REPRODUCTIVE PERFORMANCE OF HEAT-STRESSED NEW ZEALAND WHITE RABBIT BUCKS IN RESPONSE TO DIFFERENT ZINC SOURCES

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SUMMARY

This study aimed to evaluate the effect of zinc supplementation from different sources to heat-stressed New Zealand White rabbit buck diets on some reproductive traits including semen quality, conception rate and litter size. Twenty four mature bucks were randomly assigned into four groups, with six males each. The first group reared under mild condition and received the basal diet with no supplementation. The other three groups were raised under heat stress condition and received the basal diet only or the basal diet supplied with 75 mg zinc either as zinc sulphate (inorganic zinc) or as zinc picolinate (organic zinc), for the second, third and fourth groups, respectively. It was found that dietary zinc supplementation reduced the heat stress-related increases in reaction time, and seminal plasma levels of alanine transaminase (ALT) and malondialdehyde (MDA). Decreased values of serum and seminal plasma testosterone, sperm count, sperm motility and conception rate induced by heat stress conditions were restored by zinc supplementation. Seminal plasma zinc and total antioxidant capacity (TAC) were significantly increased in zinc-treated groups when compared to both control groups in different conditions. Organic source of zinc was more potent than inorganic one in restoring levels of serum testosterone, seminal plasma MDA and testosterone, and sperm motility. From these results, it could be concluded that dietary zinc addition from different sources, especially zinc picolinate (organic form), is helpful in alleviating the negative effect of heat stress on reproductive traits of New Zealand White rabbit bucks.

Keywords: *New Zealand White Rabbit, zinc sulphate, zinc picolinate and reproductive performance.*

INTRODUCTION

The breeding season of rabbits in Egypt is usually through the period from October – April, to avoid the heat stress negative effects in summer season on reproductive and productive performance (Attia et al., 2011). The thermo-neutral zone temperature in rabbits is around 18–21°C (Marai and Habeeb, 1994). Environmental temperature above 28 ºC causes heat-induced physiological stress. Heat stress is aggravated when high environmental temperature is accompanied by high ambient humidity (Marai et al., 1996). Thermoregulation in rabbits is rather poor as the most sweat glands in rabbits are not functional and perspiration is not great due to the fur (Marai *et al.*, 2002b).

Heat stress negatively affects feed intake, feed utilization, water metabolism, reproductive traits, blood parameters, enzymatic reactions, hormonal secretions and mineral imbalances (Okab *et al*., 2008). Reproductive performance is depending on the production of sex hormones, especially testosterone, which in turn suppressed when stressors interfere with such hormones (Marai *et al*., 2002b). Heat stress can reduce luteinizing hormone (LH) secretion that important for spermatogenesis (Gilad *et al*., 1993). Thermal stress adversely affects semen quality of rabbits (El-Masry *et al*., 1994) by disturbing the normal physiological balance of animal body and hormonal pattern (Marai *et al*., 1991).

Heat stress increases mobilization and excretion of minerals and vitamins, consequently leads to marginal deficiencies and increased requirements of minerals and vitamins (Siegel, 1995). Habeeb et al. (2018) stated that hot climate conditions decreased blood and seminal plasma Zn by 16 and 26%.

Zinc is an essential trace element required for the action of more than 200 metalloenzymes (Shinde et al., 2006), and plays an important role in polymeric organization of DNA and RNA (García-Contreras et al., 2011), protein synthesis and cell division (Lukac and Massanyi, 2007). Zinc participates in ribonuclease (RNase) activity which is highly active during the mitosis of spermatogonia and meiosis of spermatocytes (Cheah and Yang, 2011); plays an important role in prostate, epididymal and testicular functions (Ebisch *et al*., 2003). Zinc can function as a temporary inhibitor for sperm lipid peroxidation, sperm oxygen uptake and sperm nuclear chromatin decondensation (Stephenson and Brackett, 1999). Zinc levels in seminal plasma have been positively associated with sperm concentration and motility (Chia et al., 2000).

