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Effects of Nano Zinc Oxide-Enriched Diets on Bone Morphometry and Biomechanical Strength in Japanese Quails

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Abstract: Zinc is an essential trace element critical for numerous biological functions, including bone development, enzyme activity, and immune response. This study investigated the effects of zinc oxide (ZnO) and nano zinc oxide (NZn) supplementation on bone morphometry and biomechanical properties in Japanese quails (*Coturnix coturnix japonica*). A total of 118 one-day-old quails were divided into three groups: control (C), zinc oxide (Zn), and nano zinc oxide (NZn), with each group further subdivided into replicates. Diets were formulated to provide 75 mg/kg zinc, with additional zinc sources added to achieve the desired levels. Morphometric and biomechanical analyses were conducted using a 3-point bending test to evaluate tibiotarsus bone strength and structural properties. Results indicated that the NC group exhibited a significantly higher external mediolateral diameter (ExtMLD) compared to the Zn and C groups (P = 0.003), suggesting enhanced periosteal bone growth with NZn supplementation. However, no significant differences were observed in internal diameters or biomechanical parameters such as breaking force, moment of inertia, strength, stiffness, and elastic modulus among the groups (P>0.05). Sex-based comparisons revealed that female quails in the Zn and C groups had significantly higher breaking force and moment of inertia compared to males (P < 0.05). Still, no such differences were observed in the NZn group. These findings suggest that while NZn may positively influence specific morphometric parameters, its impact on biomechanical strength remains limited. The study highlights the need for further research to elucidate the mechanisms underlying zinc's effects on bone development.

***Keywords:** Biomechanic, Bone morphometry, Japanese quails, Zinc.*

Nano Çinko Oksit ile Zenginleştirilmiş Diyetlerin Japon Bıldırcınlarında Kemik Morfometrisi ve Biyomekanik Dayanıklılık Üzerindeki Etkileri

Özet: Çinko, kemik gelişimi, enzim aktivitesi ve bağışıklık yanıtı da dahil olmak üzere birçok biyolojik fonksiyon için kritik olan temel bir eser elementtir. Bu çalışma, çinko oksit (ZnO) ve nano çinko oksit (NZn) takviyesinin Japon bıldırcınlarında (Coturnix coturnix japonica) tibiotarsus kemiğinin morfometrisi ve biyomekanik özellikleri üzerindeki etkilerini araştırmıştır. Toplam 118 adet bir günlük bıldırcın, kontrol (C), çinko oksit (Zn) ve nano çinko oksit (NZn) olmak üzere üç gruba ayrılmış ve her grup kendi içinde alt gruplara bölünmüştür. Diyetler, 75 mg/kg çinko sağlayacak şekilde formüle edilmiş ve belirlenen düzeylere ulaşmak için ek çinko kaynakları kullanılmıştır. Morfometrik ve biyomekanik analizler, kemik dayanıklılığı ve yapısal özellikleri değerlendirmek amacıyla üç nokta eğme testi kullanılarak gerçekleştirilmiştir. Sonuçlar, NZn grubunun dış mediolateral çapının (ExtMLD) Zn ve C gruplarına kıyasla anlamlı derecede daha yüksek olduğunu (P = 0,003) göstermiştir, bu da NZn takviyesinin periosteal kemik büyümesini artırabileceğini düşündürmektedir. Ancak, gruplar arasında iç çaplar veya kırılma kuvveti, atalet momenti, mukavemet, rijitlik ve elastik modül gibi biyomekanik parametrelerde anlamlı bir farklılık gözlenmemiştir (P > 0,05). Cinsiyete bağlı karşılaştırmalarda, Zn ve C gruplarındaki dişi bıldırcınların kırılma kuvveti ve atalet momenti değerlerinin erkeklere kıyasla anlamlı derecede daha yüksek olduğu tespit edilmiştir (P < 0,05), ancak NZn grubunda benzer bir fark bulunmamıştır. Bu bulgular, NZn'nun belirli morfometrik parametreler üzerinde olumlu etkiler gösterebileceğini ancak biyomekanik dayanıklılık üzerindeki etkisinin sınırlı olduğunu ortaya koymaktadır. Çalışma, çinkonun kemik gelişimi üzerindeki etkilerini açıklığa kavuşturmak amacıyla daha ileri araştırmalara ihtiyaç olduğunu vurgulamaktadır.

