Evaluation of the effect of ZnO nanoparticles at different concentrations on reproductive characteristics and hematological parameters on male Japan's quails

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Avaliação do efeito de nanopartículas de ZnO em diferentes concentrações sobre as características reprodutivas e parâmetros hematológicos em codornas japonesas machos

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Received: 2025 Nov 12 | **Accepted:** 2025 Dec 11
Editor: Sarah Sgavioli
DOI: http://dx.doi.org/10.7213/acad.2026.2405
Rev. Acad. Ciênc. Anim. 2026;24:e2405

Abstract

This study aimed to determine the physiological and reproductive effects of nano zinc oxide added to the feed of male quails. One hundred twenty males were used and randomly distributed into four treatments with three replicates in a closed hall equipped with ventilation fans as follows: control (birds were fed a standard diet without any additives) or 40, 50, and 60 mg of nano zinc oxide/kg feed. The results showed that nano zinc oxide led to a significant increase in the blood characteristics of male birds in 60 mg/kg feed. The body weight escalated in the 50 and 60 mg/kg feed to the other treatments. The results also showed a significant increase in the relative weight of the right and left testicles, and the relative weight of the seminal vesicle and vasa in 50 and 60 mg of nano zinc oxide. There was an improvement in sperm count and the percentage of live sperm, as well as an increase in testosterone levels. Conversely, the percentage of dead and deformed sperm decreased. This study showed that treating quail with nano-zinc oxide at a concentration of 60 mg/kg of feed for 42 days gave the best results, leading to improved blood characteristics in terms of increased hemoglobin concentration, red blood cell count, and packed cell volume, in addition to increased live body weight, male reproductive organ weight, sperm count, live sperm percentage, a significant increase in testosterone levels, and a significant decrease in the number of dead and malformed sperm. Therefore, this study recommends using this concentration to improve male reproductive efficiency.

Keywords: Blood count. Body weight. Testicle. Foam gland. Sperm.

Resumo

Este estudo teve como objetivo determinar os efeitos fisiológicos e reprodutivos do óxido de zinco nano adicionado à ração de codornas machos. Cento e vinte machos foram utilizados e distribuídos aleatoriamente em quatro tratamentos com três repetições em um galpão fechado equipado com ventiladores, da seguinte forma: controle (aves alimentadas com uma dieta padrão sem aditivos) ou 40, 50 e 60 mg de óxido de zinco nano/kg de ração. Os resultados mostraram que o óxido de zinco nano levou a um aumento significativo nas características sanguíneas das aves no tratamento com 60 mg/kg de ração. O peso corporal aumentou nos tratamentos com 50 e 60 mg/kg de ração em comparação aos demais. Os resultados também mostraram um aumento significativo no peso relativo dos testículos direito e esquerdo, e no peso relativo da vesícula seminal e dos vasos deferentes nos tratamentos com 50 e 60 mg de óxido de zinco nano. Houve uma melhora na contagem de espermatozoides e na porcentagem de espermatozoides vivos, bem como um aumento nos níveis de testosterona. Por outro lado, a porcentagem de espermatozoides mortos e deformados diminuiu. Este estudo demonstrou que o tratamento de codornas com nano-óxido de zinco na concentração de 60 mg/kg de ração durante 42 dias apresentou os melhores resultados, levando à melhoria das características sanguíneas em termos de aumento da concentração de hemoglobina, contagem de glóbulos vermelhos e volume globular, além de aumento do peso corporal, peso dos órgãos reprodutores masculinos, contagem de espermatozoides, percentual de espermatozoides vivos, aumento significativo dos níveis de testosterona e diminuição significativa do número de espermatozoides mortos e malformados. Portanto, este estudo recomenda o uso dessa concentração para melhorar a eficiência reprodutiva masculina.