Traditionally, Zn is supplemented in the animal diets as inorganic salt. However, recently the use of organic Zn for animals has gained popularity because of its reported higher bioavailability than inorganic sources (Droke *et al*., 1998). Barrie et al. (1987) reported that zinc picolinate appears to be absorbed significantly better than other sources. Very rare information is available about the effect of zinc picolinate supplementation on reproductive performance of rabbit bucks reared under heat stress conditions in comparable to those reared under mild conditions. Therefore, the present work aimed to study the effect of dietary zinc supplementation from different sources on reproductive capacity of heat-stressed New Zealand White rabbit buck diets.

MATERIALS AND METHODS

Animals, experimental design, diets and husbandry:

The present study was carried out in a private rabbit farm, Dakahlia governorate, Egypt, during the hot summer months from May to July. A total number of 24 New Zealand White (NZW) rabbit bucks at 32-36 weeks of age were randomly distributed into 4 treatment groups (6 bucks per each one). All groups were nearly similar average initial weights (2950 ± 140 g). Group 1 was kept in building under controlled environmental conditions (Fan and Pad Evaporative Cooling Systems). The other three groups (group 2, 3 and 4) were kept in the traditional rabbit building under uncontrolled environmental conditions, which was naturally ventilated through wire mesh windows. Groups 1 and 2 were fed the basal diet without supplementation. Whereas, group 3 and 4 were fed the basal diet supplemented with 75 mg zinc/kg diet from inorganic source (329.67 g zinc sulphate) or organic source (354.99 g zinc picolinate), respectively. The basal diet was formulated to cover the nutrient requirements of bucks according to the recommendations of De Blas and Mateos (2010). The composition and calculated chemical analysis of the basal diet are presented in Table (1).

Table (1): Composition and calculated chemical analysis of the basal diet.

Ingredient	%
Berseem hay	36
Barely grain	10
Yellow corn	15
Soybean meal	17
Wheat bran	20
Limestone	1
NaCl	0.5
Premix*	0.5
Total	100
Calculated chemical analysis:	
Crude protein	17.97
Ether extract	3.70
Crude fiber	14.28
Ash	8.78
Nitrogen free extract	55.27

*Premix (minerals and vitamins mixture) contains per kg: vit. A, 20000 IU; vit. D3, 15000 IU; vit. E, 8.33 g; vit. K, 0.33 g; vit. B1, 0.33; vit. B2, 1.0 g; vit. B6, 0.33 g; vit. B5, 8.33 g; vit. B12, 1.7 mg; pantothenic acid, 3.33 g; biotine, 33 mg; folic acid, 0.83 g; choline chloride, 200; manganese 80 g; zinc 60 g; iron30 g; copper 4 g; iodine 0.5 g; selenium 0.1 g; and cobalt 0.1 g

Air temperature and relative humidity inside the rabbit building were daily measured between 12:00 to 14:00 p.m. using an automatic thermo-hygrometer. Temperature-humidity index (THI) was calculated using the equation modified by Marai et al. (2001) as follows:

THI = db˚C – [(db˚C – 14.4)(0.31 – 0.31 RH)]

Where: db˚C = dry bulb temperature in ˚C and RH = relative humidity percentage/100.

The obtained THI values were then classified as follows: < 27.8 = absence of heat stress, 27.8 to < 28.9 = moderated heat stress, 28.9 to < 30.0 = sever heat stress and 30 and over = very sever heat stress. Recorded air temperature values ranged from 24.2 to 27.8˚C in controlled environmental condition, and from 30.7 to 37.6 in uncontrolled environmental conditions. The range of relative humidity was 70-80% in controlled environmental condition and 50-70% in uncontrolled environmental one. Based on these values, calculated temperature-humidity index (THI) range was 23.9-26.97 in controlled conditions (mild conditions), and 28.17-35.44 in uncontrolled conditions (heat stress conditions).

The rabbits were individually housed in galvanized wired cages (50×60×35cm). Each cage was provided with feeder, automatic nipple drinker and a bowl. Dropped urine and feces from the cages to the floor were cleaned. All rabbits were kept under the same managerial and hygienic conditions during the experimental period.

