The Influence of Dietary Zinc Oxide Nanoparticles on Zootechnical Performance and Biometric Indices of Growing New Zealand White Rabbits

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1. Abstract

This study was designed to compare the effects of dietary conventional inorganic Zinc oxide (ZnO) and nano-zinc oxide (ZnO-NPs) on the zootechnical performance, selected blood serum indices, antioxidant status, general health and production of rabbit meat enriched with zinc enriched with nano-zinc in growing New Zealand White rabbits (NZW). A total of 481 newly-born rabbits produced from two natural successive mating between 36 primiparous (NZW) Rabbit does and 12 bucks by the intensive breeding system were utilized throughout this study. Rabbits does and bucks were randomly distributed into four equal groups, the control positive group G1 was fed the basal diet supplemented with premix contain 50 ppm inorganic zinc in the form of ZnO; while the control negative G2 was fed on a basal diet supplemented with Zinc Free premix, meanwhile, G3 was fed on the basal diet supplemented with premix contain 25 ppm nano zinc as zinc oxide nanoparticles, however, G4 was fed on the basal diet supplemented with 50 ppm nano zinc as zinc oxide nanoparticles in the premix, Litters after weaning continued on the same diet like their dams till the age of selling at 62 days old. The results showed that the growing rabbits in (G4) supplemented with 50 mg ZnO-NPs/kg diet surpassing significance ($P \leq 0.05$) in all groups and achieved the best result regarding (birth weight(g), weaning weight(g), sealing weight (g) moreover lower Pre-weaning mortality %, and Post-weaning mortality%). Also, G4 showed significant improvement in FCR in comparison with other groups. Furthermore, showed highest significant increase in total protein and globulin, HDL, SOD, GSH, Catalase activity, and zinc concentration in serum, liver, and thigh tissue and also showed the highest significant decrease in A\G ratio, creatinine, urea, uric acid, cholesterol, Triglyceride, LDL, VLDL, risk factor, MDA and NO level in G4 at 62 day age old, compared to other groups, while there was no significant difference in albumin level between all the experimental groups. In conclusion, supplementation of 50 mg ZnO-Nps\kg diet in the premix to the diet of growing NZW rabbit maximize the productive performance and improve FCR without any adverse effect.

Keywords: ZnO; Nano-ZnO; growth Performance; Serum profile; Antioxidnat-biomarker; growing NZW Rabbits.

2. Introduction

proper nutrition and supplementation of zinc are critical elements for minimizing the incidence of digestive disorders and diarrhea [1]. Dietary Zinc has different vital functions as it can significantly impact animal performance and immunity, making rabbit production profitable by achieving maximum weight gain within a minimum period with the best cost and maintain reproduction all the year despite the adverse condition of weather in some countries and maintains a successful economic rabbit production[2]. Also, Zinc is a component of numerous enzymes and hormones [3] and is essential for the body's proper physiological functions like normal growth,

reproduction, DNA synthesis, cell division, and gene expression photochemical processes of vision, wound healing, ossification, augmenting the immune system of the body through energy production, protein synthesis, and protection of membranes from bacterial endotoxins and lymphocyte replication and antibody production [4, 5]. The Zinc content of cereal grains and pasture varies, and is greatly influenced by soil zinc status; thus the intake of such feeds may result in a serious Zn deficiency[2], with subsequently impaired rabbit efficiency, as it reduces food consumption, weight loss, lowered hematocrit Values, the frequent appearance of alopecia and dermatosis as well as reproductive failure [6]. Therefore, a Zinc source must be added to the rabbit diet either in inorganic form or organic form however, it was shown that there was no difference in zinc bioavailability between inorganic and organic sources in rabbits [7]. Also, no recent trials on the zinc requirements of rabbits have been published in the literature, but levels of use vary between 25 and 60 mg kg-1. Zinc oxide is the most commonly used source because it is less reactive and has a higher zinc concentration than sulfate and carbonate salts[8].

High dietary zinc can lead to excess zinc in the stool, which causes environmental pollution [9]. Furthermore, affect the balance of other trace elements in the body and reduce the stability of vitamins and other nutrients, and long-term application can cause zinc residue in the animal body [10,11]. Thus, this problem opens a window for better bio-available Zn sources and if possible, to reduce the supplemental dose of Zn to the animal food [12]. In the last decade, nanotechnology has been widely used in animal husbandry to improve the utilization of trace elements in animal diets. Nano Zn, as a substitute to the conventional Zn sources, can be a good alternative in livestock feeding. Apart from being highly bioavailable, reports have pointed out the growth-promoting, antibacterial, immunomodulatory, and many other beneficial effects of ZnO-Nps [13]. The use of ZnO-Nps has shown to produce better results as compared with conventional Zn sources and also micro Zn and is also less toxic [14,15]. ZnO-Nps has been reported to enhance growth performance, improve feed utility and provide economic benefits in weaning piglets and poultry [16, 17]. Zinc oxide Nanoparticles also have a minimal adverse effects on human cells[18]. On the basis of the above-mentioned data, our research hypothesis was: to compare between two sources of Zn (inorganic zinc oxide and Zinc oxide Nanoparticles) to achieve the best rabbit production, improve growth performance and health (immune and antioxidant status). In addition to producing Zinc enriched meat a situation that of public health importance as it is considered as an effective tool to probably eliminate Zinc deficiency in humans.

