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Influence *of in-ovo* and after hatching treatment with inorganic selenium on productive performance, some hormones and antioxidant status of broiler

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Abstract: The current study aimed to evaluate the effects of inorganic selenium (sodium selenite) added to the drinking water of hatched chicks from eggs injected with it on productive performance, antioxidant status, and some hormonal parameters. 216 unsexed one-day-old broiler chicks were distributed into 6 groups (3 replicates, 12 birds/replicate) till 42 days of age, as follows: The first (1^{st}) and second (2^{nd}) groups: hatched chicks came from non-injected eggs without any substance. The third (3^{rd}) and fourth (4^{th}) groups: hatched chicks came from eggs injected with deionized water (0.2 ml/egg) in the chorioallantoic sac, while the fifth (5^{th}) and sixth (6^{th}) groups: hatched chicks came from eggs injected with 0.02 mg sodium selenite/egg in the chorioallantoic sac. Chicks of the 1^{st}, 3^{rd}, and 5^{th} groups were given tap water, while chicks of the other groups were given drinking water supplemented with 0.3 mg sodium selenite/L. Inorganic selenium significantly improved the initial and final body weight, weight gain, feed conversion ratio, and significantly reduced the feed consumption at 35 and 42 days of age. It also enhanced vitality percentage, productive index, and reduced mortality percentage, and it was significant ($P \leq 0.05$) at 42 days of age. On the other hand, *in-ovo* injection with sodium selenite reduced malondialdehyde and corticosterone values, while total antioxidant capacity and leptin increased, and continuing adding it with drinking water led to a significant decrease in malondialdehyde while leptin and thyroxine values significantly increased. In conclusion, the *in-ovo* injection with sodium selenite and continuous treatment with it after hatching improve the antioxidant status and productive performance of the broiler, and it can be recommended to use sodium selenite in early egg nutrition during incubation period and its supplementation with drinking water of chicks after hatching.

Keywords: Antioxidant, Broiler, In-ovo nutrition, Leptin, Sodium selenite, Productive performance, Thyroid hormones.

تأثير الحقن والمعاملة بعد الفقس بالسيلينيوم غير العضوي في الأداء الإنتاجي وبعض الهرمونات وحالة مضادات الأكسدة لفروج اللحم

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المستخلص: هدفت الدراسة الحالية إلى تقييم تأثير السيلينيوم غير العضوي (الصوديوم سيلينيت) المضاف إلى ماء شرب الأفراخ الفاقسة من البيض المحقون به على الأداء الإنتاجي، وبعض الهرمونات وحالة مضادات الأكسدة. وزع 216 فرخاً بعمر يوم واحد غير مجنس في 6 مجموعات (3 مكررات، 12 طائراً/ مكرر) ولغاية عمر 42 يوماً، كانت المجموعات كالآتي: المجموعة الأولى والثانية: أفراخها من بيض غير محقونة بأية مادة. المجموعة الثالثة والرابعة: أفراخها من بيض محقونة بماء منزوع الأيونات (0.2 مل/ بيضة) في الكيس المشيمي اللقانقي، أما المجموعة الخامسة والسادسة: أفراخها من بيض محقونة بـ 0.02 ملغم من الصوديوم سيلينيت/بيضة في الكيس المشيمي اللقانقي. أعطيت أفراخ المجموعة الأولى والثالثة والخامسة ماء شرب اعتيادي، بينما أعطيت أفراخ المجموعات الأخرى ماء شرب مضافاً إليه 0.3 ملغم صوديوم سيلينيت/لتر. أدى السيلينيوم غير العضوي إلى تحسن معنوي في وزن الجسم الابتدائي والنهائي وزيادة الوزن ومعامل التحويل الغذائي، وقلل معنوياً من كمية العلف المستهلكة عند عمر 35 و42 يوماً، كما تحسنت النسبة المئوية للحيوية والدليل الإنتاجي وانخفضت النسبة المئوية للهلاكات وكان هناك فرقاً إحصائياً معنوياً بمستوى (P≤0.05) عند عمر 42 يوماً. من ناحية أخرى، أدى حقن البيض بالصوديوم سيلينيت إلى انخفاض معنوي في مستوى المالوندايالديهايد والكورتيكوستيرون، في حين ارتفعت معنوياً قيم مضادات الأكسدة الكلية وهرمون اللبتين، وأدى الاستمرار في إضافة الصوديوم سيلينيت إلى ماء الشرب إلى انخفاض معنوي في المالوندايالديهايد بينما ارتفع معنوياً مستوى هرمون اللبتين وهرمون الغدة الدرقية. بشكل عام، إن حقن البيض بالصوديوم سيلينيت واستمرارية المعاملة به بعد الفقس يحسن حالة مضادات الأكسدة والأداء الإنتاجي لفروج اللحم، ويمكن التوصية باستعمال الصوديوم سيلينيت في التغذية المبكرة للبيض أثناء فترة الحضانة وتعزيز ماء شرب الأفراخ بعد الفقس.

