

# Iraqi Journal of Veterinary Sciences



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# Bioceutical role of nano and organic selenium on certain reproductive value of laying hen during force molting

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#### Article information

Article history: Received June 21, 2022 Accepted October 21, 2022 Available online February 25, 2023

*Keywords*: FSH LH Immature follicles Laying nano-Se

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Nanotechnology has proposed many new effective forms of nutritional supplement ingredients due to their low toxicity and high bioavailability, among which are nanoselenium, due to their distinctive properties of small particles and increased surface area of compounds, allowing the opportunity for biological interactions. In this study, 48 laying hens, aged 47 weeks, of a rose type were used, and the experiment lasted for two months. The birds were fed two types of diets, one standard and the other a non-standard diet, represented by the use of crushed yellow corn for molting. The birds were divided into six groups 8 birds/group, with the first group receiving a standard diet, the second group receiving only yellow tops crushed, the third group receiving organic-Se 2 g/L with a standard diet, the fourth group receiving Nano-Se 0.5 ml/L with standard stalk, the fifth group receiving organic- Se with straw 2 g/L, and the sixth group receiving nano-Se with straw 0.5 ml/L. The results showed a significant rise in ovary weight, oviduct weight, number of immature and mature follicles, FSH, LH, as well as an improvement in the histological characteristics of the shell gland in the groups treated with nano and organic-Se, indicating that nano-Se plays a critical role in restoring most reproductive and histological parameters to their normal levels, close to the baseline. This study found that organic and nano-Se are crucial in improving most reproductive and physiological parameters of laying hens exposed to molting.

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Abstract

#### Introduction

It is vital to have healthy, high-quality animal products enriched with micronutrients that support worldwide human health (1). Poultry products are the world's second most eaten food, accounting for roughly 30% of total meat output. Selenium has been identified as an essential micronutrient for boosting poultry performance, health, and antioxidant system (2). Both organic and inorganic forms of selenium are frequently utilized in animal feed, benefiting poultry productivity (3,4). However, numerous studies show that the organic form has a higher absorbance than the inorganic (5,6). Selenium is involved in the metabolism of various hormones, including thyroid hormones (7), enhancing antioxidant capacity in animals, regulating immunity, production performance, and fertility, and minimizing the adverse effects of free radicals (8). However, as the field of animal production has advanced, interest in nanotechnology has grown for feed additives in animal diets, particularly poultry diets. 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 more prominent, such as a larger surface area and higher absorption capacity, which makes it easier for them to reach the body's cells (9). The word nano comes from a Latin word that means to dwarf. Nanoparticles have sizes ranging from 1 to 100 nanometers (10). Nanotechnology is a relic of a bygone era. Over the last decade, there has been an increase in research into technologies that support nanotechnology. Many companies have specialized in manufacturing new forms of nano-sized materials concerned with poultry and livestock production systems to improve animal production efficiency. The study aimed to identify the physiological role of organic and nano selenium in hens during force molting.

#### Materials and methods

A total of 48 rose-type laying hens were employed in this study. For the length of the two-month trial, the birds were grown in an open-type auditorium and ground-bred. The birds were fed on two types of rations: a regular ration and a non-standard ration, symbolized by the use of crushed yellow maize for molting. According to NRC (11), feed and water were freely distributed using plastic troughs and manholes. The birds were divided into six groups of eight birds each. The first group represented control with a standard diet, the second group molting with yellow corn crushed only, the third group organic selenium at a rate of 2 g/L with a standard diet, and the fourth group nano-selenium was supplemented at a rate of 0.5 ml / L with standard stalk. The fifth group supplemented organic selenium with molting at 2 g/L. The sixth group was supplemented with nanoselenium with molting at a rate of 0.5 ml/L.

#### **Ethical approve**

The University of Mosul, College of Veterinary Medicine's Animal Care and Use Committee evaluated and approved each step of the experiment's protocols.