3. Materials and Methods

the current study was carried out at a private rabbit farm (Al-Qods) located in Sharkia province-Egypt. (December 2019- April 2020) and its protocol was approved by the Institutional Animal Care and Use Committee in Egypt (**V**et CU16072020173).

3.1. Preparation of ZnO-NPS

Zinc Oxide was obtained from (Allied Signal Riedel-deHaëlco. extra pure Zinc, ZnO M=81.38g\Mo) Germany, as an inorganic source of zinc. Preparation of Zn NPs was done at the Petrochemicals department, Egyptian Petroleum Research Institute (EPRI), Cairo, Egypt. Zinc oxide nanoparticles (ZnO NPs) were prepared throughout the ball milling physical route using the High Energy Ball Milling (HEBM), Planetary Ball Mill PM 400.following the method described by [19].

3.2. Characterization of the Prepared ZnO-NPs

Characterization was carried out using X-Ray Diffraction (XRD) Philips X‟ Pertdiffractometer system with Cu Kα radiation source (1.54 Å), High-Resolution Transmission Electron Microscope (HRTEM) model Tecnai G20 (FEI) microscope instrument operated at an accelerating voltage of 200 kV and magnification range up to 1,000,000 X, Dynamic light scattering (DLS) have been used to investigate and determine the structure and particle size (Ps) of the prepared material [20]. In addition, the morphology of the prepared ZnO NPs has been studied using High-resolution transmission electron microscopy (HRTEM), which had been done at the Petrochemicals department, Egyptian Petroleum Research Institute (EPRI).

3.3. Premix and diet Preparation

Four experimental diets were formulated on the basis of the actual proximate composition of their ingredients to meet the nutrient requirements of adult and growing New Zealand White (NZW) breed of rabbits, according to the single feed recommendations [21] for the intensively reared rabbits. All diets were processed in 3.5 mm pellet diameter [1]. Four types of specifically formulated rabbit premix were prepared at Misr Feed HY Mix company for feed additives to contain either, 50 mg/kg ZnO (G1), Zero ZnO /kg (G2), 25 mg ZnO-NPs /kg (G3), and 50 mg ZnO-NPs /kg (G4) were used in the corresponding experimental diets (Table 1).

3.4. Rabbits and husbandry

a total of 481 newly-born rabbits were utilized throughout this study, which was produced through double natural successive mating; the interval time between them was 20 min. by the intensive breeding system between 36 primiparous does and 12 bucks NZW Rabbit (3 does\ buck).

3.4.1. Adult husbandry

the adult 36 primiparous does were 5 months age-old and weight 3.068 ±0.24 kg while the 12 bucks were 6 months age-old and 3.326 ±0.85 kg weigh. The NZW Rabbit obtained from Al-Qods rabbit Farm and were utilized throughout this study were divided into four equal groups consist of 9 does and 3 bucks and each group, subdivided into 3 does and one buck . Each adult rabbit was individually housed in cleaned and sterilized flat-deck metal cages measuring 60×50×40 cm and provided with wire screened floor which allows feces and urine to drop. Each doe cage is supplied with nest cage at day 25 of parturition, which measure 45 ×30×35cm with the floor has drain holes spaced 0.5 cm apart made in the bottom of nest box which well-bedded by wheat straw to a depth of 2 cm so maintained the temperature at 30°C inside nest cage. Adult rabbits left for two weeks on the experimental diets for acclimatization before first mating, the experimental trial lasted for 135 days.

3.4.2. Kids husbandry

growing kids kept in controlled closed nesting cage, allow the does to suckle their kittens once a day for 5 minutes for the first 13 day of age-old then freely open the door to allow nipping of feed with their does to develop the cecum growth, then the nesting cages were removed after 23 day from kindling and allowing kids to roam in their doe's cages, kids were weaned at 28 age-old and were removed to growing cages which measuring 60×50×40 cm and provided with wire screened floor which allow feces and urine to drop, not more than 3 kg live rabbit weight in each cage. The sealing weight of growing rabbits was 62 days age-old.
The Adult and growing experimental groups have been housed in the same compartment of the building, Cooler and forced ventilation systems allowed the building air temperature to be maintained between a maximum of 23.2°C and a

minimum of 16.6 °C throughout the experiment. Relative humidity was adjusted according to the thermal index to be comfortable. The rabbits were subjected to a 16 h light/8 h dark photoperiod. Each rabbit cage was provided with a water canal and experimental feeder. All rabbits were kept under standard hygienic conditions and were vaccinated against rabbit hemorrhagic disease, bacterial diseases according to the routine prophylactic vaccination program.

3.5. Feeding regimen

3.5.1. Feeding regimen for Adult NZW Rabbit

Does and bucks during the acclimatization period were fed 150 g\day, then does during pregnancy and lactation were converted to ad-libtum feeding while bucks during breeding kept on 150/day [1].

3.5.2. Feeding regimen for growing NZW rabbit after weaning

all kids allowed to suckle colostrum at first 24 hr of kindling and then followed by controlled natural suckling, permitting does to enter the nesting cage once daily at 7:00 A.M for 6 minutes through the first 12^{th} day of their life, after the 13^{th} days of birth kids started to nipple food with their does. growing rabbit after weaning at 28 age-old introduced to feed gradually from 30g\day until reaching 165g\day in the day 58 of age-old until sealing weight at 62 age-old [1].