الكلمات المفتاحية: مضادات الأكسدة، تغذية البيض، فروج اللحم، اللبتين، الصوديوم سيلينيت، الأداء الانتاجي، هرمونات الغدة الدرقية.

INTRODUCTION.

The poultry industry faced great challenges in the world in terms of reproduction and production improvement (Zia *et al.,* 2017). Therefore, the search for natural ingredients that can be added to feed and water to achieve high production efficiency in poultry has become necessary, especially after the European Union banned the use of growth-stimulating antibiotics. Despite the positive and significant role of antibiotics in the development of the poultry industry, they are causing health damage to animals and humans due to consuming their products (Dibne and Richard, 2005). The research was focused on improving poultry production in ways that depended on adding antibiotic alternatives such as probiotics, plant extracts, and organic acids in addition to enzymes (Ahmed *et al.,* 2017; Pirgozliev *et al.,* 2015). In recent years, interest in the poultry industry has increased due to the wide development in its production, whether meat or eggs, compared to other animal products (Windhorst, 2006). The development of breeding programs for broilers also increased in order to increase the speed of growth in modern breeds, and because of genetic improvement, produced flocks were characterized by rapid growth and a high conversion coefficient. However, these flocks were characterized by rapid metabolism, which made them more susceptible to diseases and more susceptible to oxidative stress resulting from the increased generation of free radicals, which will affect the performance of poultry and therefore will need antioxidants for production (Surai, 2016).

As a result, recent reports have indicated that adding antioxidants to poultry feed can improve the antioxidant status, health, and performance of birds (Karadas *et al.,* 2014; Pirgozliev *et al.,* 2019). Selenium is one of the antioxidants added to broiler diets that can reduce the risks of oxidative stress.

Selenium is one of the mineral elements that researchers have paid attention to because it enters all cells and tissues of the body at various distribution rates. Selenium has many effects on the vital functions of both humans and animals (Zhang *et al.,* 2018; Edens and Sefton, 2016). Selenium plays an important role in the immune system function and growth of poultry (Liao *et al.,* 2012). It is also the main component of many antioxidant enzymes including glutathione peroxidase GSH-Px (Pilarczyk *et al.,* 2012), which protects the cell from free radical damage (Ighodaro and Akinloye, 2018). Selenium has an important role in the synthesis and metabolism of thyroid hormones (Brown and Arthur, 2001), also plays as cofactor of the enzyme (5 deiodinase), which is important in the synthesis of Triiodothyronine (T_3) hormone. The T_3 is important in controlling the growth of animals, especially poultry, by controlling energy and protein assimilation. Also selenium increases protein digestibility and energy utilization (Chen *et al.,* 2014; Saleh *et al.,* 2014). There are two types of selenium that can be added to poultry rations, organic selenium (selenomethionine and selenium-rich yeast, etc.), and inorganic selenium in the form of salts such as sodium selenite, which is the most widely used source of selenium (Surai and Fisinin, 2014; Yuan *et al.,* 2011).

