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INFLUENCE OF INGESTION OF NANO-SELENIUM ON GROWTH PERFORMANCE, ANTIOXIDATIVE AND MUTAGENICITY STATUS IN SOMATIC CELLS OF NEW ZEALAND WHITE RABBITS

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Heat stress is one of many problems facing the modern rabbit production. This study was planned to ascertain the effect of ingestion of different sources of selenium on alleviation of heat stress in growing rabbits, productive Performance, mutagenicity in somatic cells and oxidative status in rabbits was used as indices for the study. A total number of 60 unsexed weaned New Zealand White (NZW) rabbits, aged 6 weeks and averaged 660±3 gm of body weight were randomly distributed into four experimental groups (15 rabbits of each). The first group was used as a control and received orally 2ml of saline solution every day, while the 2nd, 3rd and 4th groups were received orally selenium three sources of nano, organic and inorganic, respectively, at level of 0.3 mg/kg body weight every day using stomach tube. The experiment continued for 6 weeks. The sources of selenium were dissolved in 2ml of saline solution. Heat stress was induced by exposure of all rabbit groups to a temperature of 38 °C for 4 hours daily.

The results demonstrated that *heat stress significantly depressed body weight gain, feed intake, feed conversion ratio, empty carcass (%), dressing % and abdominal fat weight, while feeding nano-Se or organic form clearly alleviated these negative effects of heat stress as compared to those fed inorganic Se form and control groups. Also, heat stress caused increase in chromosomal aberrations, as well as it decreased the total nucleic acids and protein contents. In addition, nano and organic selenium treatments caused to decrease in all studied parameters relative to heat stress compared to those of inorganic Se form and control groups. In addition, ingestion of nano and organic source of*

selenium by rabbits caused significant improvement in total antioxidant capacity in blood and alleviated the negative effects of heat stress via reducing the malondialdehyde content in blood.

In conclusion, *feeding of nano and organic source of selenium enhance productive performance of rabbits by improving the antioxidative properties under heat stress. This effect could be due to their ability in decreasing frequencies of chromosomal aberrations and decreasing total nucleic acids and protein contents. The use of nano-Se or organic Se form appeared to be more significant than inorganic Se in alleviation the undesirable effects of heat stress.*

Keywords: Selenium forms, oxidative stress, chromosomal aberrations,

Heat is one of the most important stressors affecting animal production. The most obvious limitation of rabbit production is in regions with a hot climate. The thermo-neutral zone temperature in rabbits is around 18–21 °C (Habeeb *et al*., 1998). Thus, when rabbits are exposed to elevated ambient temperatures (Ta) imbalances are induced in their body temperature (Habeeb *et al*., 1999), at high temperature (30°C) the appetite is depressed, the productive and reproductive performances are impaired and the resistance to disease is decreased (Okab *et al*., 2008). Heat stress is one of the wide varieties of factors, which caused oxidative stress *in-vivo* (Kumar and Kumar, 2011), during summer and/or in the tropics. Reactive oxygen species (ROS), is consider the main cause of oxidative stress, and are constantly generated *in-vivo* as an integral part of metabolism. Reactive oxygen species can damage the cells, including damage to DNA, lipid membranes and proteins, if the production of reactive oxygen species be more than usual. Selenium is a dietary essential trace mineral having many of biological functions in the living system. Selenium (Se) plays a central role in enzymatic defense pathways against oxidative damage in tissues.

Nano-Se has attracted widespread attention due to its high bioavailability and low toxicity, because nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability and low toxicity (Wang *et al*., 2007). Since surface area-to-volume ratio increases with decreasing the particle size, selenium nanoparticles have high biological activity (Zhang *et al*., 2005), including anti-hydroxyl radical property (Gao *et*

al., 2002) and a protective action against the oxidation of DNA (Huang *et al*., 2003). Nano-Se possesses higher efficiency than selenite, selenomethionine, and (Zhang *et al*., 2005) and regulating selenoenzymes and exhibit lesser toxicity (Zhang *et al*., 2001).

So, this study was designed to investigate the effect of different sources selenium on alleviation of heat stress in growing rabbits. Productive performance, chromosomal aberrations and oxidative status in rabbits were used as indices for the study.