Age(days)	g\day
28-30	30
30-37	74
37-44	102
44-51	132
51-58	147
58-65	165

3.6. Measurements, observations

3.6.1. Zootechnical performance

Live Birth weight at day one, live weaning weight at 28 days age old, and live sealing weight at 62 days age old were recorded. Moreover, feed intake in different experimental groups was recorded, and feed conversion ratios (FCR) were calculated accordingly. FCR is expressed as the ratio of the total feed consumed during the growing period divided by the weight of rabbits sold minus the weight of rabbit weaned [6]. Pre and post-weaning mortality percent of rabbits throughout the experimental period in the experimental groups were recorded on daily basis and the mortality rate% was calculated at the end of the experimental period accordingly. Pre-weaning mortality (%) = (number of littermate kids - number of kids at weaning) / number of littermate kids. Post-weaning mortality (%) = (number of kids at weaning - number of kids sold) / number of kids at weaning.

3.6.2 Serum Parameters

At the end of the experimental period, individual 10 ml blood samples were taken from marginal ear vein using 23G butterfly needle [22]of nine representatives randomly selected rabbits from each group into a zinc free vacationer tube without coagulant which maintained in a slanted position for 10 min at room temperature and centrifuged at 3000 rpm for 15 min at 4°C to separate the serum, which was then harvested and stored at -20°C until analysis[23] And used for subsequent determination of total protein, albumin [24], calculation of Globulin[25], Albumin\Globulin (A\G) Ratio, Lipid profile including total cholesterol, total glyceride, and HDL[26], LDL, and VLDL[27]. The Risk factor is the ratio between the total cholesterol to HDL-cholesterol and it were calculated as: Risk Factor =TC/HDL-c [28] .Liver enzymes including ALT, AST [26], and Alkaline Phosphatase[29].Kidney function including: total bilirubin [30],Urea, Uric acid[31], and Creatinine[32].

3.6.3. Antioxidant biomarkers in liver homogenates

Liver and pectoral muscle samples from nine rabbits in each group were collected at the end of the experiment and kept on an ice-cold surface, rinsed with ice-cold phosphate buffer saline (pH 7.2). Then, the samples were divided into aliquots and snap-frozen in liquid nitrogen. Frozen tissues and serum samples were stored at -20°C before analysis and then used for subsequent determination of homogenate total protein [24], reduced glutathione (R-GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and malonaldehyde (MDA) and R-GSH levels were carried out according to the method of [33] and [34], respectively. While, the GPx and SOD activities were determined according to[35] and [36], respectively. Nitric Oxide levels were carried out according to [37] using the diagnostic kit. Determination of catalase was carried out according to [38].

3.6.4. Determination of Zinc in serum, Liver and thigh muscle tissues at the end of the experimental period, nine rabbits randomly selected from each group were slaughtered then serum as well as liver and thigh muscle were quickly collected on an ice-cold surface and stored in a deep freezer at -20°C and then used for determination of Zinc concentration. Using methods of [39]after wet mineralization of the samples with nitric acid, Zinc concentration in liver and thigh muscle was determined by Perkin-Elmer Mod.3030 atomic absorption spectrophotometer (AAS).

3.7. Statistical analysis

The obtained data were calculated and statistically analyzed according to [40] using MiniTab software version 17 for Windows. The differences between groups were determined with variance analysis (one-way analysis of variance (ANOVA) using the probability level of 0.05 for the rejection of the null hypnosis. Significant differences among means were determined by Duncan Test. All data were recorded on an individual basis. Data were expressed as means ± SE.

4. Results

4.1. Characterization of ZnO-NPS by XRD and HRTEM techniques

the structural analyses were detected by the XRD technique for both the starting Zinc oxide powder and the generated zinc oxide NPs. The XRD pattern of the starting powder and the generated NPs revealed that all lines are belonging to zinc oxide and no other lines are detected for other impurities (Fig.1). It could be observed that the starting powder and the generated NPs have single phase polycrystalline monoclinic structure of a = 6.8491 Å and with pronounced intensity for ZnO (230) peak which matched with ICCD card no. 32-1135. The particle size (Ps) of the prepared sample has been measured and calculated by HRTEM as seen in (Fig.2) showed that the average particles was about 70 nm. Also, it could be observed that the prepared sample shows uniform shape nanoparticles with no aggregations before and after milling (Fig.3 A and B).

4.2. Impact of dietary ZnO-Nps on Zootechnical performance of Growing NZW Rabbit

In the current study data concerns the growth and the health performance summarized in (Table 2), Showed that the growing rabbits fed on the basal diet supplemented with 50 ppm ZnO-NPs G4 along the whole experimental period surpassing significantly ($P \leq 0.05$) all groups and achieved a highly significant increase in live birth weight, weaning weight at 28 days age-old, and sealing weight at 62 d age-old than G1 supplemented with conventional ZnO ,while there was no significant difference between G3 and G4. Rabbit supplemented with ZnO-Nps at different levels showed significant best FCR and a significant decrease in mortality rate in comparison

with other groups ($P < 0.01$). G2 supplemented with a zinc free premix showed a reduction in all zootechnical performance as showed a significant reduction in birth weight (g), weaning weight (g), and sealing weight (g). Also, it showed a considerable increase in FCR. Moreover, showed a highly significant increase in Pre and post-weaning mortalities (%).