Inorganic selenium has been used as a source of selenium to be added to poultry rations, as it is less expensive than the organic source and is absorbed from the intestinal lining by simple diffusion (Surai and Fisinin, 2014). Recommended concentrations for adding selenium to broiler diets are 0.15 ppm throughout the growth period, and toxic concentrations are 10-20 mg/kg feed (N.R.C., 1994). However, it was recommended (European Commission, 2014) not to allow selenium to be given more than 0.5 mg/kg feed. Accordingly, the study aimed to determine the effect of inorganic selenium on the antioxidant status and productive performance of broilers.

MATERIALS AND METHODS

This experiment was conducted in the livestock fields/College of Agriculture and Forestry/ University of Mosul. The current study aimed to evaluate the effects of sodium selenite (SS) addition to the drinking water of chick's strain Ross-308 hatched from *in-ovo* injected eggs with SS on productive performance, antioxidant status, and some hormonal parameters. The treatments were as follows:

- The 1st group: chicks hatched from non-injected eggs and reared on a standard ration and tap water.
- The 2nd group: as in the 1st group except that the chicks were reared on drinking water supplemented with SS 0.3 mg/L.
- The 3rd group: chicks hatched from *in-ovo* injected eggs with D.W. (0.2 ml/egg) in the chorioallantoic sac and reared on a standard ration and tap water.
- The 4th group was as in the 3rd group except that the chicks were reared on drinking water supplemented with SS 0.3 mg/L.

- The 5th group: chicks hatched from *in-ovo* injected eggs with SS 0.02 mg/egg inthe chorioallantoic sac and reared on a standard ration and tap water.
- The 6th group: as in the 3rd group except that the chicks were reared on drinking water supplemented with SS 0.3 mg/L.

The treatments continued to 42 days of age, bird were raised under ideal environmental conditions of ventilation and lighting and fed standard ration according to (N.R.C., 1994) containing 20.16% crude protein and 2841.6 kcal/kg metabolizable energy. At the end of the experiment, the parameters of body weight (B.wt), feed consumption (FC), feed conversion ratio (FCR), weight gain rate, productive index, mortality rate, and vitality of birds at the ages of 35 and 42 days were studied. Blood samples were collected at days of age42, then the serum was isolated and stored at -20°C and used for the determination of total antioxidant capacity (TAC), glutathione (GSH), malondialdehyde (MDA), Leptin, corticosterone, insulin like growth factor-1 (IGF-1), thyroxine (T_4) and triiodothyronine (T_3) were determined by the use of a kit made by Elabscience, USA.

Statistical analysis was conducted as a factorial experiment with two factors, and to determine the differences between the groups were determined using the Duncan's test (Gupta *et al.,* 2016) for all the measurements covered by the study, at least significant differences (P≤0.05) and using the ready-made SAS program (SAS, 2009).

RESULTS AND DISCUSSION.

Tables 1 and 2 revealed that *in-ovo* injection with sodium selenite (SS) caused a significant (P≤0.05) increase in the initial B.wt of the hatched chicks, final body weight and weight gain 35 and 42 days of age compared with the negative and positive control. There was, also a significant decrease in FC in the SS injected group compared with the negative control group, and significantly improved FCR compared with both negative and positive control.

In regard to the continuity of treatments with SS in drinking water after hatching, the addition of SS to the drinking water significantly reduces (P≤0.05) FC and FCR for the two periods (35 and 42 days) as compared with the discontinuous treatment. For the interaction effects, the *in-ovo* injection with SS regardless of the after-hatching treatment showed the highest

Table (1) Effect of *in-ovo* injection with sodium selenite (SS) and its continuity after hatching on productive performance of broiler at 35 days of age.

