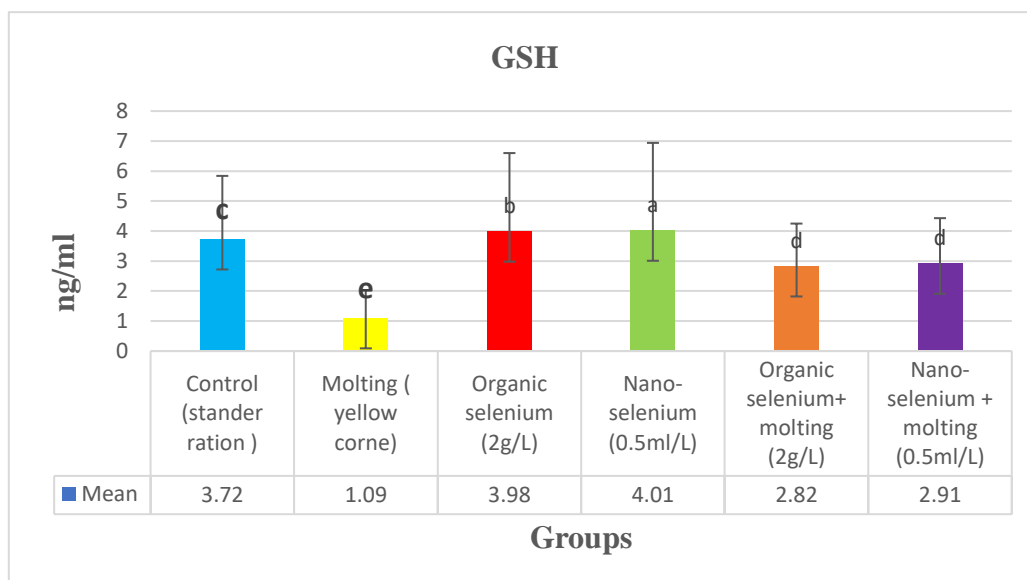


Figure (1) Impact of nano and organic selenium in glutathione in laying hens during forced molting

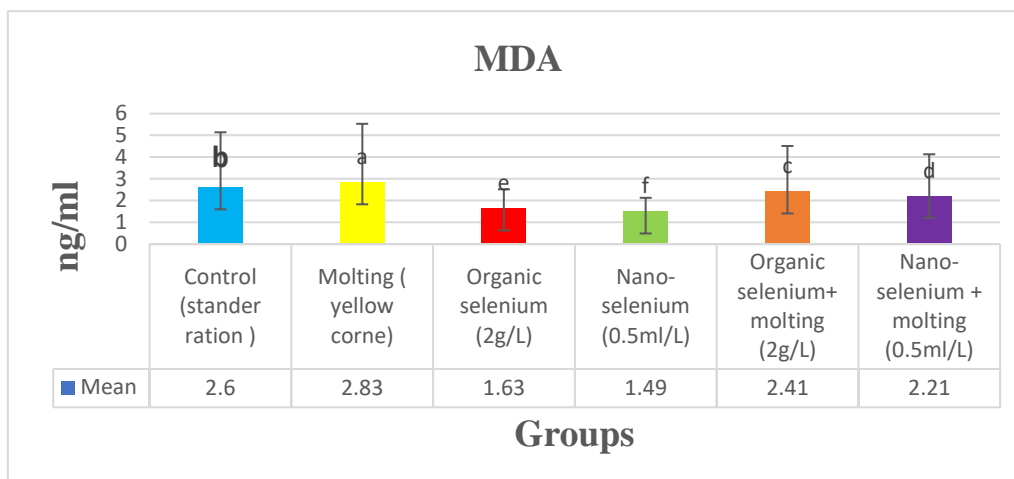


Figure (2) Impact of nano and organic selenium in malondialdehyde in laying hens during forced molting