MATERIALS AND METHODS

The present study was carried out in private rabbits farm, near El-Nobariah city, El-Beherah Province, Egypt,

Flock management

The rabbits were housed in galvanized wire (50 × 50 × 45 cm) cages provided with feeders and automatic watery system, in a well-ventilated building, where the environmental temperature ranged between 23.6 ℃ to 34. 4 ℃ while, humidity was from 40.4 to 70.2 %. A period of 14-16 hours of day light was provided. Feed and clean fresh water were available all time *ad libitum* for all rabbit groups during the experimental period. The commercial basal pelleted diet was formulated to cover the nutrient requirements and contained 17% crude protein, 13.8% crude fiber, 2.3% fat and 2500 kcal digestive energy /kg diet. All nutrients, essential amino acids, minerals and vitamins in the experimental diets were adjusted according to the rabbit requirements of NRC (1977). Manure was dropped from the cages on the floor and were collected and removed daily. All rabbits were kept under the same managerial, hygienic and environmental conditions during April and May (2017).

Preparation and characterisation of nanoselenium (Nano-Se).

The chemicals used in present study were purchased from Sigma-Aldrich Company (Los Angeles, USA). Nanoselenium was prepared using chemical co-precipitation method according to Zhang *et al*., (2011). X-ray diffraction (XRD) and transmission electron microscopy (TEM) were used for characterization of selenium-nano particles **(**Se-NPs). Transmission Electron Microscopy (TEM) showed that the size of elemental selenium was 20-50 nm.

Experimental design

A total number of 60 unsexed weaned New Zealand White (NZW) rabbits, aged 6 weeks and averaged 660±3 gm body weight were randomly distributed into four experimental groups (15 rabbits of each). Each treatment was divided into three equal replicates, each of 4 rabbits. The 1st group (control group) received 2 ml of distilled water orally every day, while the 2nd, 3rd and 4th groups were orally received three forms of selenium, nano, organic (Sel-Plex™) and inorganic (Sodium selenite) forms respectively at level of 0.3 mg/kg body weight every day using stomach tube during the experimental period. The experiment continued for 6 weeks. The test ingredient was dissolved into 2 ml of distilled water and administered orally in the morning, before feeding. Heat stress was induced through exposure of all rabbit groups to a temperature of 38 °C daily for 4 hours starting from 10.00 A.M. to 14.00 P.M. using gas heaters then the rest of the day were kept at natural temperature.

***Productive performance and Carcass measurements*:**

Animals were weighed individual at the beginning of the experiment (6 weeks) and every two weeks thereafter up to 12 weeks. Feed intake was recorded individually each 14-d intervals and feed conversion ratio was calculated. Feed conversion ratio was calculated as feed consumed (g) divided by weight gain (g) and economic efficiency was calculated according to Raya *et al*. (1991).

At the end of the experimental, three rabbits from each group were slaughtered to study characteristics of carcass. Carcass weight was considered as the weight of fore part, intermediate part and hind part. The weight of additional edible parts included the weight of liver, heart and kidneys. Dressed meat weight was obtained as the sum of the carcass weight and the weight of the edible parts. Dressing yield was calculated by dividing the dressed meat weight by preslaughter weight and expressed as a percentage. Three tissue samples from hind leg muscle of rabbit of each treatment were stored at 4°C.

The samples were subjected to the analysis for determination of moisture, crude protein, ether extract (fat) and ash according to AOAC 2001 and water holding capacity (WHC) was estimated according to Nakamura and Katoh, 1985 after centrifugation of 1g of muscle to 4 minutes at 1,500 x g. The remaining water after centrifugation was quantified by drying the sample over night at 70 ° C. The WHC was calculated as follows: (sample weight after

centrifugation – weight after drying) x 100 / initial weight. Se concentrations in meat were measured by atomic absorption spectro photometry according to the method of Brown and Watkinson (1977). The samples were prepared for measuringby ultrasound sonication with a mixture of nitric acid and hydrogen peroxide. Lipid oxidation in minced samples of hind leg meat was measured by the thiobarbituric acid method according to Piette and Raymond (1999) at 0, 6 and 12days from refrigerated time storage. Thiobarbituric acid-reactive substances (TBARS) were expressed in mg of malondialdehyde per kg muscle.