4.3. Impact of ZnO-Nps on serum parameterof Growing NZW Rabbits

Date present in (Table 3) revealed that the impact of ZnO-Nps on serum parameters of growing NZW rabbits. Groups supplemented with ZnO-Nps in the premix showed the highest significant increase in total protein and globulin ($p < 0.001$) and the highest significant decrease in A\G ratio ($p < 0.001$), while there was no significant difference in albumin level between all the experimental groups. Also growing rabbits in G4 supplemented with 50mg ZnO-NPs\kg diet in the premix showed a significant decrease ($p < 0.001$) in ALT, AST, and ALK levels than other treated groups. Moreover, Kidney function in growing NZW rabbits fed on ZnO-NPS showed highest significant decrease in creatinine, urea, and uric acid levels compared to the other groups. Moreover, there is no significance difference in T.Billirubin and creatinine level in groups supplemented with convention ZnO and ZnO-Nps groups while the lowest level were shown in G4.

4.4. Impact of dietary ZnO-Nps on serum lipid profile of Growing NZW Rabbits

The effect of dietary nano-zinc oxide supplementation on serum lipid profile of growing NZW rabbits in (table 4) showed that the rabbits of group G4 supplemented with 50 mg ZnO-NPs/kg diet in the premix surpassing all groups and achieved the best result. As it showed a considerable decrease in the cholesterol, Triglyceride, LDL, VLDL, and the Risk Factor and a significant increase in HDL, while Growing rabbits fed on basal diet supplemented with a zinc free premix G2 showed significant higher cholesterol, total glyceride, LDL, VLDL, and Risk Factor in comparison with other groups.

4.5. Impact of ZnO-Nps on antioxidant-biomarker of Growing NZW Rabbits

Effect of dietary Zinc Oxide Nanoparticles supplementation on some antioxidant biomarkers of New Zealand white rabbits is explained in (table 5). Group treated with 50 mg ZnO-Nps\kg diet showed a highest significant increase in SOD, GSH, and Catalase activity while showed significant decrease in MDA and NO level than group supplemented with conventional ZnO and free zinc groups, while there were no significant difference between G3 and G4 in MDA and NO level. Moreover, G2 showed a highly significant increase in MDA and NO levels, and also showed a significant decrease in SOD, GSH, and catalase activity.

4.6. Impact of ZnO-Nps on Zinc concentration in serum, liver, and thigh muscle of growing NZW Rabbits

Data in (Table 6) revealed the impact of different sources of ZnO in Zinc concentrations level in serum, liver, and thigh muscles of the New Zealand white rabbits at the end of the experiment. Growing rabbits in G2 showed a significant reduction in Zn concentration in serum, liver, and thigh muscle in comparison with other groups fed on basal diet supplemented with Zinc either in inorganic or Nano form. Moreover, there was a highly significant increase in zinc concentration in serum, liver, and thigh muscle tissues in group supplemented with ZnO-NPs in the premix, G4 supplemented with 50 mg ZnO-Nps\kg diet achieved the highest concentration than other groups.

5. Discussion

The positive effect of the dietary ZnO-NPs on most of the growth performance parameters is backed to the fact that the smaller the diameter of ZnO-NPs particles size results in higher absorption and easier diffusion throughout the mucous membranes, decreasing the influence of intestinal clearance mechanisms, penetrating deeply into tissues through fine capillaries, crossing epithelial lining fenestration (e.g. liver), enabling efficient uptake by cells and efficient delivery of active compounds to target sites in the body. Also nano-minerals increasing the surface and so used for enhancing the bioavailability of minerals in a livestock industry, all of these mechanisms improved the general health status and subsequently the growth performance traits. While rabbit in G2 supplemented with free zinc premix showed a reduction in growth and FCR, insure the importance of Zinc for normal growth as Zn is essential for body's proper physiological functions like normal growth, through energy production, protein synthesis, protection of membranes from bacterial endotoxins and lymphocyte replication and antibody production [41;11].

Our result comes in accordance with the observations reported by [42,43] that rabbits feed basal diet supplemented with 60 and 30 mg ZnO-NPs/kg diet had the heaviest LBW, DWG, and improved FCR of rabbits and Also showed lowest mortality rate, when compared with those groups which were fed on a basal diet with a free Zinc premix and 60 mg zinc oxide/kg diet ($P<0.001$). In the same trend [44,4]observed that daily feed intake and weight gain were significantly increased with supplementation of 30-120 ppm of ZnO-NPs to the broiler as compared to the control.