Palavras-chave: *Hemograma. Peso corporal. Testículo. Glândula espumosa. Espermatozoides.*

Introduction

Quail meat is considered an important source of animal protein, as it is an important and fundamental pillar in meeting human nutritional needs to a large extent (Lukanov et al., 2023). The quail operations have progressed markedly in recent years, resulting in substantial increases in productivity and efficiency due to advancements and applied research across several scientific domains within the sector (Hameed et al., 2021).

Antibiotics were previously used in quail feed and added to their diets, but then these additions and uses occurred as a result of the emergence of organisms that are resistant to these antibiotics and are pathogenic, as their presence in food residues was observed, and this poses a great danger to consumers and their life (Patra and Lalhriatpuii, 2020). Consequently, the quail sector has recently observed the adoption of numerous additives as substitutes for antibiotics, including probiotics, trace minerals, vitamins, organic acids, and others (Yaqoob, 2017; Ricke et al., 2020; Rasheed et al., 2021; Jasim et al., 2025).

However, as a result of the rapid development in the field of animal production, the importance of nutrition has become directed towards nanotechnology for use as feed additives in the diets of farm animals, including quail in particular, as nanotechnology is one of the new technologies in the field of scientific research, as it transforms material particles whose size ranges between 1-100 nanometers to increase the surface area and thus increase their chemical reactions. These properties make nanomaterials and elements efficient in terms of transport and absorption (Gopi et al., 2017). Nanotechnology also represents a revolutionary path in the field of technological development related to the management of materials on the nanometer scale, as nanotechnology means any technology at the nanoscale level that has many applications in the world (Nasrollahzadeh et al., 2019).

Nanoparticles are considered a large group of compounds that are either natural or engineered, and are classified into several categories, including organic and inorganic. Organic nanoparticles are also classified into metallic, such as silver and gold nanoparticles, and into metal oxide particles, such as nanoparticles of oxide and zinc oxide nanoparticles (Elmer and White, 2018; Ijaz et al., 2020).

The growth and development of most life forms depend on zinc, a trace mineral that is a cofactor for more than 300 industrial enzymes and proteins (Sahraei and Janmohammadi, 2014). Birds need zinc for metabolic activity of carbohydrates, proteins, lipids, proteins, antioxidants, and RNA and DNA catalysis (Feng et al., 2010; Hatab et al., 2022; Yusof et al., 2023).

The immune system and hormone synthesis promote many biological activities, reproduction, and immunity. Zinc is a potent antioxidant in cellular function, as it stimulates the carbonic anhydrase enzyme to eliminate free radicals (Babaei et al., 2007). Therefore, adding zinc to the diet reduces oxidative damage to cells caused by free radicals (Tupe et al., 2010; Almoteoty et al., 2022). Zinc is regarded as a facilitator of the functions of several hormones, including glucagon, insulin, and sex hormones (Chand et al., 2014). Zinc significantly contributes to fertility by safeguarding sperm DNA chromatin from damage caused by free radicals (Babaei et al., 2007). It enhances sperm production and motility in semen by functioning as an antibacterial agent and safeguarding sperm cells from injury (Kothari and Chaudhari, 2016).

Zinc significantly contributes to the maintenance of optimal testosterone levels in the bloodstream by facilitating the growth and function of the testes (Egwurugwu et al., 2013). Furthermore, it is crucial in the release of insulin-like growth factor (IGF-1) (Truong and Silkiss, 2023). Evidence suggests that IGF-1 regulates Sertoli cells during the prepubertal phase (Pitetti et al., 2013). IGF-1 further stimulates testosterone production from Leydig cells (Yoon and Roser, 2010). Zinc promotes the development of puberty in male birds consuming zinc-enriched diets by enhancing IGF-1 gene expression in the testes (Khoobbakht et al., 2020).

This study aimed to assess the impact of varying concentrations of nano-zinc oxide on the blood profile, reproductive efficiency, and testosterone levels in male quails.