Collection of blood samples

At the end of the experiment, during slaughter test, 5 ml of blood samples were taken at 09:00 am from the marginal ear vein under vacuum in clean tubes, centrifuged and serum is separated, kept on -20°C to perform some relevant analysis such as Oxidant/ antioxidant markers including malondialdehyde and total antioxidant were estimated using commercial kits (Bio Merieux, France) according to the procedure outlined by the manufacturer.

***Assays to detect chromosomal aberrations*:**

During slaughter test the bone marrow tissue was subjected to colchicines treatment (0.5 solution 0.1ml/culture), hypotonic treatment (KCI, 5.6 g /l), fixed in acetomethanol, spread, and stained by Giemsa stain, in 6.8 phosphate buffer (Evans,1987). To analyze the frequency and type of chromosomal aberrations, 100 well spread metaphases were analyzed under the microscope for each rat in either the therapeutic or double therapeutic dose-exposed animals. The chromosomal aberrations observed were classified as follows: Structural chromosomal aberrations which include gap, break, deletion and centromeric attenuation.

Determination of nucleic acid contents and total protein in tissue:

Quantitative changes in nucleic acid were determined by the method described by Bregman (1983). One gram of liver was homogenized in 5% trichloroacteic acid, centrifuged and boiled in a mixture of absolute ethanol and ethanol/ether mixture 3:1. After centrifugation, trichloroacteic acid 5% was added. The supernatant was separated to be ready to be quantified using specific reagents for DNA (DPA reagent) and RNA (orcinol reagent). Protein is estimated in tissue using commercial kits according to Lowry *et al.* (1951).

Statistical analysis:

Data were statistically analyzed according to Snedecor and Cochran (1982) using SAS (2001) Computer Program as the following fixed model:

$$Y_{ijk}=\mu + Ti + Rj + e_{ijk}$$

Where: Y_{ijk} = the observation; μ = Overall mean; Ti = Effect of treatments(i= 1,…4); Rj= Replicates (j=1,2,3); e_{ijk}= Random error component assumed to be normally distributed.

Duncan's multiple range tests was performed (Duncan, 1955) to detect significant differences among means

RESULTS AND DISCUSSION

Productive performance:

The effect of different Se sources on live body weight (LBW) and total body weight gain (TBWG) at different ages are presented in Table 1. Results show that LBW values were significantly the highest with rabbits received Nano-Se followed by the rabbits received organic Se as compared to the other treatment groups. The same trend was noted in body weight gain. These results are in agreement with Ebeid *et al*. (2012) who showed that feeding rabbits on diets supplemented with organic Se at levels from 0.15 up to 0.30 ppm had superior live body weight and daily weight gain compared with the control group. However, are in disagreement the present results, with Dokoupilova *et al*. (2007) who found that body weight gain of growing rabbits did not significantly affect by adding dietary selenium.

The improvement in body weight gain in these groups may be due to that nano-Se and organic Se are absorbed well from the gut compared to the other forms of Se. On the other side, the possible reasons are that Se nanoparticles may make selenium more effective in stimulating of the thyroid gland (T3) (Edens, 2002), where Se is an important auxiliary factor for the key enzyme of 5-deiodinase. The iodothyronine deiodinase enzyme convert the pro-hormone thyroxine (T 4) to the active form of triiodothyronine (T3). Triiodothyronine is a main hormone that regulates growth by controlling the body's energy and protein anabolism (Preter, 2000).

Feeding of different Se sources had no significant effect on total feed intake at all ages, except at 6-8 week of age. Feed conversion ratio was significantly the lowest with rabbits received nano selenium followed by the rabbits received

Table 1: Effect of different sources of dietary Se on live body weight and total weight gain of growing NZW rabbits exposed to heat stress