#### Sampling

The birds were slaughtered once the experiment was completed to get blood samples by cutting the jugular vein. Vacutest test tubes containing a gel material were employed for this purpose. The samples were allowed to clot at room temperature before being quickly discarded by a centrifuge (3000 cycles/minutes), and the serum was obtained and kept at -20°C in Eppendorf tubes for 15 minutes to measure the levels of follicle-stimulating hormone and luteinizing hormone using the ELISA technique (12-14). After the trial, each bird was anatomically characterized, with samples

obtained from the shell gland tissue to determine the thickness of the mucosal epithelium, the thickness of the cavity between the microvilli, and the number of tubular glands and goblet cells. The measurements were taken with a Color digital camera HMDC-5 coupled to an optical microscope. This camera has built-in image analysis software (Scope image-0.9) to collect these measurements (15,16).

#### **Statistical examination**

The records have been statistically tested by one-way analysis of variance (ANOVA), and Duncan's more than one variety test became used to check the importance of variations among corporations on the opportunity level ( $P \le 0.05$ ) to investigate the records the use of the SPSS program (17).

#### Results

Table 1 shows a significant decline in the weight of the ovary, the length of the oviduct, the number of developing follicles, and the number of mature follicles in the molting group compared to the control group at the probability level (P $\leq$  0.05), with a significant increase in the weight of the oviduct when compared to the control group, organic and nano-selenium treatments at concentrations of 2g/L and 0.5ml/L increased ovary weight, oviduct length, and the number of developing and mature follicles significantly. Regarding the number of growing follicles, the nanoselenium group outperformed the organic selenium group. The results of the statistical analysis showed that the organic and nano-selenium groups with molting had a significant increase in ovary weight, oviduct length, and the number of developing and mature follicles with a decrease in oviduct weight compared to the molting group alone. The nano selenium group with molting significantly increased the weight of the ovaries and the number of follicles, the developing group compared to the organic selenium with molting. The weight of the ovary and the number of developing and mature follicles reverted to normal in the group treated with nano-selenium with molting, identical to the control group.

Table 1: Effect of nano and			

Groups	Ovary weight (g/100g B.W.)	Oviduct weight (g/100g B.W.)	Oviduct length (mm)	No. immature follicles	No. mature follicles
Control	3.09±0.12 b	1.21±0.08 b	60.81±0.46 b	72.60±1.72 c	6.49±0.24 b
Molting	2.24±0.04 d	1.37±0.01 a	47.22±1.42 d	49.20±0.66 e	2.60±0.40 c
Organic selenium	3.19±0.08 a	1.17±0.01 bc	62.51±0.39 a	87.00±3.20 b	8.20±0.37 a
Nano-selenium	3.29±0.05 a	1.20±0.03 b	63.34±0.30 a	103.40±3.09 a	8.40±0.24 a
Organic selenium+ molting	2.93±0.12 c	1.06±0.04 c	53.52±0.50 c	70.89±1.87 d	5.60±0.24 b
Nano-selenium + molting	3.11±0.07 a	1.05±0.02 c	52.80±0.19 c	73.24±1.67 c	6.40±0.50 b

Small letters within the same column indicate a significant difference among the groups at  $P \le 0.05$ .

Table 2 indicates that the thickness of the cavity between the microvilli increased significantly at the probability level (P $\leq$  0.05). In contrast, the thickness of the epithelium of the mucosal layer, the number of tubular glands, and the number of goblet cells decreased significantly in the molting group compared to the control group. When compared to the control group, the organic and nano-selenium groups showed significant superiority in the thickness of the mucous layer epithelium with a significant decrease in the thickness of the cavity between micro-villi, and the nano-selenium group caused a significant difference in the thickness of the cavity between micro-villi and numbers of goblet cell compared with organic Se. In comparison to the molting group alone, the organic and nano-selenium groups with molting at 2 g/L and 0.5 ml/L showed significant superiority in the thickness of the mucous layer epithelium, the number of tubular glands

and goblet cells, and a significant decrease in the thickness of the cavity between the microvilli. When compared to the group of organic selenium with the molting, the thickness of the epithelium of the mucosal layer and the thickness of the lumen between the microvilli increased significantly. The results above show that the nano selenium with the molting group enhanced the thickness of the mucous laver epithelium, outperformed the control group, and returned it to its normal value. Although there were no significant differences in the number of tubular glands and goblet cells between the micro-villi of the nano-selenium group with molting and the control group, there was a significant decrease in the thickness of the cavity between the microvilli of the nano-selenium group with molting compared to the control group. It was mathematically close to its normal values.