Our result showed that the total protein level in the groups treated with ZnO-NPs are in the normal reference levels (5.4-7.5 g\dl) while G2 showed a significant decrease in the total protein. The total protein is a summation between Albumin and globulin, and there was no significant difference in the albumin level between all the experimental groups so hypoproteinemia in G2 is backed to the malnutrition from the Zinc free premix in the diet[45]. Increasing the serum total protein and globulin in other groups are attributed to the important role of Zn in protein synthesis and altering the immune function [46], while groups supplemented with ZnO-Nps showed higher bioavailability of nano zinc, so showed a significant increase in these parameters than the control positive group. In parallel to our result [42] showed that serum total protein, globulin, and IgG were significantly elevated in groups treated with ZnO-NPS when compared with the control zinc free and convention ZnO groups. On Contrast to our result [25] showed a highly significant decrease in serum total proteins, albumin, and globulins levels in ZnO-NPs supplemented rabbits groups in comparison with the control group. Furthermore, it was reported by [43] that nano- zinc supplementation diet did not significantly affect plasma total protein, albumin, and globulin comparing to the control ALT and AST enzyme activities indicate liver health status [47], so increasing in these enzymes is a sign of hepatocytes injury while Serum creatinine and urea levels are considered as an indicators for renal function and any rising in the level of these parameters means the kidney function fall. In the harmony with our result [48] revealed that serum levels of ALT, AST, creatinine, and urea showed a significant decrease in response to ZnO-NPs, THO, or ZnO-NPs + THO compared to control in the male rabbits. Also [49] reported that Infected rabbits with Eimeria post-treatment with 10 mg ZnO-Nps/Kg compared to the control negative group after 2 weeks of infection exhibited a significant decrease in serum ALT activity. On the other hand [25] showed a highly significant increase in serum ALT, AST,

creatinine, and urea levels activities in groups supplemented with ZnO-Nps and combination between ZnO and ZnO-Nps in comparison with the control group in the NZW rabbit, the highest value was observed in ZnO-Nps group, while [43] reported that liver enzymes ALT and AST were not significantly influenced but slightly increased by different dietary treatments with ZnO-NPs when compared with the control, Also reported that nano-zinc had no adverse effect on kidney function as plasma creatinine and urea contents were numerically similar by dietary treatment with ZnO-Nps as compared with the control group..

Zinc has an important role in enzyme action which an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption[50], Presence of Zinc in nanoparticles form gives it a potent hypolipidemic effect than a regular form which has no significant effect [51], that is why lipid profile in serum of rabbit treated with ZnO-Nps showed a significant decrease in triglycerides and total cholesterol and increase in HDL. Since the Total cholesterol consists of Small dense LDL particles which are more atherogenic than larger buoyant ones and the larger and high dense HDL particles which are considered protective so there is a strong association of Total cholesterol to HDL-cholesterol (TC/HDL-c), which indicates the risk of heart disease incidence, the higher the ratio, the higher the risk, by increasing the incidence of chronic heart disease and myocardial infarction.[52]. So supplementation rabbit diet with ZnO-Nps decrease the incidence of heart disease and thus decrease the mortality rate. Similar findings were also reported by[25, 43] who reported that serum TG, TC, and VLDL-C levels showed a highly significant decrease and Serum HDL-C level showed a highly significant decrease in ZnO-NPs supplemented rabbits group in comparison with control one. Moreover, [44] reported that supplementation with ZnO-NPs diet decreased serum triglycerides, total cholesterol, and LDL while cholesterol with no significant differences, however, HDL-cholesterol increased ($P < 0.05$) in comparison with the control diet.

Rabbits groups supplemented with Zinc showed a marked increase in activities of hepatic catalase may be due to the indirect antioxidant effect of Zinc which removes peroxide from the body and reduces the formation of free radicals and acts as an inhibitor of NADPH oxidase so protects mitochondrial membrane structure from being damaged and Zn is an essential component in Cu-Zn-SOD, and dietary Zn levels positively correlate with Cu-Zn-SOD activity and dietary zinc supplementation can modulate SOD activity in rabbits[53], it has been demonstrated that Cu-Zn-SOD is involved in cellular scavenging of free radicals and ROS. Also, it induces metallothionein-a protein with antioxidant properties and increases the protein sulfhydryl group stability [54,55]. Moreover, MDA is the main marker for lipid peroxidation and oxidative damage caused by ROS [56]. Some research revealed that rabbit fed supplemented with ZnO-NPs and combined ZnO and ZnO NPs improve antioxidant activity by significantly improve the activity of SOD enzyme and slightly elevated plasma catalase than the control group respectively[42, 43]. Also [49] found that Infected rabbit with Eimeria treated with 10 mg ZnO-NPs/Kg compared to the control negative group after 2 weeks of infection exhibited a significant increase in serum SOD and CAT activities, While [25] showed that compared with the control group rabbits groups supplemented with ZnO-NPs and combined ZnO and ZnO-Nps, hepatic MDA level was a highly significant increase, while Hepatic CAT activities showed a highly significant decrease, the lowest value was observed in ZnO NPs supplemented rabbits group. The highly significant increase in zinc concentration in serum, liver, and thigh

tissue in the group supplemented with ZnO-NPs attributed to the smaller ZnO-NPs particles size diameter in comparison with conventional ZnO which results in higher absorption and easier diffusion throughout the mucous membranes, penetrating deeply into tissues through fine capillaries, crossing epithelial lining fenestration (e.g. liver), enabling efficient uptake by cells and efficient delivery of active compounds to target sites in the body and increase the cell concentration [57, 58]. In the same trend [42, 49] revealed that the administration of ZnO-NPs to rabbits increase significantly Zinc concentration in serum and liver which partly supported better absorption of ZnO-NPs and subsequently the positive relationship between ZnO-NPs supplementation and growth performance of rabbits. Similar results were observed by [59-61] showed a significant increase in muscle and serum Zn content (ppm) in the groups supplementation with ZnO-NPs in the diet compared with the control in the broiler diet.