Discussion

According to the findings, the molting group resulted in a decrease in the bird's production parameters, such as feed consumption, egg weight, egg production rate, and percentage of egg production, with an increase in the feed conversion factor for egg production (detrimental affect) and a decrease in most of the blood parameters, as well as an elevate in oxidative damage by increasing malondialdehyde and decreasing glutathione level. The treatment with organic and nano selenium improved the negative effect of molting and returned most of the parameters to their normal position, close to the control group, which corresponds to what was mentioned Meng *et al* (2019) (16) that adding selenium in its various forms, including organic and nano, to laying hen diets led to an increase in feed consumption, egg weight, and production eggs with an improvement in the feed conversion factor for egg production in additament to an elevate in the glutathione and a decline in malondialdehyde, according to Lui *et al* (2020) (17) administering 0.3ppm selenium to laying hens resulted in an increase in glutathione and a decline in malondialdehyde levels. The special properties of selenium in particular its nano-form, can be credited to its important impact, selenium (Se) is required by animals for growth, vitality, endocrine and metabolic functions in multiple tissues (18). Even though selenium fortification has become popular for various species, new research shows that Se-nanoscale have a wide nutritive value, catalytic activity, and absorbability, but minimum toxicity when particularly in comparison to another Se sources (19). Se was already demonstrated to benefit numerous animal species by getting better their redox and overall validity, as well as their own efficiency and reproductive manifestation (20,21). Antioxidants shield the body from the damaging generation of reactive oxygen species produced throughout metabolic activities. (22). Se exerts resveratrol capabilities by increasing glutathione peroxidase vigor (23) and striving to improve immune reaction (22). It also diminishes hydroxyl radicals and hydrogen peroxides while working to improve lipid oxidative stability, thereby protecting animal health (24). The addition of nano-Se to the diet risen final weight gain, feed conversion efficiency (FCR), and regular average daily gain. (20). The treatment's improvement in blood traits may be due to

selenium, because Se is a main element of glutathione peroxidase, where it provides protection to blood cell aspects and elements from lipid peroxidation to epithelium, going to add 1 mg of nano-Se /kg of feed improves the stability of cellular membrane and immune cell effectiveness. (25) The addendum of diverse organic-Se or nano-Se sources to chicken ration resulted in a rise in the rate of RBC and Hb rate, and Se enhance the function of the blood forming tissue in order to rise the rate of RBC, Hb content, and the packet cell volume (25). Those certain results coincide to those procured by Selim *et al* (2015) (27), who discovered that use organic or nanoscale forms of selenium to the broiler ration increase RBC, hematocrit, and Hb amount when compared to inorganic selenium. El-Sheikh *et al* (2010) (28) also found that organic selenium when adding at 0.2 and 0.3 ppm statistically raise hemoglobin levels, and Mohapatra *et al* (2014) (29) noted that nano-Se is greater efficacy in raising hematological remedies than inorganic sodium selenite at 0.3 ppm, and that selenium has an antioxidant property on the red blood cell surface as the erythrocyte count and Hb rate rise, so does the maturation of erythropoiesis (30). The findings also revealed a significant lowering of MCV, which could be likened to an inverse correlation with a rise in the rate of red cells, while the dramatic elevate in the level of MCHC could be credited to the presence of a direct relation with the identified a rise in hemoglobin (31). The optimized role of selenium, boosting the immune status of the bird, and reduces the impact of oxidative stress can be credited to the importance of selenium in enhancing intrinsic environs of the chickens, as indicated by Muhammad *et al* (2021)(32) that supplementing laying hen diets with organic selenium at a dose of 0.3 mg/kg ration enhanced efficiency by excess in the length and width of the villi, an upgrade in the internal environment through a rise in the number of *Lactobacilli* with a decrease in the number of *E.coli*, and thus selenium plays a large vital role in feeding laying hens.

Conclusion

We assert from this study that the addition of organic and nano-selenium improved most of the physiological traits of laying hens during forced molting, with the prospect of going back most of the values to their normal spot close to control, as well as the importance of nanocomposites as feed ingredients due to their distinguishing properties of tiny size of their molecules and the provision of a larger surface area, facilitating digestion and absorption.

Reference

- [1]. Khan, A. Z., Kumbhar, S., Liu, Y., Hamid, M., Pan, C., Nido, S. A., & Huang, K. (2018). Dietary supplementation of selenium-enriched probiotics enhances meat quality of broiler chickens (*Gallus gallus domesticus*) raised under high ambient temperature. *Biological trace element research*, 182(2), 328-338. <https://doi.org/10.1007/s12011-017-1094-z>
- [2]. Mechora, Š., Čalasan, A. Ž., Felicijan, M., Krajnc, A. U., & Ambrožič-Dolinšek, J. (2017). The impact of selenium treatment on some physiological and antioxidant properties of *Apium repens*. *Aquatic Botany*, 138, 16-23. <https://doi.org/10.1016/j.aquabot.2016.12.002>