Table 2: Effect of nano and organic selenium on some histological values of shell gland in laying hen during forced molting

Groups	Epithelium thickness (µM)	Microvilli space (µM)	No. tubular gland	No. goblet cell
Control	46.02±2.62c	72.88±14.41b	9.20±1.15a	5.40±0.50b
Molting	29.26±2.59e	167.98±10.10a	4.00±0.70c	3.20±0.86c
Organic selenium	47.20±1.77b	66.82±5.12e	7.80±0.73ab	4.80±0.66b
Nano-selenium	48.24±3.90b	69.52±5.85d	8.20±1.39ab	10.00±0.70a
Organic selenium + molting	43.96±2.85d	68.42±5.92d	5.60±0.92b	$5.80 \pm 0.58b$
Nano-selenium + molting	59.84±9.62a	70.34±6.39c	7.00±0.83ab	5.98±0.37b

The small letters within the same column indicate a significant difference among the groups at  $P \le 0.05$ .

The results of the statistical analysis in table 3 refer to a significant decrease in the levels of follicular stimulating hormone and luteinizing hormone in the lash group compared to the control group at the level of probability ( $P \le 0.05$ ). The levels of both hormones increased significantly in the organic and nano-selenium groups with concentrations of 2g/L and 0.5ml/L, respectively, as compared to the control group, with the nano-selenium group outperforming the organic selenium group in the level of follicle-stimulating hormone. The organic and nano-selenium groups with molasses had significantly higher levels of follicle-stimulating hormone and luteinizing hormone than the

molting alone group, and the nano-selenium group with molting had significantly higher levels of the two hormones than the organic selenium group with molting. Compared to the control group, the organic and nano-selenium groups with molting restored the levels of the two hormones to their normal values, and the nano-selenium group with molting was the most effective in improving the levels of the two hormones, in addition to improving the histological features of the shell gland in compare with control group (Figure 1-6).

Table 3: Effect of nano and organic selenium on follicular stimulating hormone and luteinizing hormone

Groups	Follicular stimulating hormone (ng/ml)	Luteinizing hormone (ng/ml)
Control	2.32±1.12e	2.59±2.10d
Molting	$0.98 \pm 0.09 f$	0.52±0.01e
Organic selenium	3.82±1.73b	3.92±2.81a
Nano-selenium	3.97±1.92a	3.98±2.94a
Organic selenium+molting	2.56±1.43d	2.86±2.41c
Nano-selenium+molting	2.88±1.61c	3.01±2.62b

The small letters within the same column indicate a significant difference among the groups at  $P \le 0.05$ .

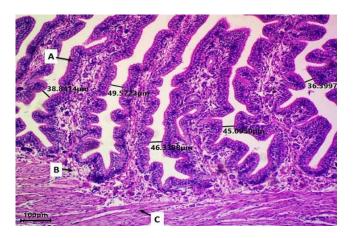


Figure 1: Histological section of the shell gland of the control group (normal diet) showing normal histological features represented by ciliated pseudo-columnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

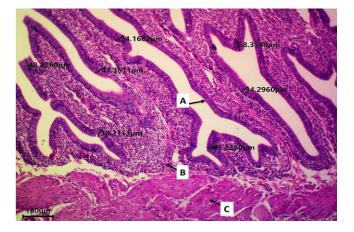


Figure 2: Histological section of the shell gland of the organic Se group showing histological features represented by ciliated pseudocolumnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

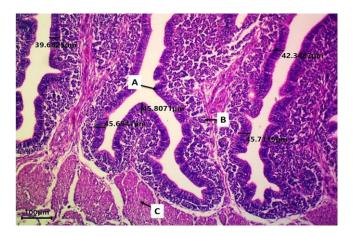


Figure 3: Histological section of the shell gland of the nano-Se group showing histological features represented by ciliated pseudocolumnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