Items	Treatment groups				
	Control	**Nano-Se**	**Organic Se**	**Na-Se**	**Sig. test**
Live body weight (g)					
6 Wks	660.10 ±1.03	658.90 ±1.59	659.60 ±1.15	659.20 ±1.38	NS
8 Wks	869.90^{c} ±5.55	959.30^{a} ±6.08	948.70^{a} ±5.03	921.70^{b} ±7.52	*
10 Wks	1135.00^{c} ±6.93	1246.90^{a} ±7.48	1227.50^{a} ±6.03	1148.90^{b} ±6.38	*
12 Wks	1381.70^{b} ±8.44	1524.40^{a} ±5.50	1495.80^{a} ±4.50	1420.20^{b} ±8.33	*
Total body weight gain (g)					
6-8 Wks	209.80^{c} ±6.25	300.40^{a} ±6.18	289.10^{a} ±8.44	262.50^{b} ±6.70	*
8-10 Wks	265.10^{b} ±6.99	287.60^{a} ±8.74	278.80^{a} ±5.29	263.20^{b} ±6.85	*
10-12Wks	246.70^{b} ±10.16	277.50^{a} ±9.36	268.30^{a} ±8.01	235.30^{b} ±10.44	*
6-12 Wks	721.60^{b} ±8.59	865.50^{a} ±10.64	836.20^{a} ±5.05	761.00^{b} ±9.58	*

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: ($P \leq 0.05$).

Organic selenium compared to the other groups (Table 2). These results are in disagreement with the findings of Ebeid *et al*. (2012) who showed that feeding rabbits on diets supplemented with organic Se at levels from 0.15 up to 0.30 ppm led to inferior feed conversion ratios compared with the control group. It has already been reported that the lifetime of the trypsin and peroxidase increases dramatically by interfacing them with nanomaterials. This ability to enhance these enzymes stability may impact the numerous biological processes such as digestion, metabolism and nutrient uptake (Sharma *et al*., 2007).From the economical point of view, the highest economic efficiency was recorded with rabbits received nano selenium followed by the rabbits received organic selenium compared to the other groups. This increment may be due to the improvement of weight gain as a result of increasing of feed intake, while the worst value was observed for those received inorganic selenium Table 3.

Table 2: Effect of different sources of dietary Se on feed intake and feed conversion of growing NZW rabbits exposed to heat stress

Items	Treatment groups				
	Control	**Nano-Se**	**Organic Se**	**Na-Se**	**Sig. test**
Total Feed intake (g)					
6 -8Wks	783..90^{b} ±26.57	1007..20^{a}±21.70	983.75^{a} ±23.56	1054.49^{a} ±21.04	
8 -10 Wks	925.52±27.36	983.26±32.69	918.01±29.40	946.93±27.84	NS
10-12Wks	893.00±31.84	897.30±39.12	877.30±42.18	864.50±42.36	NS
6-12 Wks	2602.4^{b} ±49.03	2887.8^{a}±56.11	2779.1^{ab} ±52.62	2865.3^{a} ±54.24	
Feed conversion (g feed/g gain)					
6 -8 Wks	3.75 ^{a} ± 0.12	3.36 ^{b} ±0.07	3.40 ^{b} ± 0.03	4.00 ^{a}± 0.14	*
8 -10Wks	3.49 ^{ab} ± 0.03	3.42 ^{bc} ± 0.04	3.29 ^{c} ± 0.07	3.58 ^{a} ± 0.04	*
10-12Wks	3.63 ^{a} ± 0.05	3.23 ^{b} ± 0.07	3.26 ^{b} ±0.08	3.65 ^{a} ± 0.07	*
6-12 Wks	3.60 ^{b} ± 0.04	3.33 ^{c} ± 0.04	3.32 ^{c} ±0.04	3.76 ^{a} ± 0.05	*
Economical efficiency(%)					
6-12 Wks	0.9	1.1	1.1	0.6	

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: (P≤ 0.05).

Carcass traits:

The effect of different Se sources on carcass traits is shown in Table 3. No significant differences in the percentages of heart, kidney, liver, dressed weight, fore part, intermediate part and hind part were found among all groups except the abdominal fat percentages. Carcass weight percentages (the weight of fore part and intermediate part) in rabbits received organic selenium and nano-Se were slightly higher in these rabbit groups except hind part with inorganic selenium and the control groups. The improvement in carcass weights could be attributed to the increase in the live body weight of rabbits. Abdominal fat was lower in rabbits received organic selenium followed by the animals received nano-Se as compared to inorganic selenium (sodium selenite) and the control groups. These results are in agreement with Ebeid *et al*. (2012) who show that feeding rabbits on diets supplemented with organic Se at levels from 0.15 up to 0.30 ppm led to an increasing in both hot carcass weight and dressing percentage compared with the control group. Saleh *et al*. (2013) also, reported that carcass weight, dressing percentage and liver weight were increased; while, abdominal fat weight was decreased by dietary