Semen collection:

The bucks were trained for artificial collection of semen by using the artificial vagina using a female teaser rabbit to study the semen traits. The temperature of the artificial vagina inner rubber sleeve was adjusted to 42- 46 ˚C and the inner sleeve was lubricated by white Vaseline. After three weeks from the beginning of the experiment, semen was collected every two weeks at 8:00 a.m. from each buck. A total of 96 ejaculates (four ejaculates from each buck) were collected through 4-10 weeks of experimental period. Libido was estimated by observing the reaction time which elapsed between introducing the female to the male till ejaculation.

Serum sampling:

At the end of the experimental period (After 10 weeks), blood samples were collected from marginal ear vein of buck in clean centrifuge tube without anticoagulant. Tube was placed in sloped manner for 30 min. to coagulate at room temperature, and then centrifuged for 20 min. at 3000 rpm to obtain the serum which kept in deep freezer at -20˚C until biochemical analysis of testosterone.

Investigated measurements:

Biochemical analysis of serum and seminal plasma: Semen hydrogen ion (pH) was measured using pH meter model 6010m (JENCO, San Diego, CA, USA). Total protein, albumin, total lipids, creatinine, Aspartate aminotransferase AST, Alanine Aminotransferase (ALT), alkaline phosphatase (ALP), zinc and initial fructose were estimated with non-enzymatic colorimetric methods. While, triglycerides, total cholesterol, HDL cholesterol, glucose and urea were determined with enzymatic colorimetric methods. Serum globulin was calculated by subtracting serum albumin from serum total protein. Albumin/globulin ratio (A/G ratio) was calculated by dividing serum albumin by serum globulin. Serum VLDL cholesterol was calculated by dividing triglycerides by 5. LDL cholesterol was calculated by subtracting serum HDL plus VLDL cholesterol from total cholesterol (Friedewald et al., 1972). Oxidant-antioxidant biomarkers were determined according to Koracevic et al. (2001) for total antioxidant capacity (TAC), and Ohkawa et al. (1979) for Malondialdehyde (MDA). Serum and semen testosterone was estimated by using Radio-Immuno-Assay (RIA) technique according to Abraham (1977).

Semen physical traits: Immediately after recording ejaculate volume without gel mass, the ejaculate was kept individually at 37°C in a water bath to sperm concentration, sperm motility (%), abnormal sperms (%) and dead spermatozoa (%).Motility percentage was estimated by using a microscope provided with a hot stage according to WHO (2010). Assessment of dead and abnormal spermatozoa was performed using an eosin-nigrosine blue staining mixture according to Blom (1950). Sperm cell concentration (×106/ml) was determined by the direct cell count using the improved Neubauerhaemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, Germany) according to Smith and Mayer (1955). The remaining whole semen was centrifuged at 3000 rpm for 10 minutes, and the supernatant (seminal plasma) was removed and stored in a deep freezer at -20˚C for biochemical analysis.

Conception rate and litter size: Each buck was randomly mated with 10 untreated does reared in mild conditions through the experimental period. The conception rate (%) for each buck was calculated as follows: Number of fertilized does/number of mated does×100. Litter size at birth was recorded per each doe and the average value was calculated per each buck.

Statistical Analysis:

Data were statistically analyzed with one-way ANOVA test by using the following statistical model: $Yij = \mu + Ti + eij$; where: Yij= observation, μ= the overall mean, Ti= treatment effect (mild condition, heat stress condition, and dietary inorganic and organic zinc), and eij= the experimental error. Duncan's Multiple Range Test (Duncan, 1955) was applied to compare the significant differences among the means. Statistical analysis tests were performed by using version 17 of SPSS software Statistical analysis program (SPSS, 2008).