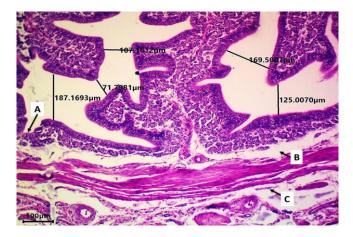


Figure 4: Histological section of the shell gland of molting group (yellow crone) showing abnormal histological features represented by ciliated pseudocolumnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

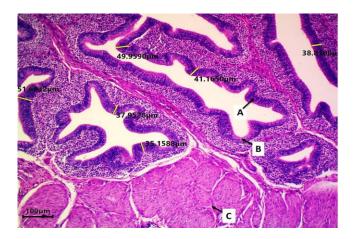


Figure 5: Histological section of the shell gland of the organic Se + molting group showing histological features represented by ciliated pseudocolumnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

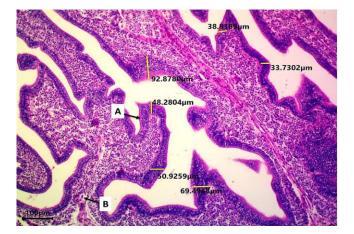


Figure 6: Histological section of the shell gland of the nano Se + molting group showing histological features represented by ciliated pseudocolumnar epithelial cells in the mucosal layer (A), submucosal layer (B), muscular layer (C), thickness measurements of the epithelium. H&E. X100.

#### Discussion

The process of molting causes morphological and physiological changes in the reproductive system of laying hens, as well as a difference in hormone levels due to their effect on the pituitary gland (18), with the results leading to a decrease in the weight of the reproductive organs, as well as morphological changes in the histological structure of the shell gland. The reason for this is that during the molting, the hen enters a state of oxidative imbalance, which causes a difference in reproductive hormone secretion as well as atrophy of the reproductive system organs and follicles (19,20). In this study, nano and organic selenium were used to combat the adverse effects of baldness, and the addition of selenium increased the weight of the genitals, as well as increased the levels of follicle-stimulating hormone and luteinizing hormone, as well as an improvement in the histological parameters of the cortex gland when compared to the control group and molting. Organic and inorganic forms of selenium are considered one of the most important sources of selenium in poultry feed. Both forms have good benefits on manufacturing performance, although according to reports, the organic form has a higher absorbency than the inorganic form (21). Organic selenium's beneficial effects can be linked to its role in boosting metabolism by lowering the detrimental effects of free radicals, enhancing avian immunity, raising poultry productivity and fertility, and selenium plays a significant part in the metabolism of several hormones (22,23). Selenium is one of the most significant microelements in producing glutathione peroxidase, an antioxidant enzyme (24,25). Although organic selenium supplementation has significant benefits, new evidence suggests that selenium nanoparticles play a critical role in increasing the weight of reproductive organs and the levels of follicle-stimulating hormone and luteinizing hormone. It also improves the histological structure of the cortex, as shown in this study's findings. Except for the relevance of organic selenium in impacting the gene expression of the cortex gland in hens (26), there has been no past studies into the effect of nanoscale selenium in the shell gland. The positive role of nano selenium can be attributed to the properties of nanoparticles (27). The relevance of selenium in enhancing the glandular features of the cortex can also be credited to selenium's importance in raising the number of erythrocyte and Hb, which offers a boost in the supply of O<sub>2</sub> to the cells and tissues of the body, thus regulating the cellular metabolism of nutrients within the body (28-30). It works to increase the surface area of compounds as a result of the small size of its molecule, which allows for biological reactions and prolongs the survival of compounds in the digestive system and gives a broader scope for enzymatic reactions, and reduces the effect of the intestinal excretion mechanism, resulting in a more extended period for digestion and absorption (31). It also ensures that active chemicals are absorbed by cells and delivered to the target organs efficiently (32). Estrogen is the key reproductive hormone regulates the reproductive system's that growth, development, maturity, and function (33-35). The nanoselenium contributes significantly to the increase in estrogen levels in rabbit diets. Nano-selenium at 0.3 mg/kg of feed increased estrogen (36). When given at a concentration of 4 ml/kg of body weight, selenium improves the effectiveness of the ovaries, estrogen, and progesterone (37). The number of tubular glands and goblet cells increased, which increased the thickness of the epithelium of the mucous layer of the cortical gland and an increase in the lumen between the microvilli, the antioxidant role of selenium in improving the internal environment of the bird through its antioxidant role and protection from the harmful effects of free radicals resulting from metabolism, increasing glutathione activity, and lowering the level of malondialdehyde can be attributed to the important role of selenium in improving the internal environment of the bird through its antioxidant role and protection from the harmful effects of free radicals resulting from metabolism (38,39). According to the findings, selenium supplementation in both organic and nano forms enhanced most of the reproductive and histological features of laying hens, with nano selenium outperforming organic selenium in restoring most of the values to normal. Furthermore, nanoparticles' superior efficacy is due to their small particle size, vast surface area, increased mucosal permeability, and improved intestinal absorption (40).

