Research Article

Effect of Dietary Supplementation of Either Zinc Oxide or Nano-Zinc Oxide on Growth Performance, some Serum Biochemical Parameters, Androgen Receptor Gene Expression and Microscopical Structure of the Testis in Growing V-Line Male Rabbits

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Abstract | This study is directed to assess the impacts of either zinc oxide (ZnO) or nano-zinc oxide (n-ZnO) dietary supplementation on the growth trials, some serum biochemical parameters, and testicular functions in growing V-line male rabbits. A total of 45 V-line were randomly separated into 3 equal groups; the first group (acted as control) was fed on a plain ration, and the two other groups (second and third groups) were nourished for 3 months on a plain diet supplemented with 60 mg ZnO/ kg and 60 mg n-ZnO/ kg, respectively. Growth performances; body weight (BW), body weight gain (BWG), food consumption (FC), and food conversion ratio (FCR), as well as serum levels of superoxide dismutase (SOD), catalase (CAT), triiodothyronine (T_3) and thyroxine (T_4), and testosterone were recorded monthly. The study also extended to examine androgen receptor (AR) gene expression and microscopical structure of testicular tissue at the end of the 5th-month-old. Results revealed that the n-ZnO group exhibited significant ($P \leq 0.05$) improvement over that of the ZnO group on all growth performances and serum biochemical parameters compared with the control group except for FC, T_3, and T_4, which were insignificantly changed. Moreover, AR gene expression and microscopical findings of the testis exhibited a significant improvement in the 2nd and 3rd groups compared to the control group. It could be concluded that either ZnO or n-ZnO dietary supplementation acts as an ameliorative tool for production and reproduction in growing V-line male rabbits however, the n-ZnO is more effective.

Keywords | Antioxidants, Androgen receptor gene, Rabbits, Testosterone, Zinc oxide.

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INTRODUCTION

Rabbits are considered a good supply of highly animal protein sources. The economy of the rabbit industry depends on improving its productivity and reproductive efficiency (Krupova et al., 2020). In this concern, Hambidge (1986) and El-Masry et al. (1994) recorded that early puberty and high fecundity improve the reproductive efficiency of rabbits. These criteria are considered good bases for economic selection. Gupta et al. (2002) reported that growth performance in post-weaning rabbits is affected by several inherent and non-inherent factors. Inherent factors include disease tolerance, efficiency of feed conversion, genera and nonstandard growth rates, however, non-inherent factors incorporate factors like nourishment, management, control of disease and surrounding environment (Gupta et al., 2002; Alemneh and Getabalew, 2019).

Zinc supplementation in animal diet is reported to play a crucial role in regulating many physiological functions relating to reproductive and productive efficiency in farm animals (Hassan et al., 2017; Kamel et al., 2020). In this respect, zinc supplementation is beneficial in combating stress (Kamel et al., 2020), enhancing antioxidant activities (Jarosz et al., 2017), improving immune response (Chrastinova et al., 2015) and production of many hormones, including testosterone (Hambidge, 1986), thyrotrophic releasing hormone (TRH), thyroid stimulating hormone (TSH) and thyroid hormones (Brandao et al., 2006). Moreover, zinc is considered an anti-inflammatory agent by influencing the production and signaling of numerous inflammatory interleukins such as interleukin (IL-1 and IL-6) and tumor necrosis factor (TNF) (Jarosz et al., 2017). Besides, it enters the structure of various enzymes that regulate gene expression RNA polymerases, alcohol dehydrogenase, carbonic anhydrase, glutamic dehydrogenase, carboxypeptidase, lactic dehydrogenase, and alkaline phosphatase (Chrastinova et al., 2015).