- [3]. Liu, H., Yu, Q., Tang, X., Fang, C., Chen, S., & Fang, R. (2020). Effect of selenium on performance, egg quality, egg selenium content and serum antioxidant capacity in laying hens. *Pakistan Journal of Zoology*, 52(2),635. <https://doi.org/10.17582/journal.pjz/20190424040448>
- [4]. Elnaggar, A. S., Ghazalah, A., Elsayed, A. H., & Abdelalem, A. (2020). Impact of selenium sources on productive and physiological performance of broilers. *Egyptian Poultry Science Journal*, 40(3), 577-597. <https://doi.org/10.21608/epsj.2020.112468>
- [5]. Liu, L., He, Y., Xiao, Z., Tao, W., Zhu, J., Wang, B., ... & Wang, M. (2017). Effects of selenium nanoparticles on reproductive performance of male Sprague-Dawley rats at supranutritional and nonlethal levels. *Biological trace element research*, 180(1), 81-89. <https://doi.org/10.1007/s12011-017-0980-8>
- [6]. Yousaf, M. S.; Rahman, Z. U.; Sandhu, M. A.; Bukhari, S. A.; Yousaf, A. (2009). Comparison of the fast-induced and high dietary zinc-induced molting: trace elements dynamic in serum and eggs at different production stages in hens (*Gallus domesticus*). *Journal of Animal Physiology and Animal Nutrition* 93, 35–43. <https://doi.org/10.1111/j.1439-0396.2007.00775.x>
- [7]. Yousaf M and Ahmad N. (2006). Effects of housing systems on productive performance of commercial layers following induced molting by aluminium oxide supplementation. *Pak Vet J* 26(3): 101-104.
- [8]. Biggs PE, Douglas MW, Koelkebeck KW and Parsons CM (2003). Evaluation of nonfeed Removal Methods for Molting Programs. *Poultry Science* 82(5): 749–753. <https://doi.org/10.1093/ps/82.5.749>
- [9]. Webster AB (2003) Physiology and behavior of the hen during induced molt. *Poultry Science* 82(6): 992–1002. DOI: 10.1093/ps/82.6.992. <https://doi.org/10.1093/ps/82.6.992>
- [10]. L. N. Balai, G. K. J. A. K. S. (2022). Investigations on PAPR and SER Performance Analysis of OFDMA and SCFDMA under Different Channels. *International Journal on Recent Technologies in Mechanical and Electrical Engineering*, 9(5), 28–35. <https://doi.org/10.17762/ijrmee.v9i5.371>
- [11]. Nowack, B. (2010). Nanosilver revisited downstream. *Science*, 330(6007), 1054-1055. <https://doi.org/10.1126/science.1198074>
- [12]. Horie, M., & Tabei, Y. (2021). Role of oxidative stress in nanoparticles toxicity. *Free Radical Research*, 55(4), 331-342. <https://doi.org/10.1080/10715762.2020.1859108>
- [13]. National Research council (N.R.C).(1994). Nutrient requirement of poultry 9th revisited National Academy Press, Washington DC. USA.
- [14]. Hameed, H. M., Maty, H. N., & Hassan, A. A. (2022). Effect of dietary BHA supplementation on certain physiological values in broiler chicken. *Iraqi Journal of Veterinary Sciences*. <https://doi.org/10.33899/ijvs.2022.132202.2068>
- [15]. Agarwal, D. A. . (2022). Advancing Privacy and Security of Internet of Things to Find Integrated Solutions. *International Journal on Future Revolution in Computer Science & Communication Engineering*, 8(2), 05–08. <https://doi.org/10.17762/ijfrcsce.v8i2.2067>