Parameters / Treatments	Initial body weight of hatched chicks (gm)	Final body weight (gm)	Total weight gain (gm/bird)	Total feed consumption (gm/bird)	Feed conversion ratio (gm/bird)
Effect of *in-ovo* injection of Sodium selenite (During Incubation)					
Control (non- injected eggs)	44.16±0.26 b	1863.22±35.3 b	1891.06±35.60 b	3079.65±76.52 a	b71.70±0.0
*Positive Control (Eggs injected with deionized water)	43.87±0.38 b	1691.17±35.26 c	1647.30±35.15 c	3024.75±47.81 ab	1.83±0.03 a
**Eggs injected with Inorg. Se. (Sodium selenite)	a346.21±0.2	1982.61±25.96 a	a61936.40±25.9	2956.68±18.36 b	1.52±0.02 c
Effect of Continuity of Sodium selenite Treatment After Hatching					
Discontinuous Inorg. Se. (Water without Inorg. Se.)	44.78±0.48 a	1845.46±46.46 a	1800.68±46.04 a	3101.19±42.93 a	1.73±0.06 a
Continuous Inorg. Se. (Water with Inorg. Se.)	44.72±0.39 a	1845.87±52.33 a	1801.15±52.22 a	2939.53±26.24 b	1.64±0.05 b
Effect of The Interactions					
Non-injected Eggs + Tap Water	44.66±0.22 b	1787.33±3.05 b	1742.67±3.02 b	3224.15±52.66 a	a31.85±0.0
Non-injected Eggs + Water with Inorg. Se.	43.66±0.22 b	1939.11±22.11 a	1895.44±22.19 a	2935.14±74.97 b	1.55±0.05 b
Eggs injected with D.W. + Tap Water	43.41±0.65 b	1734.17±44.98 bc	1690.76±44.35 bc	3120.63±29.58 a	1.84±0.05 a
Eggs injected with D.W. + Water with Inorg. Se.	44.33±0.33 b	1648.17±48.41 c	1603.83±48.20 b	2928.87±36.93 b	±0.05 a31.8
Eggs injected with Inorg. Se. + Tap Water	46.27±0.47 a	2014.89±40.56 a	1968.62±40.47 a	2958.77±24.13 b	1.50±0.04 b
Eggs injected with Inorg. Se. + Water with Inorg. Se.	46.16±0.19 a	1950.34±26.16 a	1904.17±26.16 a	2954.59±33.15 b	1.55±0.008 b

- Values (Means ±Standard Errors) with different letters vertically indicate presence of least significant difference at probability level (P≤0.05).
- *Injecting deionized water 0.2 ml/egg in the chorionic sac on the tenth day of incubation.
- **Sodium selenite is injected on the tenth day of incubation at a dose of 0.02 mg/egg and 0.2 ml/egg in the chorioallantoic sac.

- Continuous treatments: Sodium selenite was added to the drinking water of the hatched chicks at a dose of 0.3 mg/L until 42 days of age.

Table (2) Effect of *in-ovo* injection with sodium selenite (SS) and its continuity after hatching on productive performance of broiler at 42 days of age.

Parameters / Treatments	Initial body weight of hatched chicks (gm)	Final body weight (gm)	Total weight gain (gm/bird)	Total feed consumption (gm/bird)	Feed conversion ratio (gm/bird)
Effect of *in-ovo* injection of Sodium selenite (During Incubation)					
Control (non- injected eggs)	44.16±0.26 b	2393.67±22.12 b	2349.50±22.03 b	4553.77±66.89 b	1.94±0.07 b
*Positive Control (Eggs injected with D.W.)	43.87±0.38 b	±32.82 00.4235 b	2309.95±33.01 b	4758.86±45.99 a	2.06±0.04 a
**Eggs injected with Inorg. Se. (Sodium selenite)	a346.21±0.2	2615.33 ±44.22 a	2569.12±44.36 a	4402.33±61.92 c	1.71±0.02 c
Effect of Continuity of Sodium selenite Treatment After Hatching					
Discontinuous Inorg. Se. (Water without Inorg. Se.)	44.78±0.48 a	2435.89±36.15 a	2390.99±35.98 a	4672.95±101.36 a	1.96±0.06 a
Continuous Inorg. Se. (Water with Inorg. Se.)	44.72±0.39 a	2472.78±58.14 a	2428.06±57.83 a	4470.35±80.58 b	1.85±0.05 a
Effect of The Interactions					
Non-injected Eggs + Tap Water	44.66±0.22 b	2390.67±33.49 b	2346.00±33.27 b	4921.86±20.05 a	2.10±0.02 a
Non-injected Eggs + Water with Inorg. Se.	43.66±0.22 b	2396.67±36.28 b	2353.00±326.16 b	4185.68±48.41 d	1.78±0.03 b
Eggs injected with D.W. + Tap Water	43.41±0.65 b	2371.33±41.45 b	2327.57±42.10 b	4813.37±65.06 ab	2.07±0.06 a
Eggs injected with D.W. + Water with Inorg. Se.	44.33±0.33 b	2336.67±58.03 b	2292.33±58.02 b	4704.35±85.08 b	2.05±0.07 a
Eggs injected with Inorg. Se. + Tap Water	46.27±0.47 a	2545.67±60.92 a	2499.39±61.30 a	4283.63±45.33 d	1.71±0.05 b
Eggs injected with Inorg. Se. + Water with Inorg. Se.	46.16±0.19 a	2685.00±34.82 a	2638.84±34.96 a	4521.03±55.04 c	1.71±0.06 b