Table 3: Effect of different sources of dietary Se on carcass traits of growing NZW rabbits exposed to heat stress

Items	Treatment groups				
	Control	Nano-Se	Organic Se	Na-Se	Sig. test
Pre slaughter Wt.	1370.00^{d}±7.12	1553.33^{a}±5.81	1510.00^{b}±5.77	1472.33^{c}±6.22	*
Heart ,%	0.294 ±0.001	0.297±0.002	0.299 ±0.002	0.298 ±0.002	NS
Liver, %	2.75±0.01	2.77±0.01	2.76±0.01	2.75±0.02	NS
Kidney, %	0.75 ± 0.01	0.76 ±0.01	0.78 ± 0.01	0.78± 0.01	NS
Abdominal fat, %	2.35 ^{a}± 0.01	1.96 ^{bc} ±0.11	1.85 ^{c} ± 0.09	2.13 ^{ab} ±0.05	*
Dressed weight,%	58.21 ± 3.97	58.05 ± 3.94	58.11 ± 3.95	58.08 ± 3.94	NS
Fore part %	30.67 ± 3.14	31.25 ± 4.25	34.53 ±4.03	32.43± 5.60	NS
Intermediate part, %	30.41±1.43	31.20±2.44	30.90±2.17	29.27±3.24	NS
Hind part , %	38.92±3.32	37.55±4.76	34.57±4.35	38.30±4.43	NS

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: ($P \leq 0.05$).

supplementation of organic selenium. Dokoupilova *et al*. (2007), reported that dressing-out percentage of growing rabbits did not affect by adding Se (Se-enriched yeast). The improvement in the performance of rabbits due to Se supplementation may be due to that decreases the oxidative stress and helps in the scavenging of ROS from the tissues, because Se is a cofactor for many antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase (El-Batal *et al*., 2012).

The effect of different Se sources on chemical composition (moisture, protein percentages, fat and ash) of hind leg meat is shown in Table 4. The results show significant differences for fat and protein percentage. Treatment with different Se sources caused significant increase in protein percentage as compared with control. The hind leg meat of rabbits fed Se contained less fat than that of control rabbits. These results are in agreement with the findings of Tatli and Seven, (2009). Dietary selenium intake may changes the energy metabolism through AMP-activated protein kinase (AMPK) which is a sensor of energy status that controls cellular energy homeostasis (He *et al*., 2016), therefore, high intake of energy led to increase total body protein deposition rate. Se supplementation had no effect on the moisture and ash concentration of hind leg meat (Table 4).

The effect of different Se sources on water holding capacity (WHC) of hind leg meat is shown in Table 4. The treatment groups with different Se sources caused significantly to increase in WHC as compared with control.

Table 4: Effect of different sources of dietary Se on chemical composition and meat quality of rabbit exposed to heat stress

Items	Treatment groups				
	Control	**Nano-Se**	**Organic Se**	**Na-Se**	**Sig. test**
Mosture %	70.72 ±0.12	70.67 ±0.13	70.65 ±0.11	70.75 ±0.11	NS
Crude protein %	19.43 c ±0.24	21.12 a ±0.13	20.96 a ±0.09	20.51 b ±0.08	*
Ether Extract %	6.57 a ±0.11	4.26 c ±0.08	4.47 bc ±0.11	4. 64 b ±0.10	*
Ash %	1.86 ±0.54	1.92 ±0.22	1.91 ±0.18	2.05 ±0.18	NS
Water-holding capacity %	50.72 c ± 0.12	60.83 a ±0.20	60.66 a ± 0.17	55.94 b ± 0.15	*
Selenium concentration µg/g	0.09 c ± 0.001	0.54 a ±0.01	0.52 a ± 0.01	0. 47 b ± 0.01	*
mg MDA/kg at 0d	2.31 a ± 0.04	1.22 b ±0.09	1.29 b ± 0.11	1. 41 b ± 0.12	*
mg MDA/kg at 6d	2.70 a ± 0.12	1.50 c ±0.10	1.62 bc ± 0.04	1. 84 b ± 0.07	*
mg MDA/kg at 12d	3.28 a ± 0.14	1.96 b ±0.17	2.03 b ± 0.17	2. 29 b ± 0.20	*

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: ($P \leq 0.05$).