- [16]. Campbell TW. Avian hematology and cytology. 2nd ed. Iowa: Iowa State University Press; 1995. 176-198 p. (available at)
- [17]. Steel RD, Torrie JH, Dickey DA. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. New York: McGraw-Hill Book Co; 1997. DOI: 10.4236/blr.2014.54024
- [18]. Meng, T., Liu, Y. L., Xie, C. Y., Zhang, B., Huang, Y. Q., Zhang, Y. W., & Wu, X. (2019). Effects of different selenium sources on laying performance, egg selenium concentration, and antioxidant capacity in laying hens. *Biological trace element research*, 189(2), 548-555. <https://doi.org/10.1007/s12011-018-1490-z>
- [19]. Liu, H., Yu, Q., Tang, X., Fang, C., Chen, S., & Fang, R. (2020). Effect of selenium on performance, egg quality, egg selenium content and serum antioxidant capacity in laying hens. *Pakistan Journal of Zoology*, 52(2), 635. <https://doi.org/10.17582/journal.pjz/20190424040448>
- [20]. Sharma, A. (2022). Some Invariance Results for Isometries. *International Journal on Recent Trends in Life Science and Mathematics*, 9(2), 10–20. <https://doi.org/10.17762/ijlsm.v9i2.131>
- [21]. Mohapatra P, Swain RK, Mishra SK, Behera T, Swain P, Behura NC. (2014). Effects of nano-selenium Supplementation on the performance of layer grower birds. *Asian J Anim Vet Adv*. 9(10):641–52; <https://doi.org/10.3923/ajava.2014.641.652>
- [22]. Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay- Nedecky B, Peng Q, et al.(2018). Nano-selenium and its nanomedicine applications: a critical review. *Int J Nanomed* .13:2107–28; <https://doi.org/10.2147/IJN.S157541>
- [23]. Abdel-Wareth AAA, Ahmed AE, Hassan HA, Abd El-Sadekd MS, Ghazalah AA, Lohakare J.(2019). Nutritional impact of nano-selenium, garlic oil, and their combination on growth and reproductive performance of male Californian rabbits. *Anim Feed Sci*. 249:37– 45; <https://doi.org/10.1016/j.anifeedsci.2019.01.016>
- [24]. Boostani A, Sadeghi A, Mousavi S, Chamani M, Kashan NE.(2015). Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. *Livest Sci*.178:330–6.<https://doi.org/10.1016/j.livsci.2015.05.004>
- [25]. Wang HL, Zhang JS, Yu HQ.(2007). Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radic Biol Med*.42:1524–33. <https://doi.org/10.1016/j.freeradbiomed.2007.02.013>
- [26]. Rebelo, D., Machado, I., Nunes, T., Tavares, L., Gil, S., & Almeida, V. (2022). Characterization of Acute Infections by *Leptospira* Spp. in 53 Dogs Admitted to a Biological Isolation and Containment Unit. *Revista Electronica De Veterinaria*, 55 - 67. Retrieved from <https://www.veterinaria.org/index.php/REDVET/article/view/129>
- [27]. Ebeid TA. (2012). Vitamin E and organic selenium enhances the antioxidative status and quality of chicken cockerel semen under high ambient temperature. *Br Poult Sci* . 53:708–14; <https://doi.org/10.1080/00071668.2012.722192>

- [28]. Grossi S, Rossi L, De Marco M, Sgoifo Rossi CA. (2021). The effect of different sources of selenium supplementation on the meat quality traits of young charolaise bulls during the finishing phase. *Antioxidants* .10(4): 596. <https://doi.org/10.3390/antiox10040596>
- [29]. Pelyhe, C., & Mézes, M. (2013). Myths and facts about the effects of nano selenium in farm animals—mini-review. *Eur Chem Bull*, 2(12), 1049-1052. <https://doi.org/10.12737/21360>
- [30]. Tayeb, İ and Qader, G. (2012). Effect of feed supplementation of selenium and vitamin E on production performance and some hematological parameters of broiler. *KSÜ Doğa Bilimleri Dergisi*, 15(3), 46-56.
- [31]. Selim, N. A., Radwan, N. L., Youssef, S. F., Eldin, T. S., & Elwafa, S. A. (2015). Effect of inclusion inorganic, organic or nano selenium forms in broiler diets on: 2- Physiological, immunological and toxicity statuses of broiler chicks. *International Journal of Poultry Science*, 14(3), 144. <https://doi.org/10.3923/ijps.2015.144.155>
- [32]. El-Sheikh, A. M. H., Abdalla, E. A., & Maysa, M. H. (2010). The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. 2-Immune system and some physiological aspects in Bandarah chicks affected by organic selenium. *Egyptian Poultry Science Journal*, 30(2), 517-533. <https://doi.org/10.17221/1715-cjas>
- [33]. Mohapatra, P., RK. Swain, S.K. Mishra, T. Behera, P. Swain, S.S. Mishra; N.C. Behura, S.C. Sabat, K. Sathy, K. Dhama and P. Jayasankar. (2014). Effects of dietary nano-selenium on tissue selenium deposition, antioxidant status and immune functions in layer chicks. *Int. J. Pharmacol.*, 10: 160-167. <https://doi.org/10.3923/ijp.2014.160.167>
- [34]. Rizk, Y. S. (2018). Effect of dietary source of selenium on productive and reproductive performance of Sinai laying hens under heat stress conditions. *Egyptian Poultry Science Journal*, 37(2), 461-489.
- [35]. Abdul-Majeed AF, Abdul-Rahman SY.(2021). Impact of breed, sex and age on hematological and biochemical parameters of local quail. *Iraqi J Vet Sci.*35(3):459-464. DOI: 10.33899/ijvs.2020.126960.1432
- [36]. Muhammad, A. I., Mohamed, D. A., Chwen, L. T., Akit, H., & Samsudin, A. A. (2021). Effect of selenium sources on laying performance, egg quality characteristics, intestinal morphology, microbial population and digesta volatile fatty acids in laying hens. *Animals*, 11(6), 1681. <https://doi.org/10.3390/ani11061681>.