RESULTS AND DISCUSSION

Serum testosterone and biochemical analysis of seminal plasma:

As shown in Table (2), serum testosterone levels were significantly decreased ($p<0.01$) in control group exposed to heat stress condition when compared to mild control one. The addition of zinc from the two sources increased significantly the decreased levels of testosterone due to heat stress, and this effect was maximized by organic zinc treatment to be comparable to mild condition control group. Seminal plasma pH and contents of total protein, albumin, total lipid, cholesterol and initial fructose, and activities of AST and ALP and were not differed significantly among trial groups. While, heat stress condition significantly increased ALT activity and MDA level, but significantly decreased seminal plasma Zn and testosterone concentrations, in comparable to mild control group. Insignificant increase in seminal plasma TAC level in heat stressed control group when compared to mild control one. Zinc addition to heat-stressed buck diets significantly reduced the high ALT activity and high MDA level, and increased significantly the low level of testosterone. Noteworthy, these values were similar with those in mild condition. TAC and Zn levels in seminal plasma were statistically higher in Zn supplemented groups than both control groups. It is worth mentioning that organic Zn administration was more effective than inorganic one in modulating these parameters in seminal plasma.

Our results, concerned with heat stress, agree with Habeeb *et al*. (2018) who found that serum and seminal plasma contents of testosterone and zinc were lower in bucks reared under hot summer season than under those reared under mild climate. El-Masry et al. (1994) stated that, during hot summer, there were significant rises in seminal plasma cholesterol, total lipids and transaminase enzymes (AST and ALT) levels, while total protein concentrations were significantly decreased. Soren *et al.* (2016) recorded significant increases in seminal MDA and TAC of bulls reared under tropical climatic conditions. El-Tohamy *et al.* (2012) stated significant decrease and increase in seminal plasma TAC and TBARS, respectively, in male rabbits reared in summer compared with those reared in winter.

Regarding to zinc effect, the present results are similar to those of El-Masry *et al.* (1994) and El-Speiy and El-Hanoun (2013) who found that treating male rabbits, reared in hot summer conditions, with 3 ml zinc sulfate increased significantly serum testosterone and seminal plasma testosterone and antioxidant enzyme activities, but significantly decreased transaminase enzymes and TBARS. El-Hawary et al. (2018) found that feeding heat-stressed Friesian Bulls on organic zinc significantly decreased seminal plasma AST, ALT and ALP, and significantly increased serum testosterone and seminal plasma antioxidant enzymes and fructose.

Low serum testosterone induced by heat stress in the present study may attribute to the depression in hypothalamic hormone releasing factors and consequently the pituitary hormones like luteinizing hormone (LH) (Gilad *et al*., 1993; Marai and Habeeb, 1997). The extreme changes occur in biological functions in animals like depression in feed intake and utilization as well as disturbances in water, protein, energy and mineral metabolism may be responsible for reducing testosterone secretion (Habeeb et al., 2018). Hunt et al. (1992) reported that low Zn levels have a negative effect on serum testosterone concentration. Zn has a main role in the 5α-reductase enzyme that is necessary for the trans-formation of testosterone into a biologically active form, 5α dihydro testosterone (Ali *et al*., 2007).

Table (2): Mean ± SD of serum testosterone and some biochemical characteristics of seminal plasma of heat stressed rabbit bucks as affected by different dietary zinc sources.