6. Conclusion

Based on the results of this study, we can conclude that intake of a diet supplemented with ZnO in modified form as ZnO-Nps having a positive effect in animals even at half doses than the conventional sources. Supplementation with ZnO as nano form improved the growth, digestive efficiency, increasing birth weight, weaning weight, sealing weight, and FCR. Also altered serum protein and lipid profile biochemical parameter with favorable effects. ZnO-Nps improved immunity antioxidant status by decreasing the pre-waning and post-weaning mortality rate also immunity, and other performance of a different group of animals. Regarding Zn concentration in serum, liver, and thigh, Nano Zn supplementation also showed a higher Zinc concentration. It can be concluded that ZnO-Nps can replace ZnO in the rabbit dietary system with positive effects on growing rabbit productive performance.

7. References

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Table 1: Physical and chemical compositions of experimental diets of rabbits (%)

Groups / Ingredient	G 1 50 ppm ZnO	G 2 0 ZnO	G 3 25 ppm ZnO-NPs	G 4 50 ppm ZnO-NPs
Yellow corn	9.5	9.5	9.5	9.5
Barely	22	22	22	22
Wheat bran	19.5	19.5	19.5	19.5
Barsem Hay	26.1	26.1	26.1	26.1
SBM(44%CP)	17	17	17	17
Corn g meal 60%	0.7	0.7	0.7	0.7
Soya oil	2	2	2	2
D-L Methionine	0.4	0.4	0.4	0.4
Lime stone	1	1	1	1
Di Ca Phosphate	1	1	1	1
Sod. Chloride	0.4	0.4	0.4	0.4
Antitoxin	0.01	0.01	0.01	0.01
Premix*****	0.3	0.3	0.3	0.3
Anticolostridea	0.05	0.05	0.05	0.05
Anticoccoidea	0.04	0.04	0.04	0.04
Calculated analysis				
D E Kcal\Kg	2669.3	2669.3	2669.3	2669.3
Crude protein %	18.00	18.00	18.00	18.00
Crude Fiber %	11.00	11.00	11.00	11.00
E.E %	2.40	2.40	2.40	2.40
Crude Ash %	5.40	5.40	5.40	5.40
Lysine %	0.67	0.67	0.67	0.67
Methionine %	0.7	0.7	0.7	0.7
Meth+cyst %	0.54	0.54	0.54	0.54
Ca %	1.10	1.10	1.10	1.10
Total P %	0.69	0.69	0.69	0.69
Available P	0.28	0.28	0.28	0.28

*Each 3 Kg of Premix contain:vit. A 6000000 IU, D3 3000000 IU, E 40000 mg, K3 2000 mg, B1 2000 mg, B2 4000 mg,B6 200 mg, B12 10 mg, Pantothenic 10000 mg, Nicotenic 50000 mg, Folic acid 3000 mg, Biotein 50 mg, Choline chloride 250 mg, Manganase8.5mg, *(Zinc, G1 50 mg Zinc Oxide, G2 0 mg Zinc Oxide, G3 25 mg zinc oxide nanoparticles and G4 50 mg zinc oxide nanoparticles), Iron 50mg, Copper 5mg, Iodine 0.2mg, Selenium 0.1mg, Cobalt 0.1mg, Magnesium Ox.300mg, Caco3Added up to 3kg.

Table 2: The overall zootechnical performance parameters of growing NZW Rabbit

Groups / Item	G1 50 ppm ZnO	G2 0 ZnO	G3 25 ppm ZnO-NPs	G4 50 ppm ZnO-NPs
Birth weight(g)	61.67 ^{b} ± 5.53	56.06 ^{c} ± 4.10	66.21^{ab} ± 5.68	71.66^{a} ± 4.21
Weaning Weight (g)	602.39^{b} ±34.77	536.88 ^{c} ± 28.08	644.60 ^{a} ± 37.06	657.72 ^{a} ± 23.33
Sealing Weight (g)	1790.38 ^{b} ± 117.57	1635.00 ^{c} ± 114.29	1894.42 ^{a} ± 134.75	1943.61 ^{a} ± 131.38
Pre-Weaning Mortality %	21.28 ^{b} ± 2.28	30.68 ^{a} ± 6.88	14.65 ^{c} ± 1.82	12.75 ^{c} ± 0.98
Post-Weaning Mortality %	5.23 ^{b} ± 0.42	23.80 ^{a} ± 1.32	0.83 ^{c} ± 0.04	2.89 ^{bc} ± 0.16
FCR	3.06 ^{b} ± 0.16	3.47 ^{a} ± 0.26	2.88 ^{bc} ± 0.10	2.69 ^{c} ± 0.23

• Values in the same row with different superscripts are significantly different at P ≤ 0.05

a-c…Means bearing different superscripts within the same row are significantly different (P<0.05).