Recently, substantial development has been achieved in the employment of nanotechnology science in many fields, particularly mineral nutrition (Hassan et al., 2017). Nanominerals, such as nano-zinc oxide (n-ZnO), are characterized by small size, high bioavailability, and broad surface area, which allow more cellular reactions (Hafez et al., 2020; Goma et al., 2021). In this context, n-ZnO supplementation in rabbit ration with different levels as 30 mg/kg n-ZnO (Kamel et al., 2020), 50 to 400 mg /kg n-ZnO Selim et al. (2012) causes improvement in growth performance and reproduction. It has been reported that the n-ZnO can enhance immune responses, antioxidant activities, and lipid metabolism (Hafez et al., 2020; Kamel et al., 2020). Nevertheless, several reports revealed the negative influence of either zinc oxide (ZnO) or n-ZnO on antioxidant enzymes activity (Goma et al., 2021), T_3, T_4 (Luabi et al., 2019), testosterone (Goma et al., 2021), growth (Gabr et al., 2016) as well as semen picture (Halo et al., 2021).

Based on the aforementioned studies, it seems that studies concerning the influences of ZnO and n-ZnO supplementation on animal production and reproduction are conflicting and determined by various factors such as Zn source (Attia et al., 2015), dose (Attia et al., 2015; Goma et al., 2021), duration of application (Taha and Ismail, 2023), breed (Kamel et al., 2020; Al-Sagheer et al., 2020), species (Hassan et al., 2017; Goma et al., 2021) and age (Alikwe et al., 2011; Kamel et al., 2020) of treated animal. Consequently, more investigations are warranted to clarify such conflict. Therefore, the current research aimed to declare the influence of ZnO and n-ZnO dietary supplementation at a dosage of 60 mg/kg ration on a complex set of growth and reproductive performance parameters in growing V-line male rabbits. The growth performance trials involve BW, BWG, FC, and FCR. Besides, serum levels of SOD, CAT, T_3, and T_4 were also investigated. Reproductive performance trials include the determination of testosterone levels in serum, examination of AR expression and microscopical structure of testicular tissue.

MATERIALS AND METHODS

The current research procedures were conducted in the lab animal unit of the Department of Physiology, Faculty of Veterinary Medicine, Beni-Suef University.

Ethical statement

The ethics of the handling and euthanizing of the animals were done under Approval No. 021-182. The approval was obtained from the Institutional Animal Care and Use Committee at Beni-Suef University. All animal experiments were subjected to the proper housing and maintenance conditions.

Animals

A total of 45 V-line breed clinically healthy growing male rabbits, obtained from a local supplier, were involved in these experiments. Their ages were around 6 weeks old, with a mean BW of 1.5 – 2 Kg. They were left for 2 weeks for acclimatization to the laboratory environment. Rabbits were individually housed in wired cages (40 x 50 x 50 cm) supplemented with a feed hopper and automatic watering system with nipples. Throughout this study, rabbits were kept under standard circumstances (Marai et al., 2001). Animals were kept on a balanced pelleted diet and water ad libitum. The plain ration included yellow corn, soybean, gluten, mineral salts, molasses, and premix of vitamins. The estimated chemical constituents of the ration were crude fiber (12.6%), digestible energy (2500) kcal /kg ration, and crude protein (17%), as described by NRC (1991).

Chemicals

ZnO was purchased from Alpha Chemika, INDIA, as a white powder nearly insoluble in water. ZnO nanoparticles were biosynthesized and characterized (Farghali et al., 2007) in the lab of Nano-materials, Faculty of Postgraduate Studies, Beni-Suef University, Egypt.

Experimental design

Animals were equally divided, randomly, into 3 comparable groups (15 rabbits/group) and received the following corresponding diets throughout the experimental period that extended for 3 months:-

a- 1st Group (Control): received plain diet.

b- 2nd Group: received a plain diet mixed with 60 mg ZnO/kg dry matter (Hassan et al., 2017).

c- 3rd Group: received a plain diet mixed with 60 mg

n-ZnO/kg dry matter (Tag-El Din, 2019).

Growth performance

BW, BWG, FC, and FCR were determined monthly (at the end of the 3rd, 4th, and 5th month of age). Experimental animals were deprived of feed for 12 hours before being subjected to weight to evade any intestinal content from the weighting process (Al-Dobaib et al., 2007).