Item	Mild control group	Heat-stressed groups			*p-value*
		Control	Zinc sulfate	Zinc picolinate	
Serum testosterone (ng/ml)	2.76 ± 0.14^{a}	2.11 ± 0.27^{c}	2.42 ± 0.07^{b}	2.71 ± 0.23^{ab}	<0.01
Semen pH	7.32 ± 0.34	7.25 ± 0.38	7.00 ± 0.34	6.91 ± 0.34	0.178
Seminal plasma:					
Total Protein (g/dl)	4.12 ± 0.22	3.99 ± 0.08	3.98 ± 0.05	4.01 ± 0.08	0.388
Albumin (g/dl)	2.26 ± 0.13	1.99 ± 0.18	2.04 ± 0.10	2.20 ± 0.17	0.078
Total lipids (mg/dl)	159 ± 30.5	184 ± 22.4	157 ± 23.6	153 ± 12.6	0.262
Cholesterol (mg/dl)	57.5 ± 4.82	59.5 ± 6.96	62.3 ± 5.39	57.2 ± 4.08	0.539
AST (U/ ml)	28.0 ± 6.00	33.7 ± 4.86	28.8 ± 5.72	30.4 ± 3.48	0.439
ALT (U/ml)	23.7 ± 2.16^{b}	29.4 ± 3.31^{a}	20.3 ± 5.01^{b}	22.9 ± 3.06^{b}	<0.05
ALP (U/ L)	68.5 ± 19.3	50.8 ± 9.82	58.0 ± 8.61	48.2 ± 12.0	0.179
TAC (mmol/L)	0.65 ± 0.14^{b}	0.79 ± 0.04^{ab}	0.83 ± 0.09^{a}	0.94 ± 0.09^{a}	<0.01
MDA (nmol/ml)	0.91 ± 0.09^{b}	1.13 ± 0.09^{a}	1.01 ± 0.09^{ab}	0.99 ± 0.08^{b}	<0.05
Zn (µg/ml)	2.83 ± 0.18^{b}	1.98 ± 0.28^{c}	3.19 ± 0.44^{ab}	3.32 ± 0.23^{a}	<0.001
Testosterone (ng/ml)	1.97 ± 0.22^{a}	1.59 ± 0.13^{b}	1.71 ± 0.11^{ab}	1.96 ± 0.25^{a}	<0.05
Initial Fructose (mg/dl)	192 ± 11.8	222 ± 35.2	199 ± 44.9	217 ± 57.4	0.687

Means in the same column bearing different superscript letters are significantly different at P<0.05.
AST: Aspartate aminotransferase; ALT: Alanine Aminotransferase; ALP: alkaline phosphatase; TAC: total antioxidant capacity; MDA: Malondialdehyde.

High activity of transaminase enzymes is used as indicator of the degree membrane damage of spermatozoa due heat stress. This damage is induced by high lipid peroxidation, as indicated by high levels of MDA under heat stress condition (Table 2). In the present study, zinc treatment alleviated the effect of heat stress on ALT activity. This effect may attributed to the high concentration of zinc ions (Table 2) that may had a protective action to reduce the damage of cell membrane of sperms (Pursel *et al*., 1968), This hypothesis is supported by high levels of TAC and low levels of MDA in seminal plasma of zinc-treated groups (Table 2).

Reaction time, semen physical traits, conception rate and litter size:

Reaction time of rabbit males exposed to stressful conditions, with or without dietary zinc supplementation, was longer than (p<0.001) those exposed to mild condition. Heat stress conditions did not influence significantly ejaculate volume, abnormal and dead sperm percentages, and litter size at birth. While, sperm count, motility percentage and conception rate were decreased significantly under heat stress condition, in comparable to mild condition. Dietary zinc supplementation, with different sources, alleviated the negative effect of heat stress on reaction time and sperm count, but these values still worse than those in mild control group. Only organic form of Zn supplementation significantly increased motility percentage in heat-stressed bucks. Zinc treated groups had intermediated conception rate values, which had no significant differences with both control groups in mild and heat stress conditions.

Table (3): Mean ± SD of reaction time, semen physical characteristics, conception rate and litter size of heat stressed rabbit bucks as affected by different dietary zinc sources.

Items	Mild control group	Heat-stressed groups			*p-value*
		Control	Zinc sulfate	Zinc picolinate	
Reaction time (s)	14.5 ± 1.70^{c}	25.7 ± 3.16^{a}	19.2 ± 1.93^{b}	18.9 ± 1.08^{b}	<0.001
Ejaculate volume (ml)	0.66 ± 0.10	0.60 ± 0.08	0.59 ± 0.14	0.61 ± 0.02	0.553
Sperm count ($\times 10^{6}$/ml)	285 ± 19.6^{a}	195 ± 31.2^{c}	232 ± 27.8^{b}	240 ± 24.5^{b}	<0.001
Motility (%)	76.4 ± 11.5^{a}	55.7 ± 4.40^{b}	65.5 ± 3.58^{ab}	69.4 ± 5.90^{a}	<0.01
Abnormal sperms (%)	18.8 ± 2.66	16.1 ± 2.77	19.9 ± 2.75	15.9 ± 2.38	0.138
Dead sperms (%)	15.7 ± 5.30	19.3 ± 8.77	18.8 ± 8.51	17.7 ± 2.48	0.800
Conception rate (%)	73.3 ± 5.16^{a}	65.0 ± 5.48^{b}	70.0 ± 6.32^{ab}	71.7 ± 4.08^{ab}	<0.05
Litter size at birth	5.91 ± 0.21	6.33 ± 0.62	5.74 ± 0.38	5.98 ± 0.16	0.090