- Values (Means ±Standard Errors) with different letters vertically indicate presence of least significant difference at probability level ($P \leq 0.05$).
- *Injecting deionized water 0.2 ml/egg in the chorionic sac on the tenth day of incubation.

- **Sodium selenite is injected on the tenth day of incubation at a dose of 0.02 mg/egg and 0.2 ml/egg in the chorioallantoic sac.
- Continuous treatments: Sodium selenite was added to the drinking water of the hatched chicks at a dose of 0.3 mg/L until 42 days of age.

hatched chick B.wt (Table 1 and 2). Whereas the chicks hatched from the *in-ovo* injection with SS and those hatched from *in-ovo* injection eggs with SS recorded a significant increase in B.wt and W.G at 35 days of age, and those hatched from *in-ovo* injection eggs and treated after hatching with SS showed a significant increase in B.wt and GW at 42 days of age at ($P \leq 0.05$). They also showed a significant improvement in FCR at 35 and 42 days of age. On the other hand, the chicks hatched from the non-injected eggs recorded the highest FC values at 35 and 42 days of age ($P \leq 0.05$). These results agreed with the results of Wang *et al.* (2021) as it was noted that the addition of inorganic selenium at two different levels led to a significant increase in live body weight and a significant improvement in the feed conversion factor, as well as a decrease in the total mortality rate. The results of the current study agreed with the results of Ghazalah *et al.* (2020) as it was reported that the Final body weight, rate of weight gain and the improvement of the food conversion ratio when adding inorganic selenium compared with the control treatment. The reason for the improvement in product performance may be due to the fact that selenium is a necessary complement and activator of key enzymes in the manufacture and activity of T_3 hormone such as deiodinase, and that T_3 is an important regulator of growth in poultry by regulating energy production and protein metabolism, so selenium can increase growth by improving the digestion and assimilation of protein and energy consumption (Chen *et al.*, 2014 : Saleh *et al.*, 2014). Our results did not agree with the results of Prasoon *et al.* (2018) as no significant differences were observed in body weight, feed consumption, or feed conversion among all treatments.

Table 3 revealed that *in-ovo* injection with SS enhance significantly ($P \leq 0.05$) the productive index at 35 and 42 days of age. In addition, the addition of SS to drinking water after hatching (continuous treatment) reduced mortality percentage and increased vitality percentage and the productive index significantly at 42 days of age. Concerning the effects of interaction, the addition of SS to the drinking water of chicks hatched from *in-ovo* injected or non-injected eggs, and chicks hatched from *in-ovo* injected eggs with SS. Whether treated with SS with drinking water or not, reduced significantly mortality percentage at 35 and 42 days of age, and enhance significantly vitality% and productive index at 35 and 42 days of age ($P \leq 0.05$).

Table 4 showed that the *in-ovo* injection with sodium selenite SS improved the antioxidant status as represented by the significant reduction in MDA and the significant increase in TAC as compared with other treatments. Whereas the addition of SS to the drinking water of hatched chicks reduced the MDA significantly at ($P \leq 0.05$). For the effects of interaction, the *in-ovo* injection with SS and the addition of SS to the drinking water of non-injected eggs (negative control) and to chicks hatched from D.W. *in-ovo* injected eggs showed the lowest MDA values, whereas the chicks hatched from *in-ovo* injected eggs with SS showed the highest TAC values at P≤0.05. On the other hand, GSH values are not affected by the type of treatments or continuity of treatments or by interaction.