Material and methods

Sixty male *Coturnix japonica*, aged 30 days, were randomly allocated into four treatments (15 birds per treatment) with three replicates per treatment (5 birds each replication). The weights of the birds varied from 170 to 190 grams. They were raised in a closed *C. japonica* hall equipped with extractors to ventilate the hall. Wood sawdust was used as a floor bedding with a thickness of 4-5 cm.

The lighting period was 16 hours using artificial lamps with a period of darkness of 8 hours daily. Feed and water were provided *ad libitum*, reared on standard ration supplemented with 40 and 60 mg nanozinc oxide/kg, ratio and feed the birds on a ration (National Research Council, 1994) (Table 1). All birds were placed in cages (50 × 50 × 50 cm). A thermometer was installed inside the cages to monitor the environmental conditions; the recorded temperature ranged from 20 to 35 ºC, while the relative humidity levels varied between 45 and 77%.

Table 1 - The components of the ration used in the study

Ingredients	Percentage of ingredients (%)
Yellow corn	40.0
Wheat	22.0
Proteins center	10.0
Soybean meat	25.0
Sun flower oil	2.0
Calcium	0.9
Salt	0.1
Calculated chemical analysis	
Protein ratio	23.89
Energy (kcal/kg)	2,971

Note: Computed based on National Research Council (1994).

The treatments were control (birds were fed a standard diet without any additives) or 40, 50, and 60 mg of nano zinc oxide/kg feed. Nanozinc oxide were obtained from a specialized company (US Research Nanomaterials, Inc®, USA, Purity 99.8).

At 60 days of age, the birds were weighed and slaughtered. The birds were subjected to 12 hour fasting period to slaughter as part of the standard procedures to ensure the accuracy of the results and to improve the quality of the experimental assessments. Blood samples were collected directly at slaughter with tubes containing an anticoagulant (EDTA). Blood tests were conducted to quantify red blood cells and packed cell volume as specified by Campbell (1995). The hemoglobin concentration was evaluated using the technique developed by Drabkin and Austin (1935) with the kit from Biosystems, Spain, and concentrations were computed as outlined by Abdulmajeed et al. (2012).

The mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were calculated as reported in Campbell (1995), and as described by Abdul-Majeed and Abdel-Rahman (2021). Blood smears were stained with Kimsa stain to determine the number of leukocytes, as mentioned in Campbell (1995). Concentrations were calculated as described by Al-Rahawi (2021). The relative weights of the testicles, the foam gland, and the carrier vessel were also calculated, and the reproductive efficiency/group was evaluated according to what was shown (Al-Sanafi, 1990). Live and dead sperm and the percentage of abnormalities were calculated. A ready-made test kit using ELISA technology was used to estimate the level of the testosterone.

Statistical analysis

A one-way completely randomized design (C.R.D.) was used and the data were analyzed using SAS software (SAS Institute, 2009). Differences between groups were determined using Duncan's test (Steel and Torri, 1960), with significance level of $p \leq 0.05$.

Ethical statement

All experimental procedures involving animals were approved by the Northern Technical University/ Technical Research Center ethics committee (approval process No. 0016/2025/2/23), and performed in accordance with the World Health Organization and the International Committee of Medical Journal Editors.

Results and discussion

The statistical analysis results in Table 2 demonstrate that the addition of varying concentrations of nano zinc oxide significantly elevated the counts of red and white blood cells, hemoglobin concentration, and the volume of packed blood cells in the treatment with 60 mg nano zinc oxide/kg feed, relative to the other experimental treatments. There was a significant reduction in MCV and MCH in the 60 mg/ kg treatment, indicating smaller erythrocytes with a lower absolute hemoglobin content. However, MCHC increased due to the higher hemoglobin concentration per unit of cell volume.