The results are in agreement with Cardinali *et al*. (2015) who reported that supplementation of some antioxidants to rabbit rations increased meat WHC. This marked increase in WHC was probably related to the positive effect of Se as antioxidants on the integrity of muscle fibers, thereby enhancing their capability to retain water (Stanley 1991).

The effect of different Se sources on selenium concentration of hind leg meat is shown in Table 4. Selenium concentration was higher significantly in all groups of received selenium than in the control group. These results are in agreement with the results of Dokoupilová *et al.* (2007) who concluded that rabbits fed with organic Se was effective in retain of selenium in the meat tissues. These effects could act as a positive factor in order to improve rabbit meat consumption, turning it into a healthy food for human (Hernanández and Gondret, 2006). Selenium feeding as nano-Se and organic forms results in

higher tissue selenium concentrations than selenium feeding in the form of selenite. This has also been shown in pigs (Zhan *et al*., 2007) and chickens (Payne and Southern, 2005).

The effect of different Se sources on lipid oxidation of hind leg meat at different time storage is shown in Table 4. Treatment groups with different Se sources caused significantly to decrease in TBARS levels as compared with control group.

Thiobarbituric acid-reactive substances (TBARS) production was measured in the hind leg meat, which is more susceptible to oxidative deterioration due to its higher fat content. Lipid oxidation and discoloration are storage believed to be major causes of quality deterioration in meat during refrigerated storage.

The level of oxidation after refrigerated storage of rabbit leg meat has been studied by Hernández *et al*. (2008). They found that oxidative products were not very high in rabbit meat after refrigerated storage. However, there has been an increasing interest in using antioxidants in rabbit feeds to produce meat with high oxidative stability. These observations are consistent with those of López-Bote *et al*., (1998) who found that meat lipid oxidative stability was improved by increasing the level of antioxidant efficiency oats in rabbit diet. Also, vitamin E has also been effective in reducing lipid oxidation during refrigerated and frozen storage of meat (Lo Fiego *et al*., 2004).

Serum antioxidative status

Rabbits exposure to heat stress resulted in significant decrease in serum total antioxidant capacity and elevated serum malondialdehyde (MDA) which was obtained in the control group. However, rabbits receiving different sources of Se in the present study appeared to antagonize the effect of heat stress (Table 5). The total antioxidant capacity in blood serum of rabbits received nano-Se and those received organic selenium was significantly increased compared to the control and Se selenite groups. However, these treatments significantly reduced lipid peroxidation in serum expressed as serum MDA in comparison with the other groups. Similar to our finding, several studies showed that heat stress causes oxidative stress into tissue via increasing lipid peroxidation (MDA) Kim *et al*., (2010). Also, dietary organic selenium at different levels in rabbits showed a positive effect on total antioxidant capacity compared with control group (Abdulkareem, 2011). Also, the finding of this study is consistent with findings

of Zeweil *et al*. (2016) who showed that exposing growing rabbits to high temperature conditions during summer season resulted in significant decrease in serum total antioxidant capacity which was obtained in the control group, These results could be suggested that dietary addition of selenium decrease the oxidative stress level caused by heat stress.

Total DNA, RNA, and total protein contents:

The effect of Se source on total DNA, RNA, and total protein contents in rabbit blood are summarized in Table 5. The current results in the present study show that the values recorded for total DNA, RNA, and protein contents were significantly reduced in the control group compared with animals receiving different sources of Se. A highly significant increase was observed in nano-Se group followed by organic selenium group as compared to control and inorganic Se groups. These results are in agreement with those of Mostafa *et al*. (2009) who reported that hyperthermia has been shown to induce a number of effects in mammalian cells including inhibition of DNA, RNA, and protein synthesis. This finding could be explained by the over production of ROS free radical chain reactions which can cause oxidative damage to proteins and DNA (Stocker and Keaney 2004).