***Anahtar Kelimeler:** Biyomekanik, çinko, Japon bıldırcını, kemik morfometrisi.*

Introduction

Zinc is a crucial trace element involved in over 2,000 transcription factors, essential for maintaining structural and functional integrity. It plays vital roles in bone development, enzyme activity, hormonal regulation, reproduction, growth, immune response, and more (Abbasi et al., 2017; McDowell, 2003). Zinc deficiency can adversely affect health and productivity in livestock (Suttle, 2010).

Nanotechnology involves manipulating matter at the nanoscale (1-100 nm), leading to innovative physical, chemical, and biological properties (Feng et al., 2009; Patil et al., 2012). Nano minerals, produced through physical, chemical, or biological methods, are widely used in agriculture, livestock, and food systems. They offer advantages such as enhanced absorption, reduced excretion, and multifunctional effects like growth promotion, immunomodulation, and antibacterial activity, even at lower doses than conventional minerals (Swain et al., 2015). Studies have demonstrated the efficacy of nano zinc, nano selenium, and nano chromium in animal nutrition (Swain et al., 2015).

Bones contain a significant amount of zinc, essential for normal bone development. Increased dietary zinc levels have been reported to improve bone strength (Bahtiyarca et al., 2007). While some studies have demonstrated that organic zinc minerals positively influence nutrition and growth, others have found no such effect (Ammerman et al., 1995; Cao et al., 2000). The mineral requirements of animals and their bioavailability depend on various factors, including species, breed, age, sex, growth rate, production type, and level, the chemical form and solubility of the mineral, diet composition (nutrient balance, presence of anti-nutritional factors), interactions between minerals, ambient temperature, and the criteria used to assess needs (e.g., maximum growth or bone mineralization) (Yazgan, 1990).

The Japanese quail (Coturnix japonica) has been widely used as an experimental model in various disciplines within the life sciences for decades (Padgett et al., 1959; Minvielle, 2004). As an animal avian model, the Japanese quail is frequently used in studies on the toxicology of chemical compounds and the effects of environmental endocrine disruptors or to study physiological processes in birds (Donaldson et al., 2015; El-Kholy et al., 2019; Tomaszewska et al., 2018,). Quails are also used as an animal model to study bone formation and development in both pre-hatch and post-hatch studies (Hiyama et al., 2019; Kawai et al., 2018; Miller and Bowman, 1981; Ohashi and Kusuhara, 1991; Pourlis et al., 1998; Simmons and Pankovich, 1963; ˇSkrob́anek et al., 2005; Zibŕ ın et al., 2003).

A comprehensive assessment of bone tissue's mechanical properties requires analysis of geometric parameters and implementation of standardized mechanical testing protocols. The biomechanical parameters obtained from these tests, including force-displacement relationship, stiffness, ultimate strength, and elastic modulus, are crucial for characterizing the structural and material properties of bone tissue.

The slope of the elastic region of the force-displacement curve represents the extrinsic. Stiffness or rigidity of the structure. The elastic modulus is a measure of the intrinsic stiffness of the material. The maximum stress the bone can sustain is referred to as the ultimate strength; these strength values are independent of the size and shape of the bone. However, the force required to break the bone differs from intrinsic strength because this breaking load or fracture force varies with bone size. It is crucial to keep this distinction in mind because intrinsic strength and breaking load can exhibit different trends in drug studies, especially if the drug affects the size of the bone (Turner and Burr, 1993).

Limited information is available regarding the effects of zinc sources on bone structure and development in quails (Kolaş et al., 2013).

This study aimed to compare the effects of zinc oxide and nano zinc oxide supplementation in different diets on bone strength.

Material and Methods

This study was carried out with the permission of Aydın Adnan Menderes University, Animal Experiments Local Ethics Committee, number 64583101/2023/46.

Animal Material

This study utilized a total of 118 one-day-old Japanese quails (Coturnix coturnix japonica). The quails were allocated into three groups with similar body weights: a control group, a zinc oxide group, and a nano zinc oxide group (Table 1). Experimental diets were formulated in accordance with the standards of the National Research Council (NRC). To achieve a zinc level of 75 mg/kg in the diets, an additional 60 mg/kg of zinc oxide or nano zinc oxide containing 72% elemental zinc was incorporated into the basal diet, accounting for the zinc present in the feed ingredients.