Blood sampling

Blood was individually collected monthly from the ear vein at 8.00-9.00 am to obtain sera, which were kept at -20°C till biochemical analysis (Al-Dobaib et al., 2007).

Serum biochemical analysis

SOD (U/ml), CAT (U/L), T_3 (ng/ml), T_4 (ng/ml) and testosterone (ng/ml) were determined in rabbit sera using commercial kits; SOD kit (CAT. No. SD2521, bio-diagnostic Co., EGYPT), CAT kit (Cat. No. CA 25 17, bio-diagnostic Co., EGYPT), T_3 ELISA kit (Cat. No. KET0006, Abbkine Co., China), Rabbit T_4 ELISA kit (Cat. No. MBS777396, MyBioSource Co., USA) and Rabbit testosterone ELISA kit (Cat. No. CSB-E06927Rb, Cusabio Co., USA), respectively.

Tissue sampling

Immediately after the end of 5th month, 5 rabbits randomly selected from each group were euthanized randomly following the last blood sample collection, and the testes were harvested. The left testicle of each euthanized rabbit was immersed in formalin at 10% concentration, then sectioned, and subjected to staining with hematoxylin and eosin stain (HE) and inspected microscopically (Bancroft and Layton, 2013). The right testis was isolated and kept in liquid nitrogen for AR gene expression:

Determination of AR gene expression: Total RNA was separated from rabbit testis and complementary DNA (cDNA) was synthesized by one-step reverse transcription- RT-qPCR protocol using GoTaq® 1-Step RT-qPCR System kit, Promega, USA. The primer sequence of AR gene and ß-actin were F: 5′-TCC ACC TCC TCC AAG GAC AGT-3′, R: 5′-CCA ACG CCT CCA CAC CCA A-3′ and F: 5′-TCC TTC CTG GGC ATG GAG TC-3′, R: 5′-GGA TGT CCA CGT CGC ACT TC-3′ 5′-GGA TGT CCA CGT CGC ACT TC-3′, respectively (El-dawy et al., 2016). Analysis of RT-qPCR results were determined according to Livak and Schmittgen (2001) and Yuan et al. (2006).

Statistical assessment

All data were stated as average ± standard error. One-way analysis of variance (ANOVA) followed by Turkey's and Duncan's tests were used to signify values among the tested groups exploiting statistical software program (SPSS for Windows, version 25, USA). Alterations considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Table (1) displayed that BW and BWG were significantly high ($P \leq 0.05$) in both ZnO and n-ZnO dietary treated groups comparable to the control group. While FCR, decreased significantly ($P \leq 0.05$) in ZnO and n-ZnO groups compared to the control one. Moreover, the n-ZnO group exhibited significant ($P \leq 0.05$) improvement over that of ZnO in previously estimated growth performance trials. The results of FC, throughout the experimental period, were nearly similar in all groups. Such beneficial effects on growth performances consisted with previous researches (Alikwe et al., 2011; Al-Sagheer et al., 2020; Kamel et al., 2020). Zinc is essential for biological functions inside the animal body. It is vital for growth, normal feeding, antioxidant function, and for the activities of various metalloenzymes (Tako, 2019). The positive effects of Zn may be attributed to its crucial role in the normal formation and organization of important macromolecules such as DNA and RNA, which have a principal role in the development of bones and the synthesis of somatic proteins (Chrastinova et al., 2015). On the contrary, other studies did not report any significant beneficial effect on the growth of rabbits nourished on a ration supplemented with ZnO at a dosage of 50, 100, 200, and 400 mg ZnO /kg ration (Selim et al., 2012) and zinc bacitracin at a dosage of 83 mg/per day for 81 days (Attia et al., 2015). The findings were attributed to the possibilities that zinc absorption inside the intestinal lumen may be inhibited by some antagonists such as excess amounts of Ca, Fe, unabsorbed fat, and dietary phytate that are found in plant-based diets such as soybean meal, wheat bran, and cottonseed meal (Ognik et al., 2016).