Table 5: Effect of different sources of dietary Se on serum antioxidative status, tissue protein and nucleic acid of growing NZW rabbits exposed to heat stress

Items	Treatment groups				
	Control	**Nano-Se**	**Organic Se**	**Na-Se**	**Sig. test**
Total antioxidant capacity mM/L	0.91 [b] ±0.01	1.56 [a] ±0.11	1.38 [a] ±0.13	0.99 [b] ±0.01	*
Malondialdehyde (MDA) nmol/ml	6.87 [a] ±0.22	3.62 [b] ±0.16	3.70 [b] ±0.11	6.02 [a] ±0.19	*
DNA in tissue (mg/g)	0.37 [c] ±0.02	0.42 [a] ±0.01	0.41 [a] ±0.02	0.39 [b] ±0.04	*
RNA in tissue (mg/g)	0.22 [c] ±0.007	0.27 [a] ±0.001	0.27 [a] ±0.001	0.26 [b] ±0.01	*
Total protein in tissue (g/g)	5.70 [b] ± 0.14	7.09 [a] ±0.31	7.04 [a] ± 0.20	7.03 [a] ± 0.22	*

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: ($P \leq 0.05$).

Aggregation of oxidative damaged DNA could lead to a reduced mRNA expression and protein production during heat stress (Shin *et al*., 2008). It well known that RNA is necessary for protein synthesis. The treatment of Se improved total DNA content, which could be attributed to the mechanism of chemo protection of selenium that related to its antioxidant properties as well as its ability to interfere with DNA repair pathways (Kara *et al*., 2007).

Chromosomal aberrations of bone marrow cells:

The effects of Se source on chromosomal aberrations of bone marrow cells in rabbit are summarized in Table 6. Data in Table 6 show various types of structural chromosomal aberrations which consisted of gaps, breaks, deletions and centromeric attenuations, fragmentation and toral aberration as affected by heat stress. The percentage of aberrations in bone marrow was significantly cause to reduce in all groups of rabbit treated with selenium. Our results in agreement with Yamada *et al*. (1989) and Waissenbourn and Obe, (1992) who reported that high temperature (41.5°C and 43 °C respectively) led to structural chromosome aberrations (breaks, stickiness, fragmentation). Heat may induce DNA base damage indirectly via protein damage (Takahashi, *et al*., 2004) and changes in enzyme complexes for DNA synthesis and repair (Streffer, 1995). The compounds that have antioxidant properties are known to produce anti-genotoxic effect by reducing toxic free radicals (Dusinska *et al*., 2003). Furthermore, the protective effect of selenium against the mutagenic effect, and the protective role of selenium might be related to its antioxidant effect.

In this study, although nano-Se followed by organic selenium could alleviate the negative effect of heat stress, sodium selenite at the same concentration (0.3 ppm) was less efficiency in this concern.

This difference might be due to the difference in absorption efficiency of nano-Se and sodium selenite. Hu *et al*. (2012) showed that absorption of nano-Se from the intestinal lumen into the body was higher than that of sodium selenite, while intestinal retention of nano-Se was lower than that of sodium selenite. The authors added that the organic selenium in selenium yeast is actively absorbed from the intestine via the Na+ dependent neutral amino acid pathway (Schrauzer, 2000).

Generally, the Se nanoparticles used in the present study were spherical in shape and had a size less than 100 nm according to Eszenyi *et al*. (2011). The nanoparticles of this size show high antioxidant activity (Torres *et al.*,

Table 6: Effect of different sources of dietary Se on chromosomal aberrations in bone marrow cells of rabbits exposed to heat stress

Items	Treatment groups				
	Control	Nano-Se	Organic Se	Na-Se	Sig. test
Gap	2.28^{a} ±0.04	1.58^{c} ±0.08	1.71^{bc} ±0.09	1.94^{b} ±0.08	*
Break	2.87^{a} ±0.05	1.53^{c} ±0.08	1.64^{bc} ±0.01	1.84^{b} ±0.07	*
Centromere attenuation	2.95^{a} ±0.07	1.47^{c} ±0.10	1.49^{c} ±0.10	1.89^{b} ±0.07	*
Deletion	0.75^{a} ±0.01	0.26^{c} ±0.01	0.28^{bc} ±0.01	0.31^{b} ±0.007	*

a-c Means within a row with different superscripts are significantly different.
Nano-Se: Nanoselenium, Na-Se : Sodium selenite, NS: Not significant, *: ($P \leq 0.05$).

2012) and have an increased ability to trap free radicals with greater antioxidant effect (Huang *et al*., 2003), and have an increased adsorptive ability due to interactions between the nanoparticles and NH, C=O, COO-, and C-N functional groups of proteins (Zhang *et al*., 2004). Additionally, nano-Se can act as a chemopreventive agent when administered at a smaller particle size (Peng *et al*., 2007).