Table 3: The impact of dietary ZnO-Nps supplementation on serum parameters of Growing NZW rabbits at 62 d age-old

Groups / Item	G1 50 ppm ZnO	G2 0 ZnO	G3 25 ppm ZnO-Nps	G4 50 ppm ZnO-Nps
Total protein (g\dl)	4.36 ^{b} ± 0.140	4.08^{c} ± 0.04	5.02^{a} ± 0.15	5.03 ^{a} ± 0.13
albumin (g\dl)	1.78± 0.03	1.83 ± 0.06	1.83 ± 0.06	1.78 ± 0.05
globulin (g\dl)	2.58 ^{b} ± 0.13	2.25 ^{c} ± 0.05	3.19 ^{a} ± 0.15	3.24 ^{a} ± 0.13
A.G.Ratio	0.69 ^{b} ± 0.03	0.81 ^{a} ± 0.04	0.57 ^{c} ± 0.03	0.55 ^{c} ± 0.03
ALT (IU\ml)	14.67 ^{b} ± 1.53	20.33 ^{a} ± 1.53	12.33 ^{b} ± 0.58	8.67 ^{c} ± 1.53
AST (IU\ml)	22.00 ^{b} ± 2.00	30.00 ^{a} ± 1.00	20.00 ^{bc} ± 1.00	18.67 ^{c} ± 1.53
Alk-Ph (IU\ml)	52.33 ^{b} ± 2.52	62.33 ^{a} ± 2.52	44.00^{c} ± 1.00	42.00 ^{c} ± 2.65
Total bilirubin (mg\dl)	0.92 ^{ab} ± 0.13	1.08 ^{a} ± 0.12	0.81 ^{b} ± 0.08	0.73 ^{b} ± 0.11
Urea (mg\dl)	18.33 ^{b} ±0.58	21.67 ^{a} ± 0.58	17.00 ^{c} ± 1.00	14.67^{d} ± 0.58
Creatine (mg\dl)	0.58 ^{b} ± 0.05	1.33 ^{a} ± 0.06	0.52 ^{bc} ± 0.03	0.47 ^{c} ± 0.05
Uric Acid (mg\dl)	3.50 ^{a} ± 0.17	3.65 ^{a} ± 0.13	3.20 ^{b} ± 0.20	3.00 ^{b} ± 0.10

• Values in the same row with different superscripts are significantly different at P ≤ 0.05.

a-c Means bearing different superscripts within the same row are significantly different (P<0.05).

Table 4: The impact of dietary ZnO-Nps supplementation on serum lipid profile of Growing NZW rabbits at 62 d age-old

Groups / Item	G1 50ppm ZnO	G2 0 ZnO	G3 25ppm ZnO-Nps	G4 50ppm ZnO-Nps
Cholesterol (mg\dl)	126.33 [a] ± 3.22	128.33 [a] ± 2.89	116.67 [b] ± 2.89	108.67 [c] ± 1.53
Total Glyceride (mg\dl)	97.33 [b] ± 2.08	110.00 [a] ± 2.00	88.00[c] ± 2.65	80.33[d] ± 4.51
HDL(mg\dl)	31.00 [b] ± 1.00	26.67[c] ± 1.53	33.00[b] ± 1.00	37.00 [a] ± 1.73
LDL (mg\dl)	75.87 [b] ± 2.72	79.67 [a] ± 3.36	66.07[b] ± 2.76	57.27 [c] ± 4.66
Risk Factor	4.08 [b] ± 0.05	4.82 [a] ± 0.33	3.54 [c] ± 0.14	2.94[d] ± 0.13
VLDL (mg\dl)	19.47 [b] ± 0.42	22.00[a] ± 0.40	17.6 [c] ± 0.53	16.07 [c] ± 0.90

• Values in the same row with different superscripts are significantly different at P ≤ 0.05.

a-c Means bearing different superscripts within the same row are significantly different (P<0.05).

Table 5: The impact of dietary ZnO-NPs supplementation on the antioxidant biomarkers of Growing NZW rabbits at 62 d age old.

Groups / Item	G1 50 ppm ZnO	G2 0 ZnO	G3 25 ppm ZnO-Nps	G4 50 ppm ZnO-Nps
GSH nmol\L	27.33 [c] ± 3.79	10.73 [d] ± 0.45	32.00 [b] ± 1.00	38.83 [a] ± 1.26
SOD nmol\L	2.88 [c] ± 0.13	2.27 [d] ± 0.21	4.10 [b] ± 0.10	4.50 [a] ± 0.26
catalase nmol\L	8.17 [c] ± 0.15	3.00 [d] ± 0.10	10.17 [b] ± 0.15	11.30 [a] ± 0.3
MDA nmol\L	1.21 [a] ± 0.08	1.31 [a] ± 0.09	0.85 [b] ± 0.05	0.80 [b] ± 0.03
NO nmol\L	1.91 [b] ± 0.10	3.80 [a] ± 0.26	1.367 [c] ± 0.08	1.17 [c] ± 0.06

• Values in the same row with different superscripts are significantly different at P ≤ 0.05.

a-c Means bearing different superscripts within the same row are significantly different (P<0.05).

Table 6: The impact of dietary ZnO-NPs supplementation on the Zinc concentrations in serum, liver, and thigh muscles of growing NZW rabbits at 62 d ages-old.