Moreover, the significant ($P \leq 0.05$) improvement in estimated growth performance trials in the n-ZnO treated group over that of the ZnO was supported by the record that zinc nanoparticles had a marked contribution as a mineral feed additive more than their bulk form (Swain et al., 2016). These findings are compatible with previous reports on different breeds of rabbits using various doses (Hassan et al., 2017; Tag-El Din, 2019; Kamel et al., 2020). It was suggested that the positive biological effect of nano-zinc over that of ZnO may be attributed to its properties such as wide surface area, bioactivity, and potential adsorbing action (Hassan et al., 2017).

In the current study (Table 2), ZnO and n-ZnO supplementation provoked a significant rise in serum levels of SOD and CAT as compared to the control group, and

Table 1: Effect of ZnO and n-ZnO dietary supplementation on growth performance trials in growing V-line male rabbits (Mean ± SE).

Parameter	Age (Months)	Control group	ZnO group	n-ZnO group
BW (g)	3	2284.33±14.31 ^{A}	2355.73± 19.97 ^{B}	2419.86±15.03 ^{C}
	4	2833.53±11.03 ^{A}	2978.80± 16.11 ^{B}	3119.33± 12.89 ^{C}
	5	3413.27±8.72 ^{A}	3613.00± 17.42 ^{B}	3878.00±19.56 ^{C}
BWG (g)	3	493.40± 10.11^{A}	552.13±11.05 ^{B}	615.87±17.39 ^{C}
	4	549.20 ±10.22 ^{A}	623.07± 17.29 ^{B}	699.47 ±12.11 ^{C}
	5	579.73 ± 8.29 ^{A}	634.20± 15.49^{B}	758. 67 ± 21.35 ^{C}
FC (g)	3	1822.42±26.09^{A}	1818.72±22.09^{A}	1801.97±17.39^{A}
	4	2120.89 ±9.11^{A}	2115.36 ±11.68^{A}	2112.09±11.88^{A}
	5	2236.37 ±15.84^{A}	2220.77±17.04^{A}	2216.33 ± 12.96^{A}
FCR	3	3.71± 0.08^{A}	3.31 ± 0.07^{B}	2.96 ± 0.09^{C}
	4	3.88± 0.06^{A}	3.43 ± 0.09^{B}	3.03 ± 0.06^{C}
	5	3.87 ± 0.06^{A}	3.53± 0.09^{B}	2.96 ± 0.09^{C}

-In the same raw, values having different capital letters differ significantly (P ≤ 0.05).
ZnO = Zinc Oxide, n-ZnO = nano-Zinc Oxide, BW = body weight, BWG = body weight gain, FC = food consumption, FCR = food conversion ratio

Table 2: Effect of ZnO and n-ZnO dietary supplementation on serum biochemical parameters and AR gene expression in growing V-line male rabbits (Mean ± SE).

Parameter	Age (Months)	Control group	ZnO group	n-ZnO group
SOD (U/ml)	3	1.33 ±0.04 ^{A}	1.61 ±0.04 ^{B}	1.79 ± 0.06 ^{C}
	4	1.23 ± 0.02 ^{A}	1.74 ±0.03 ^{B}	1.89 ± 0.04 ^{C}
	5	1.33± 0.03 ^{A}	1.81 ±0.02 ^{B}	1.93 ± 0.05 ^{C}
CAT (U/L)	3	284.4± 8.86 ^{A}	351.33± 6.92 ^{B}	379.2 ± 5.63 ^{C}
	4	257.00± 4.01 ^{A}	357.00 ± 3.75 ^{B}	381.6 ± 4.89 ^{C}
	5	260.40± 4.61 ^{A}	362.80± 2.54 ^{B}	397.20± 4.87 ^{C}
T_3 (ng/ml)	3	1.28 ± 0.09 $^{A`}$	1.30 ± 0.09 ^{A}	1.43 ± 0.1 ^{A}
	4	1.53 ± 0.04 ^{A}	1.54± 0.03 ^{A}	1.59± 0.03 ^{A}
	5	1.07 ± 0.02 $^{A`}$	1.12 ± 0.03 ^{A}	1.14 ± 0.03 ^{A}
T_4 (ng/ml)	3	5.97 ± 0.13 ^{A}	6.10 ± 0.11^{A}	6.27 ± 0.16 ^{A}
	4	6.07 ± 0.14 ^{A}	6.26± 0.16 ^{A}	6.39 ± 0.06^{A}
	5	5.03 ± 0.12 ^{A}	5.33 ± 0.17 ^{A}	5.43 ± 0.12^{A}
Testosterone (ng/ml)	3	1.22± 0.02 ^{A}	1.70 ± 0.03 ^{B}	1.97± 0.03 ^{C}
	4	2.08 ± 0.02 ^{A}	2.31 ± 0.03 ^{B}	2.73 ± 0.06 ^{C}
	5	2.39 ± 0.02 ^{A}	2.72 ± 0.02 ^{B}	2.97 ± 0.05 ^{C}
AR gene (fold change)	5	1.02 ± 0.03 ^{A}	1.89± 0.05 ^{B}	2.05 ± 0.03 ^{C}