CONCLUSION

This study clearly indicates that heat stress has a pro-oxidant characters and its administration is associated with induction of oxidative stress by the generation of free radicals that affect anti-oxidative system, as well as, decreased total antioxidant capacity with increase in MDA content leading to cytotoxicity. In addition, heat stress decreased total nucleic acids and liver protein contents. Co-administration of organic or inorganic selenium or selenium nano- particles (SeNps) ameliorates these disturbances and reduces the damage resulting from interaction between ROS and protein, RNA and DNA molecules.

These results could be attributed to their antiradical /antioxidant efficacy. Moreover, the data showed that the use of nano-Se appeared to be more effective than organic or inorganic selenium in attenuating the undesirable effects of heat stress and it has attracted a wide spread attention due to its high bioavailability, low toxicity and antioxidant activity.

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تاثير النانو سيلينيوم على كفاءة النمو والحالة التاكسدية والوراثة الخلوية فى الارانب النيوزليندى الابيض

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الإجهاد الحراري هو واحد من العديد من المشاكل التي تواجه انتاج الأرانب. تم استخدام مؤشرات كفاءة النمو والطفرات في الخلايا الجسدية والحالة التاكسدية كعوامل للدراسة. تم استخدام مصادر مختلفة من السيلينيوم لبيان تخفيف للإجهاد الحراري على الأرانب. تم تقسيم عدد 60 من الأرانب النيوزيلندية البيضاء ، عند أعمار 6 أسابيع ومتوسط الوزن 3± 660 جم بشكل عشوائي إلى أربع مجموعات (15 أرانب لكل مجموعة). تلقت المجموعة الأولى (المجموعة الضابطة) 2 مل من محلول ملحي عن طريق الفم كل يوم ، في حين تلقت المجموعات الثانية والثالثة والرابعة السيلينيوم عن طريق الفم بثلاثة مصادر نانو وعضوية وغير عضوية على التوالي ، عند مستوى 0.3 ملجم / كجم من وزن الجسم كل يوم باستخدام انبوبة خاصة. تم حل هذه المصادر في 2 مل من محلول ملحي.وتم تحفيز الإجهاد الحراري من خلال تعرض مجاميع الأرانب لدرجة حرارة 38 درجة مئوية يوميًا لمدة 4 ساعات واستمرت التجربة لمدة 6 أسابيع.

أظهرت النتائج أن الإجهاد الحراري أدى إلى انخفاض ملحوظ في وزن الجسم، كمية الغذاء المستهلك ، كفاءة التحويل الغذائى ، النسبة المئوية للذبيحة الفارغة، نسبة التصافى، ووزن الدهون في منطقة البطن، بينما المعاملة بالنانو-سيلنيوم يليها المعاملة بالسيلنيوم العضوي، خفف من حدة هذه التأثيرات السلبية للإجهاد للحرارى مقارنة مع تلك المعاملة بالسيلنيوم غير العضوي (فى صورة سلينات الصوديم) ومجموعة الكنترول. أيضا تسبب الإجهاد الحراري زيادة في الشذوذ الكروموسومى، فضلا عن أنه أدى إلى

انخفاض تركيز الحمض النووي والبروتين فى الخلايا. بالإضافة إلى ذلك، فقد خفضت المعاملة بالنانو سيلنيوم والسيلينيوم العضوي تاثيرالإجهاد الحراري مقارنة مع تلك المعاملة بالسيلنيوم الغير عضوي ومجموعة الكنترول.بالإضافة إلى ذلك، تسبب المعاملة بالنانو-سيلنيوم والمعاملة بالسيلنيوم العضوي للأرانب زيادات كبيرة في السعة التأكسيدية الكلية في الدم وتخفيف الآثار السلبية للإجهاد الحراري عن طريق الحد من محتوى malondialdehyde في الدم.

التوصية: نخلص من هذه الدراسة ، بان المعاملة بالنانو-سيلنيوم او المعاملة بالسيلنيوم العضوي تخفف من تاثير العبء الحرارى على الأرانب متمثلا فى تحسين كفاءة النمو وخفض الشذوذ الكرومسومى وتركيز الاحماض النووية والبروتين والحالة التاكسدية.