Table 2 - Effect of different concentrations of nano zinc oxide on blood count

Treatments	Parameters						
	RBC	WBC	HC	PCV	MCV	MCH	MCHC
Control	3.06 ± 0.24^{b}	10.60 ± 0.28^{b}	12.53 ± 0.28^{b}	44.33 ± 0.33^{b}	158.67 ± 12.86^{a}	45.01 ± 3.87^{a}	28.22 ± 0.46^{b}
NZO40	3.00 ± 0.19^{b}	11.00 ± 0.27^{b}	12.33 ± 0.27^{b}	43.40 ± 0.44^{b}	154.56 ± 11.25^{a}	43.94 ± 3.32^{ab}	28.38 ± 0.28^{b}
NZO50	3.26 ± 0.20^{b}	10.66 ± 0.31^{b}	12.53 ± 0.32^{b}	44.40 ± 0.41^{b}	145.67 ± 11.25^{a}	41.38 ± 3.81^{ab}	28.17 ± 0.49^{b}
NZO60	4.06 ± 0.16^{a}	13.80 ± 0.32^{a}	15.60 ± 0.42^{a}	48.60 ± 0.84^{a}	107.62 ± 4.41^{b}	34.56 ± 1.62^{b}	32.03 ± 0.40^{a}
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0043	< 0.1188	< 0.0001

Note: RBC = red blood cells ($10^6/mm^3$); WBC = white blood cells ($10^6/mm^3$); HC = hemoglobin concentration (g/dl); PCV = packed cell volume (%); MCV = mean corpuscular volume (μ3); MCH = mean corpuscular hemoglobin (Pg); MCHC = mean corpuscular hemoglobin concentration (g/dl); NZO = nano zinc oxide (40, 50 and 60 mg/kg). Values represent mean ± standard error. Different letters in one column indicate significant differences at the probability level ($p \leq 0.05$).

The results of our study agreed with the results of Abbasi et al., 2017, who noted an elevation in the quantity of red and white blood cells, hemoglobin concentration, and volume of packed blood cells following the administration of nano zinc oxide at a concentration of 40 mg/kg feed in comparison to the control treatment.

The findings reported by El-Sawy et al. (2021) were consistent with the results of our experiment as the use of nano zinc oxide into broiler diets at a concentration of 200 mg/kg resulted in an elevation of red and white blood cell counts, hemoglobin concentration, and packed cell volume. The findings concurred with those of Abdul-Majeed et al. (2022), who reported that the supplementation of zinc in the drinking water of broiler chickens resulted in a notable increase in red blood cell count, hemoglobin concentration, and packed cell volume; however, no significant differences were noted in mean corpuscular volume, mean corpuscular hemoglobin, and the percentage of mean corpuscular hemoglobin.

Zinc supplementation is crucial for the synthesis of red blood cells, with iron and vitamin B12, and its incorporation enhances blood constituents (Chen et al., 2016). Zinc influences numerous essential physiological functions, including thyroid function and the release of T3 and T4 hormones (Baltaci et al., 2019). Zinc sulfate nutritional supplements play a crucial role in erythropoiesis by stimulating and activating the formation of red blood cells, resulting in an increase in both red blood cell count and hemoglobin levels (Ahmed and Mohammed, 2020).

The importance of zinc in increasing the level of hemoglobin may be due to its active role in heme synthesis, as zinc acts as a cofactor in iron metabolism, in turn as a cofactor for the alpha-amylulinic acid dehydratase enzyme, which has a role in heme synthesis (Abdelhaleim et al., 2019).

The decrease in the average corpuscular volume and hemoglobin rate may be due to the existence of an inverse relationship between these measures and the number of red blood cells, as their decrease leads to an increase in the number of red blood cells and vice versa (Hameed et al., 2022). The ele-vation in glomerular hemoglobin concentration is ascribed to the expansion of packed blood cells and the hemoglobin level.