Table (3) Effect of *in-ovo* injection with sodium selenite (SS) and its continuity after hatching on mortality, vitality and product index of broiler at 35 and 42 days of age.

Parameters / Treatments	Mortality (%)	Vitality (%)	Product index	Mortality (%)	Vitality (%)	Product index
	35 days			42 days		
Effect of *in-ovo* injection of Inorganic Selenium (Sodium selenite) (During Incubation)						
Control (non- injected eggs)	5.09±1.32 a	94.90±1.32 a	302.20±23.19 b	6.94±1.18 a	93.05±1.18 a	276.08±14.39 b
*Positive Control (Eggs injected with D.W.)	4.17±0.94 a	95.83±0.94 a	252.71±8.43 c	4.63±1.17 a	95.36±1.17 a	259.93±9.09 b
**Eggs injected with Inorg. Se. (Sodium selenite)	2.31±0.85 a	97.63±0.85 a	366.39±11.11 a	4.17±0.62 a	95.83±0.62 a	348.58±11.36 a
Effect of Continuity of Sodium selenite Treatment After Hatching						
Discontinuous Inorg. Se. (Water without Inorg. Se.)	4.94±0.89 a	95.06±0.89 a	295.48±21.67 a	6.48±0.80 a	93.51±0.80 b	280.89±16.31 b
Continuous Inorg. Se. (Water with Inorg. Se.)	2.78±0.80 a	97.22±0.80 a	316.29±18.36 a	4.01±0.81 b	95.98±0.81 a	308.85±15.03 a
Effect of The Interactions						
Non-injected Eggs + Tap Water	7.40±0.92 a	92.59±0.92 b	255.21±2.49 b	8.33±1.60 a	91.60±1.60 b	248.81±10.29 d
Non-injected Eggs + Water with Inorg. Se.	2.78±1.60 b	97.22±1.60 a	349.18±21.84 a	5.56±1.60 ab	94.4±1.60 ab	303.34±13.66 bc
Eggs injected with D.W. + Tap Water	5.56±0.00 ab	94.44±0.00 ab	254.62±13.59 b	6.48±0.92 ab	93.51±0.92 ab	255.90±9.56 d
Eggs injected with D.W. + Water with Inorg. Se.	2.78±1.60 b	97.22±1.60 a	250.80±12.93 b	2.78±1.60 b	97.22±1.60 a	263.96±14.83 cd
Eggs injected with Inorg. Se. + Tap Water	1.85±0.92 b	98.14±0.92 a	377.12±21.38 a	4.63±0.92 ab	95.36±0.92 ab	337.92±22.80 ab
Eggs injected with Inorg. Se. + Water with Inorg. Se.	2.78±1.60 b	97.22±1.60 a	355.65±6.68 a	3.71±0.92 b	96.24±0.92 a	359.25±3.49 a

- Values (Means ±Standard Errors) with different letters vertically indicate presence of least significant difference at probability level (P≤0.05).

- *- Injecting deionized water on the tenth day of incubation 0.2 ml/egg in the chorioallantoic sac.
- ** Sodium selenite is injected on the tenth day of incubation at a dose of 0.02 mg/egg and 0.2ml/egg in the chorioallantoic sac.
- Continuous treatments: Sodium selenite was added to the drinking water of the hatched chicks at a dose of 0.3 mg/L until 42 days of age.

Table (4) Effect of *in-ovo* injection with Sodium selenite (SS) and its continuity after hatching on antioxidant of broiler at 42 days of age.