-In the same raw, values having different capital letters differ significantly (P ≤ 0.05).
ZnO = Zinc Oxide, n-ZnO = nano-Zinc Oxide, SOD = superoxide dismutase enzyme, CAT = catalase enzyme, T_3 = triiodothyronine, T_4 = thyroxine, AR = androgen receptor gene expression

their values were greater (P ≤ 0.05) in the 3rd group than 2nd group, throughout the experimental period. Likewise, zinc (Kuckova et al., 2021) and n-ZnO (Abdel-Monem et al., 2021) supplementation in animal feed leads to high antioxidant activities of CAT, GSH-Px, and SOD. In this context, zinc is considered a component of more than 240 enzymes and an essential component in SOD (Prasad, 2008). Furthermore, the significant (P ≤ 0.05) potent effects of n-zinc over ZnO in the present study (Table 1) can be attributed to the higher absorption and bioavailability of n-ZnO that obviously declared in several previous studies (Hafez et al., 2020; Kamel et al., 2020).

It is worth mentioning that thyroid hormones play a sig-

nificant role in the improvement of growth performance (Zearah et al., 2016), ROS production (Guerrero et al., 1999) and consequently antioxidant activities (Guerrero et al., 1999; Mancini et al., 2016) . Therefore, it was expected that there would be an increase in serum levels of T_3 and T_4 in association with the improvement in growth performance and serum levels of SOD and CAT, but it was not the matter. Results of the current work (Table 2) didn't exhibit any significant change in the serum levels of T_3 and T_4 among different groups during the experimental period. In this context, Zn (Teixeira et al., 2020), as well as nano-zinc (Hameed et al., 2023), were reported to play an valuable role in maintaining normal levels of TRH, TSH, T_3, and T_4 and improve metabolism due to its stimulating effect on the activity of pituitary and hypothalamus. It was hypothesized that Zn deficiency is linked with motivated the activity and expression of hepatic T_4-5'-monodeiodinase enzyme, which catalyzes the thyroid hormone inhibition (El-sisy et al., 2008). However, consumption of high amounts of zinc (Aihara et al., 1984) and nano-zinc (Luabi et al., 2019) may cause destructive effects on thyroid hormones.

It was also shown that ZnO and n-ZnO supplementation induced a significant ($P \leq 0.05$) increase in serum testosterone level in comparison with the control group, and their values were higher ($P \leq 0.05$) in the 3rd group over that of the 2nd group throughout the experimental period (Table 2). Data concerning the correlation between Zn sources, particularly n-Zn supplementation and serum testosterone levels in growing rabbits, seem to be scanty. However, a direct relationship between zinc supplementation and serum testosterone was reported in mature rabbits (El-Masry et al., 1994) and mature male rats (Goma et al., 2021). It was suggested that zinc is essential for the production and secretion of testosterone from the Leydig cells and gonadotrophin releasing hormone (Hambidge, 1986; Saaranen et al., 1987). On the other hand, zinc nanoparticles (3rd group) showed a greater potential ($P < 0.05$) on serum testosterone levels than the conventional sources (2nd group), suggesting higher intestinal absorption, biocompatibility, and bioavailability of nano-zinc, which was in agreement with previous researches (Hafez et al., 2020; Kamel et al., 2020).