Means in the same column bearing different superscript letters are significantly different at P<0.05.

It is known that libido is controlled by various factors such as sexual pheromones, nervous system, hormones, among which, the hypothalamus-pituitary-testis axis is very important (Yang et al., 2005). Testosterone and estradiol, act synergistically to stimulate male sexual behavior and improve the copulatory behavior (Cross and Roselli, 1999; Gado *et al*., 2015). In the present study, reduced serum testosterone level (Table 2), under heat stress condition, is paralleled with reduced libido (Table 3). This result was confirmed by El-Tohamy *et al.* (2012) and Abdulrashid and Juniper (2016) who reported that heat stressed-male rabbits reared in tropical conditions showed higher reaction time. In contrast, Marai et al. (2002a) found no significant change in reaction time of male rabbits between hot and mild conditions.

Heat exposure depresses the hypothalamic hormone releasing factors and consequently, the pituitary hormones are decreased (Habeeb *et al*., 2018). Changes in FSH and LH levels in exposed male rabbits to heat stress may be involved in such alterations in semen quality (El-Sherry *et al*., 1980). Accessory gland secretion and spermatogenesis are controlled by testosterone hormone (Hammond *et al*., 1983), which was significantly decreased in heat stress condition (Table 2). The decrease in sperm concentration after exposure to high temperature may be due to degeneration of germinal epithelium and partial atrophy in seminiferous tubules (Chou *et al*., 1974; Marai *et al*., 1996). The alteration in sperm motility may be attributed to changes in seminal biochemical component levels (El-Masry *et al*., 1994). This explanation is confirmed in the present study (Table 2). These results are consistent with those of Marai et al. (2002a), Attia *et al.* (2011), Pei *et al.* (2012), and Abdulrashid and Juniper (2016) who reported that exposing male rabbits to thermal-stress in tropical conditions significantly decreased sperm motility and density, but insignificantly affected semen volume and sperm livability. While, El-Tohamy *et al.* (2012) reported that subjecting male rabbits to hot summer conditions significantly decreased semen volume, and sperm livability, motility and abnormalities, but sperm concentration was not affected significantly.

The significant improvements in reaction time, sperm concentration and sperm motility in heat stressed male rabbits treated with zinc (organic and in organic) were similar with the results obtained by El-Tohamy *et al.* (2012), El-Speiy and El-Hanoun (2013) and Baiomy *et al.* (2018). El-Hawary *et al.* (2018) noted that zinc administration significantly decreased reaction time and significantly increased semen volume and sperm motility, Livability and concentration of Friesian Bulls reared in hot summer conditions.

Increased libido, in terms of decreased reaction time, in zinc-treated groups may be explained by higher blood testosterone (Table 2) which capable to stimulate male sexual behavior and improve the copulatory behavior (Gado *et al*., 2015). The increase in sperm concentration in response to zinc treatment under heat stress condition may be attributed to the incorporation of sufficient Zn amount into sperm, during the final stage of maturation, which is essential for DNA synthesis and its stability (Evenson *et al*., 1993), cell division, maintenance of spermatogenesis and survival of the germinal epithelium (El-Masry *et al*., 1994). In the present study Zn supplementation improved significantly blood testosterone levels (Table 2) that control spermatogenesis processes (Hammond *et al*., 1983). The improvement in sperm motility of heat stressed male rabbits treated with different zinc sources may returned to the higher testosterone levels (Table 2), improved seminal biochemical component (Table 2) and the action of zinc on both testicular tubules and seminal enzymes (Underwood, 1977).