Group / Item	G1 50 ppm ZnO	G2 0 ZnO	G3 25 ppm ZnO-Nps	G4 50 ppm ZnO-Nps
Serum Zinc, ppm	0.32 [b] ±0.05	0.07 [c] ±0.01	0.58 [a] ±0.06	0.67 [a] ± 0.07
Liver Zinc, ppm	0.48 [b] ±0.04	0.02 [c] ±0.00	0. 86 [a] ±0.08	0.89 [a] ±0.04
Thigh Zinc, ppm	0.82 [c] ±0.06	0.08[d] ±0.03	0.95 [b] ±0.07	1.10 [a] ±0.08

• Values in the same row with different superscripts are significantly different at P ≤ 0.05.

a-c Means bearing different superscripts within the same row are significantly different (P<0.05).

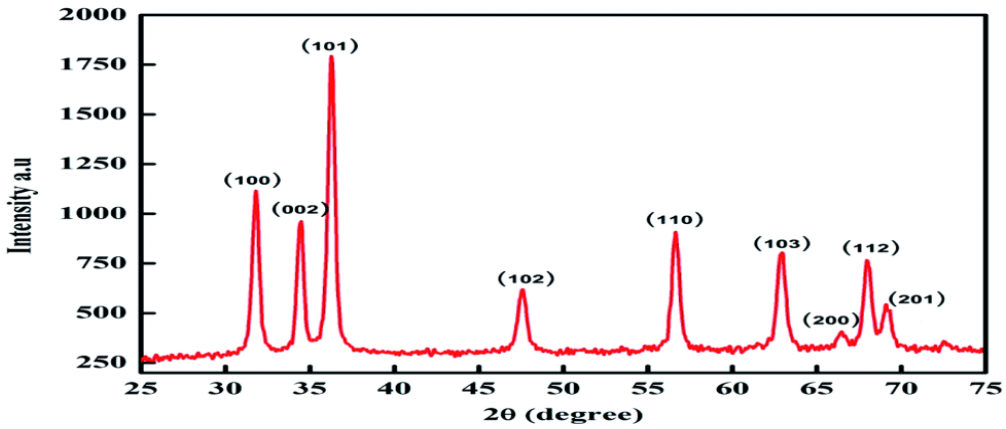


Fig.1 XRD pattern of starting Zinc Oxide powder and generated ZincOxide NPs.

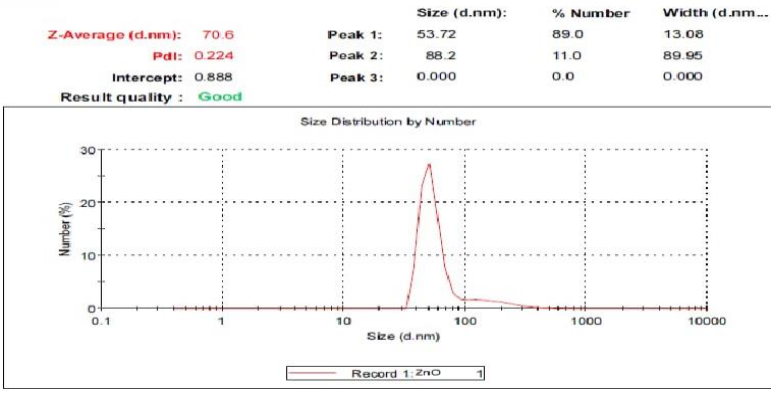
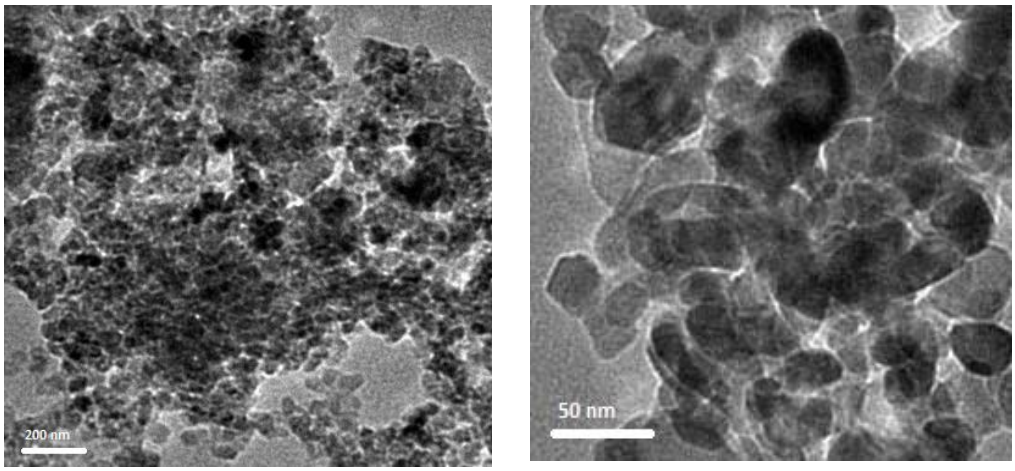


Fig.2 The average Ps was calculated from the HRTEM images and it was about 70.0 nm



(A) **(B)**

Fig .3. Zinc oxide before Milling (A) and Zinc Oxide after Milling by HTMR (B)