Concerning the influence of ZnO and n-ZnO dietary supplementation on AR gene expression at the 5th month of age, it appeared that AR gene expression significantly amplified in the supplemented groups when compared with the control group with the greatest value ($P \leq 0.05$) in n-ZnO group (Table 2). Within the available literature, there is no data concerning the influence of ZnO and n-ZnO dietary supplementation on AR gene expression in male rabbits. It is known that AR is a single nuclear receptor. It belongs to the nuclear receptor superfamily and is considered an active member of the steroid hormone that controls androgen actions via acting as a ligand-dependent transcription factor (Mangelsdorf et al., 1995). The positive effects of ZnO on AR gene expression in the present study might be due to zinc being considered an antioxidant deactivating lipid, protein, and DNA peroxidation, consequently preventing down-regulation and inhibition of gene expression (Kulbacka, et al., 2009). On the other side, the significant influences of nano-zinc over zinc might be regarded as the higher physicochemical characteristics of n-ZnO absorption, bioavailability, and surface area (Hafez et al., 2020; Kamel et al., 2020).

At the end of 5th month of age, microscopical findings of V-line rabbit testicular tissue revealed that seminiferous tubules (ST) belonging to ZnO group Figure 1 (B) showed a limited number of spermatozoa, most of which are attached to Sertoli cells (early maturation). While ST of control group, Figure 1 (A) showed normal histological structures in which spermatogonial cells exhibited mitotic activity, incomplete spermatogonial cycle (immature) and interstitial cells of Leydig (LC) were dispersed in between. On the otherside, ST of n-ZnO group Figure 1 (C) showed spermatogenic activity with spermatid and spermatozoa in higher density than ZnO group.

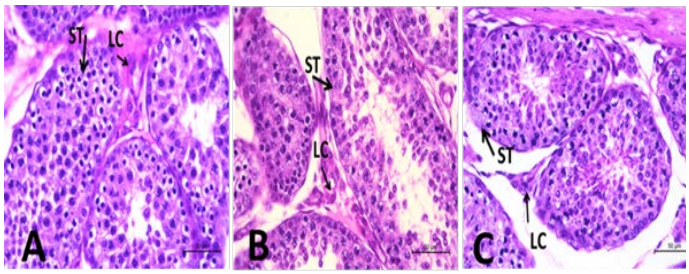

Figure 1 (A): Seminiferous tubules (ST) of the control group shows normal histological structure in which spermatogonial cells show mitotic activity, incomplete spermatogonial cycle (immature), and Leydig cells (LC) are dispersed in between (X 400). **(B)**: ST of the ZnO group shows mitotic activity, immature spermatogonial cycle, and LC are dispersed in between. Few ST tubules show a limited number of spermatozoa most of which are attached to Sertoli cells (early maturation) (X 400). **(C)**: ST of the n-ZnO group shows spermatogenic activity including spermatid and spermatozoa in high density (X 400).

Within the available literature, there is no data concerning the influence of dietary ZnO or n-ZnO supplementation on the microscopical picture of the testis in rabbits. However, the positive impacts of either ZnO or n-ZnO on the microscopical picture of the testis may be assigned to the improvement in antioxidant properties, serum testosterone level, and AR gene expression in the present study. Besides,

the physicochemical properties of n-ZnO exert superior efficacy compared with organic or inorganic Zn (Hafez et al., 2020; Goma et al., 2021). These results were compatible with previous researches declaring that Zn is essential in regulating many physiological processes stimulating spermatogenesis (Jarosz et al., 2017; Bao et al., 2009).

CONCLUSION

Conclusively, ZnO and n-ZnO dietary supplementation at a dose of 60 mg/kg ration act as an ameliorative tool of production and reproduction in growing V-line male rabbits, however, n-ZnO is more effective.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design of the research, methodology, analysis of results, and writing of the manuscript.

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