The adverse effect of heat stress on the conception rate of rabbit males was similar with the results obtained by Marai *et al.* (2002a), (El-Speiy and El-Hanoun, 2013) and Baiomy *et al.* (2018). Such effect is related to the decline in sperm concentration and motility (Table 3). The positive effect of dietary zinc supplementation on conception rate of rabbit male reared under heat stress may be returned to its ability to alleviate the negative effect of heat stress on sperm count and motility (Table 3).

CONCLUSION

From results of the present work, it could be concluded that dietary zinc addition from different sources at level of 75 mg/kg diet is helpful in alleviating the negative effect of heat stress on serum testosterone, semen quality and consequently conception rate of New Zealand White rabbit bucks. Organic zinc form as zinc picolinate was more effective than in organic one in ameliorating the deleterious effects of heat stress.

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الإستجابة التناسلية لذكور أرانب النيوزيلندي الأبيض المجهدة حراريا لمصادر مختلفة من الزنك

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استهدفت هذه الدراسة تقييم تأثير إضافة الزنك من مصادر مختلفة إلى علائق ذكور الأرانب النيوزيلندي الأبيض المجهدة حرارياً على بعض الصفات التناسلية كجودة السائل المنوي ومعدل الإخصاب وعدد الخلفات في البطن ، حيث تم توزيع أربعة وعشرين ذكر أرانب ناضج بشكل عشوائي في 4 مجموعات احتوت كل منها على عدد 6 ذكور وكانت تلك المجموعات على النحو التالي: المجموعة الأولى تم تربيتها في ظروف معتدلة وتغذت على العليقة الأساسية بدون أي إضافات غذائية ، أما باقي المجموعات فقد تم تربيتهم تحت ظروف الإجهاد الحراري وتغذت على العليقة الأساسية فقط (المجموعة الثانية) أو على العليقة الأساسية مضاف إليها 75 مجم زنك غير عضوي في صورة كبريتات الزنك (المجموعة الثالثة) أو على العليقة الأساسية مضاف إليها 75 مجم زنك عضوي في صورة بيكولينات الزنك (المجموعة الرابعة). وكانت أهم النتائج المتحصل عليها على النحو التالي: أدت إضافة الزنك لعلائق ذكور الأرانب إلى تقليل الأثر الضار للإجهاد الحراري على الرغبة الجنسية. انخفض نشاط إنزيم ALT وتركيز MDA في بلازما السائل المنوي عند إضافة الزنك لعلائق الذكور المعرضة للإجهاد الحراري. أدت إضافة الزنك لعلائق الذكور إلى زيادة قيم التستوستيرون (في الدم والسائل المنوي) وعدد الحيوانات المنوية والقدرة الحركية للحيوانات المنوية ومعدل الإخصاب والتي انخفضت نتيجة التعرض لظروف الإجهاد الحراري. ارتفع محتوى السائل المنوي من الزنك ومن السعة الكلية لمضادات الأكسدة (TAC) في المجاميع التي تغذت على الزنك وذلك بالمقارنة بمجموعتي الكنترول تحت الظروف الجوية المختلفة. وكان استخدام الزنك العضوي في صورة بيكولينات الزنك أكثر فاعلية من المصدر الغير عضوي في صورة كبريتات الزنك في تقليل أثر الإجهاد الحراري على محتوى مصل الدم من التستوستيرون ومحتوى بلازما السائل المنوي من التستوستيرون و MDA وعلى القدرة الحركية للحيوانات المنوية. وقد خلصت نتائج الدراسة الحالية إلى أن الإضافة الغذائية للزنك من مصادر مختلفة يمكن أن تساعد في تخفيف التأثير السلبي للإجهاد الحراري على الكفاءة التناسلية لذكور الأرانب النيوزيلندي الأبيض، وكان تأثير المصدر العضوي للزنك في صورة زنك بيكولينات أكثر كفاءة من المصدر الغير عضوي في تخفيف هذا الأثر.