Parameters / Treatments	Malondialdehyde (MDA) (nml/mL)	Total Antioxidant Capacity (U/ml)	Glutathione (GSH) (µmol/L)
Effect of *in-ovo* injection of Inorganic Selenium (Sodium selenite) (During Incubation)			
Control (non- injected eggs)	11.02±0.4 a	11.20±0.55 b	0.627±0.005 a
*Positive Control (Eggs injected with deionized water)	10.52±0.45 a	11.69±0.82ab	0.615±0.008 a
**Eggs injected with Inorg. Se. (Sodium selenite)	9.05±0.25 b	13.66±0.66 a	0.618±0.009 a
Effect of Discontinued and Continuity of Inorganic Selenium (Sodium selenite) Treatment After Hatching			
Discontinuous Inorganic Selenium (Water without Inorganic Selenium)	11.53±0.49 a	12.75±0.69 a	0.616±0.007 a
Continuous Inorganic Selenium (Water with Inorganic Selenium)	8.86±0.30 b	11.61±0.49 a	0.625±0.006 a
Effect of The Interactions			
Non-injected Eggs + Tap Water	12.12±0.26 b	11.44±0.97 ab	0.633±0.01 a
Non-injected Eggs + Water with Inorganic Selenium	9.92±0.32 c	10.95±0.62 b	0.621±0.005 a
Eggs injected with deionized water + Tap Water	13.32±0.25 a	12.46±1.58 ab	0.601±0.01 a
Eggs injected with D.W. + Water with Inorganic Selenium	7.72±0.30 d	10.93±0.47 b	0.630±0.012 a
Eggs injected with Inorganic Selenium+ Tap Water	9.16±0.35 c	14.37±0.69 a	0.613±0.014 a
Eggs injected with Inorganic Selenium+ Water with Inorg. Se.	8.94±0.39 c	12.95±1.12 ab	0.623±0.014 a

- Values (Means ±Standard Errors) with different letters vertically indicate presence of least significant difference at probability level ($P \leq 0.05$).
- *Injecting deionized water 0.2 ml/egg in the chorionic sac on the tenth day of incubation.

- **Sodium selenite is injected on the tenth day of incubation at a dose of 0.02 mg/egg and 0.2 ml/egg in the chorioallantoic sac.
- Continuous treatments: Sodium selenite was added to the drinking water of the hatched chicks at a dose of 0.3 mg/L until 42 days of age.

The results of the current study agreed with the results of several researchers Gul *et al.* (2021); Ghazalah *et al.* (2020) and Li *et al.* (2018) those who confirmed that adding inorganic selenium to the diet of broilers reduced the level of malondialdehyde (MDA). In addition, the results agreed with the findings of Ghazalah *et al.* (2020) who reported that the addition of selenium led to an increase in the activity of total antioxidants. The improvement in antioxidant status analyses were related to the increased GSH-Px activity when inorganic selenium was added to the broiler diet (Okunlola *et al.*, 2015; Moslehi *et al.*, 2019) which may be reflected in the decrease in MDA levels and may enhance the TAC, as is integral of about thirty enzymes as GSH-Px (Zia *et al.*, 2016), and the elimination of free radicals in the animal body depend on the enzymatic antioxidant system consisting of GSH-P, CAT and SOD, as well as the non-enzymatic antioxidants like GSH (Galano and Alvarezidaboy, 2011).

Table 5 showed that *in-ovo* injection with SS significantly increased leptin hormone values as compared with the negative and positive control groups, whereas the *in-ovo* injection with D.W. significantly increased corticosterone values as compared with the negative control and SS injection ($P \leq 0.05$). Also, the addition of SS to drinking water after hatching (continuous treatment) significantly increased leptin and T_4 values as compared with the discontinued group. For interaction effects, the *in-ovo* injection with SS and the after hatching treatment with SS with drinking water showed the highest values in leptin and T_3, whereas the chicks hatched from *in-ovo* injected eggs with D.W. showed the highest corticosterone values regardless of the after hatching treatment with SS. On the other hand, the chicks that hatched from SS *in-ovo* injected eggs and the chicks that hatched from non-injected eggs (negative control) and those that hatched from D.W. *in-ovo* injected eggs (positive control) and treated with SS after hatching recorded the highest T_4 values at ($P \leq 0.05$).

The results agreed with Ghazalah *et al.* (2020) if it was noticed that the addition of selenium led to a significant increase in the concentration of thyroxine hormone in the treatment of adding sodium selenite. The results did not agree with Choupani *et al.* (2014) if it was noticed that the addition of selenium led to a significant increase in the concentration of T3 hormone in the treatment of adding selenium to the broiler diet.