Table 3 presents the impact of varying concentrations of nano zinc oxide on the body weight and the relative weights of the testes, foam gland, and transport vessel in male quail. The incorporation of nano zinc oxide resulted in an elevation of live body weight in the third and fourth treatments relative to the first and second treatments. An increase was also observed in the relative weight of the right testicle and the left testicle, as well as the relative weight of the foam gland and the weight of the transport vessel in the two treatments of adding nano zinc oxide at concentrations of 50 and 60 mg/kg feed compared with the control treatment and the treatment of adding 40 mg of nano zinc oxide/kg feed.

Table 3 - Effect of different concentrations of nano zinc oxide (NZO) on body weight and relative weight of the right and left testicle, foam gland, and transport vessel of male *Coturnix japonica* at the age of 60 days

Treatments	Parameters				
	Body weight (g)	%WRT	%WLT	%FGW	%CW
Control	218.27 ± 2.89[c]	1.96 ± 0.05[c]	1.93 ± 0.05[c]	1.31 ± 0.05[c]	1.02 ± 0.06[c]
NZO (40 mg/kg)	218.00 ± 3.28[c]	2.07 ± 0.00[c]	1.94 ± 0.08[c]	1.27 ± 0.08[c]	1.31 ± 0.09[b]
NZO (50 mg/kg)	268.40 ± 7.08[b]	2.26 ± 0.06[b]	2.26 ± 0.06[b]	1.80 ± 0.06[b]	2.04 ± 0.13[a]
NZO (60 mg/kg)	318.33 ± 8.85[a]	3.15 ± 0.11[a]	2.88 ± 0.13[a]	2.86 ± 0.07[a]	2.13 ± 0.08[a]
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Note: WRT/WLT = weight of the right/left testicle (g/100g of bw); FGW = foam gland weight (g/100g bw); CW = conveyor weight (g/100g bw); bw = body weight. Values represent mean ± standard error. Different letters in one column indicate significant differences at the probability level ($p \leq 0.05$).

The results are consistent with those of El-Katcha et al. (2017), who indicated that nanozinc oxide at concentrations of 30, 45, and 60 IU/kg feed led to improved growth parameters and increased live body weight. Lee et al. (2022) also observed that incorporating zinc oxide into broiler diets at a concentration of 80 mg/kg feed resulted in an increase in live body weight and enhanced some productive features of the birds. Zinc is one of the essential nutrients in the diet of *C. japonica* birds and has many important effects within the body because it acts as a cofactor for many enzymes necessary in the metabolism of nutrients such as carbohydrates and proteins, which helps increase growth and improve the productive qualities of the bird (Hidayat et al., 2019; Al-Hamadani et al., 2025).

Table 4 illustrates the impact of incorporating various quantities of nano zinc oxide into the diets of male *C. japonica* on sperm count, the percentages of live and dead sperm, the percentage of deformed sperm, and the hormone testosterone. It was shown that adding nano zinc oxide at a concentration of 60 mg led to an increase in the number of sperm compared to the rest of the treatments. However, a decrease in the number of sperm was observed in the second treatment when zinc oxide was added at a concentration of 40 mg compared to the control treatment. Table 4 also indicates an increase in the percentage of live sperm in the fourth treatment, reaching 73.20% compared to the first, second, and third treatments (64.73%, 74.73%, and 65.40%, respectively). A reduction in the proportion of dead and malformed sperm was noted in the fourth treatment relative to the other experimental treatments. The addition of zinc oxide at a concentration of 60 mg resulted in an elevation of testosterone levels compared to the other treatments.