#### Conclusion

We infer that nano-selenium increase fertility, histological structure of the cortical gland, and reproductive hormones in laying hen exposed to molting. Because of the relevance of nano-complexes in poultry feeding and their importance in chicken feeding, nano-complexes may be used to replace nutritional supplements.

#### **Conflict of interest**

There are no conflicts of interest associated with the publication of this paper, according to the researcher.

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## الدور الحيوي للسلينيوم النانوي والعضوي في بعض القيم التناسلية للدجاج البياض خلال القلش الإجباري

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#### الخلاصة

في الوقت الحاضر ، اقتر حت تقنية النانو العديد من الأشكال الفعالة الجديدة . لمكونات المكملات الغذائية نظرًا لسميتها المنخفضة وتوافرها الحيوي العالى من بينها جزيئات السلينيوم النانوي، بسبب خصائصها المميزة المتمثلة بصغر جزيئاتها وزيادة المساحة السطحية للمركبات مما يتيح الفرصة للتفاعلات البيولوجية. استخدم في هذه الدراسة ٤٨ طائر من الدجاج البياض بعمر ٤٧ أسبوع من نوع روز استمرت التجربة لمدة شهرين. غذيت الطيور على نوعين من العلائق هما عليقة قياسية والأخرى هي عليقة غير قياسية المتمثلة باستخدام مجروش الذرة الصفراء لغرض إحداث القلش قسمت الطيور بصورة عشوائية لستة مجاميع بواقع ٨ طائر / مجموعة حيث مثلت المجموعة الأولى السيطرة مع عليقة قياسية، الثانية شملت مجموعة القلش مع مجروش الذرة الصفراء فقط، الثالثة مجموعة السلينيوم العضوي بتركيز ٢ غم / لتر مع عليقة قياسية، الرابعة مجموعة السلينيوم النانوي بتركيز ٥, •مل/لتر مع عليقة قياسية، الخامسة مجموعة السلينيوم العضوي مع القلش بتركيز ٢غم/لتر، السادسة مجموعة السلينيوم النانوي مع القلش بتركيز ٥٠ مل/لتر. أظهرت النتائج زيادة معنوية في وزن المبيض، وزن قناة البيض، أعداد الجريبات النامية والناضجة مع زيادة في مستوى هرمون محفز الجريبات والهرمون الوتيني إضافة الى تحسن في الصفات النسجية لغدة القشرة للمجاميع المعاملة بآلسلينيوم النانوي والعضوي مع الدور الحيوي المهم للسلينيوم النانوي في إعادة معظم المعايير التناسلية والنسجية الى مستواها الطبيعي المقارب لمجموعة السيطرة. استنتج من هذه الدراسة بان السلينيوم العضوي والنانوي لهما دور مهم في تحسين معظم الصفات التناسلية والفسلجية للدجاج البياض المعرض للقلش الإجباري.