Type 1 of iodothyronine deiodinase, which catalyzes the conversion of T_4 to active T_3, requires selenium for production (Jianhua *et al.*, 2000). Plasma T_3 concentration is produced by 5¯deiodination of thyroxin in non-thyroidal tissue, particularly the liver and kidney (Beckett *et al.*,1987). Burk. (2002) and Fairweather-Tait *et al.* (2011) concluded that selenium regulate the activities of IGF-1 hormone and thyroid hormones and enhancing the assimilation of fat, sugar and proteins which is essential to improve body growth.

Table (5) Effect of *in-ovo* injection with sodium selenite (SS) and its continuity after hatching on some hormones of broiler at 42 days of age.

Parameters / Treatments	Leptin (ng/ml)	Corticosterone (ng/ml)	Like growth factor (IGF-1) (ng/ml)	Tri-iodothyronine (T_3) (ng/dl)	Thyroxine hormone (T_4) (ng/dl)
Effect of *in-ovo* injection of Inorganic Selenium (Sodium selenite) (During Incubation)					
Control (non- injected eggs)	275.06±12.43 c	152.56±5.25 b	41.36±0.82 a	2.62±0.06 a	14.86±0.63 a
*Positive Control (Eggs injected with deionized water)	332.43±19.52 b	181.59±2.77 a	42.71±1.48 a	2.77±0.10 a	15.17±0.70 a
**Eggs injected with Inorg. Se. (Sodium selenite)	638.25±72.08 a	150.35±5.53 b	43.42±0.52 a	2.79±0.10 a	15.47±0.47 a
Effect of Discontinued and Continuity of Inorganic Selenium Treatment After Hatching					
Discontinuous Inorganic Selenium (Water without Inorganic Selenium)	322.30±21.55 b	162.93±5.68 a	41.91±0.95 a	2.64±0.07 a	14.02±0.39 b
Continuous Inorganic Selenium (Water with Inorganic Selenium)	508.20±66.24 a	160.07±4.95 a	43.07±0.71 a	2.81±0.07 a	16.31±0.38 a
Effect of The Interactions					
Non-injected Eggs + Tap Water	257.43±17.38 c	150.33±9.00 b	41.70±1.16 a	2.63±0.13 ab	13.35±0.60 b
Non-injected Eggs + Water with Inorganic Selenium	292.68±15.42 c	154.80±6.36 b	41.02±1.26 a	2.61±0.04 b	16.38±0.55 a
Eggs injected with deionized water + Tap Water	285.12±20.97 c	183.23±3.08 a	41.13±2.70 a	2.71±0.14 ab	13.30±0.34 b
Eggs injected with deionized water + Water with Inorganic Selenium	379.74±12.48 b	179.95±4.05 a	44.28±1.16 a	2.82±0.17 ab	17.05±0,58 a
Eggs injected with Inorganic Selenium + Tap Water	424.33±11.65 b	155.23±9.55 b	42.91±0.62 a	2.56±0.13 b	15.42±0.64 a
Eggs injected with Inorganic Selenium + Water with Inorganic Selenium	852.17±19.09 a	145.47±5.86 b	43.92±0.85 a	3.01±0.08 a	15.52±0.77 a

- Values (Means ±Standard Errors) with different letters vertically indicate presence of least significant difference at probability level ($P \leq 0.05$).

- *Injecting deionized water 0.2 ml/egg in the chorionic sac on the tenth day of incubation.
- **Sodium selenite is injected on the tenth day of incubation at a dose of 0.02 mg/egg and 0.2 ml/egg in the chorioallantoic sac.
- Continuous treatments: Sodium selenite was added to the drinking water of the hatched chicks at a dose of 0.3 mg/L until 42 days of age.

CONCLUSIONS.

In conclusion, *in-ovo* injection and continued addition of inorganic selenium to drinking water improved product performance and enhanced antioxidants in the blood serum of broiler.

RECOMMENDATION.

it can be recommended to use sodium selenite in egg nutrition during incubation period and its supplementation with drinking water of chicks after hatching in order to improves the physiological and productive performance of broiler.

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