Table 4 - Effect of different concentrations of nano zinc oxide (NZO) on sperm numbers, percentages of live and dead sperm, sperm abnormalities, and testosterone level

Treatments	Parameters				
	Number of sperm x 10^6 sperm/ml	%Live sperm	%Dead sperm	%Deformed sperm	Testosterone level (ng/ml)
Control	3.13 ± 0.19^b	64.73 ± 1.25^b	35.26 ± 1.25^a	39.80 ± 1.01^a	3.40 ± 0.23^b
NZO (40 mg/kg)	2.46 ± 0.20^c	64.73 ± 1.03^b	35.26 ± 1.03^a	38.60 ± 1.55^a	3.72 ± 0.2^b
NZO (50 mg/kg)	2.80 ± 0.22^{bc}	65.40 ± 0.92^b	34.60 ± 0.92^a	39.40 ± 1.46^a	3.63 ± 0.20^b
NZO (60 mg/kg)	5.53 ± 0.23^a	73.20 ± 0.85^a	26.80 ± 0.85^b	28.60 ± 1.26^b	5.96 ± 0.20^a
p-values	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Note: Values represent mean ± standard error. Different letters in one column indicate significant differences at the probability level ($p \leq 0.05$).

Zinc is crucial for the metabolic processes of carbohydrates and proteins, as well as the catalysis of RNA and DNA (Feng et al., 2010), facilitating several biological functions, reproduction, and immune responses. It is a potent antioxidant in cellular function, as it stimulates the carbonic anhydrase enzyme to eliminate free radicals (Babaei et al., 2007). Consequently, incorporating zinc into the diet mitigates oxidative cellular damage induced by free radicals (Tupe et al., 2010). Zinc enhances the activity of some hormones, including glucagon, insulin, and sex hormones (Chand et al., 2014). Zinc is crucial for enhancing fertility by safeguarding sperm DNA chromatin from damage caused by free radicals (Babaei et al., 2007). This was in line with that recorded by Abbasi et al. (2022), as zinc oxide improved the fertility in quails. It enhances sperm production and motility in semen by functioning as an antibacterial agent and safeguarding sperm cells from harm (Kothari and Chaudhari, 2016). In addition, Matty et al. (2024) recorded that 40 mg of nano zinc particles increased testosterone hormone level and the concentration of living sperm and decreased the concentration of dead and abnormal sperm, which was in line with the current results.

Zinc significantly contributes to the maintenance of appropriate blood testosterone levels by facilitating testicular growth and function (Egwurugwu et al., 2013). Moreover, it is crucial in the release of insulin-like growth factor (IGF-1) (Truong and Silkiss, 2023). Evidence suggests that IGF-1 regulates Sertoli cells during the prepubertal phase (Pitetti et al., 2013). IGF-1 further stimulates testosterone production from Leydig cells (Yoon and Roser, 2010). The current findings were in line with that recorded by Goma et al. (2021), as zinc oxide enhanced the characteristics of semen, sexual attitude and pup's rendering, hormone productivity, as well as increasing the antioxidant activity of testis. In addition, the current results were compatible with that recorded by Amorezae et al. (2016), as zinc oxide enhanced the performance of reproductivity of male quails.

According to Khoobbakht et al. (2020), zinc facilitates the development of puberty in male pigeons consuming zinc-enriched diets by enhancing IGF-1 gene expression in the testes. There are other reports on various elemental supplements globally (Zahmatkesh et al., 2020; Roitman et al., 2021; Mir Rasekhian et al., 2022).

Conclusion

The inclusion of nano zinc oxide at 40, 50, and 60 mg/kg caused no adverse effects on the physiological or reproductive parameters of male quail. Instead, the 60 mg/kg level notably improved hematological traits, reproductive organ development, semen quality, and testosterone levels. Therefore, nano zinc oxide is a promising supplement for enhancing male reproductive performance. Further large-scale and long-term studies are still needed to confirm safety and determine the optimal dosage across different production systems.

Acknowledgments

This study was funded by the Northern Technical University/Technical Research Center and the University of Mosul/College of Agriculture and Forestry, affiliated with the Ministry of Higher Education and Scientific Research, Mosul, Iraq.

Authors´contributions

The empirical research was conducted by AYJ. Data analysis and references were written by AMS and GAR. All authors reviewed and approved the published version of the research.

Data availability statement

The research data are not publicly available.

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