Table 1. Body and carcass weight values according to gender and groups.

	C		Zn		NZn	
	Female (n=22)	Male (n=18)	Female (n=22)	Male (n=17)	Female (n=27)	Male (n=12)
Body weight (gr)	239.91±17.63	198.56±19.44	232.50±26.60	192.82±17.02	233.70±28.28	196.17±27.04
Carcass weight (gr)	153.01±12.96	140.42±14.58	150.02±13.23	135.10±12.30	152.61±18.66	136.69±18.60

Nano zinc oxide, with a particle size of <50 nm, was procured from US Research Nanomaterials, Inc. (Houston, Texas). The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University.

Three-point bending test

Tibiotarsus bones were thawed at room temperature prior to mechanical testing. The midpoint of each bone was determined using a digital calliper and designated as the load point. The mediolateral and craniocaudal outer diameters of the bone were measured at the identified load point. The Zwick/Roell Z0.5 mechanical testing device, located at the TARBIYOMER facility of Adnan Menderes University, was utilized for mechanical tests (Figure 1).

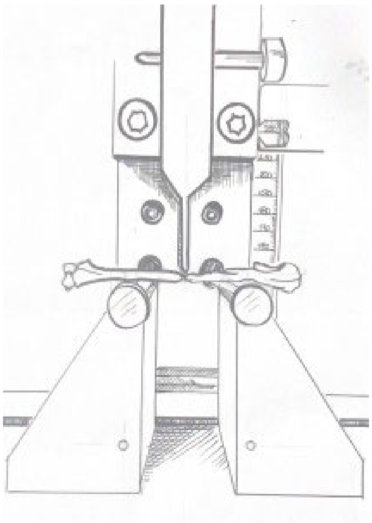

Figure 1. The Three-point bending mechanical testing device.

The distance between the support points for the three-point bending test was determined based on the length and diameter values of the bones examined. During the test, a 2 N preload was applied, followed by a load at a 1 mm/min rate until the bones fractured. A force (N)-deformation (mm) graph was generated for each bone. After testing, mediolateral and craniocaudal inner diameters were measured on the fractured bones. The cross-sectional moment of inertia was calculated using the endosteal and periosteal diameters.

Stiffness was determined from the linear regression of the force-displacement graph using the testXpert software (Zwick/Roell, Ulm, Germany). Ultimate strength and elastic modulus were calculated using stiffness, the moment of inertia, bone diameter, and the distance between the support points, based on the formulas outlined in the references (An and Draughn, 2020; ASABE Standards, 2007; Sharir et al., 2008; Turner and Burr, 1993).

Statistical Analysis

Statistical analyses were performed using the SPSS statistical package program (version 22.0, SPSS Inc., Chicago, Il, US) and R Studio software (version 4.4.2, Inc, Boston, MA, USA). The normal distribution of the data was checked using the Shapiro-Wilk test. Paired t-tests were used for gender comparisons of dependent variables when the data were parametric, while the Wilcoxon test was applied for non-parametric data. The homogeneity of variances was checked using the Levene test. For comparisons between groups, one-way analysis of variance (ANOVA) was utilized for parametric data, and the Kruskal-Walli's test was employed for non-parametric data. The homogeneity of variances was checked with the Welch test. If variances were homogeneous post-hoc Bonferroni test results were used. If variances were not homogeneous post-hoc Tamhane's T2 test results were used. The data are presented as mean value ± Standard deviation (MV±SD) and 95% confidence intervals in the tables. Statistical significance (P) was accepted as P<0.05.

Table 2. Abbreviations of measurements.

Abbreviation	Unit	Description
$Ext_{ML}D$	mm	Medio-lateral external diameter
$EXT_{CrCd}D$	mm	Crani-caudal external diameter
$Int_{ML}D$	mm	Medio-lateral internal diameter
$Int_{CrCd}D$	mm	Crani-caudal internal diameter
CI_{ML}	%	$[(Ext_{ML}D- Int_{ML}D)/ Ext_{ML}D]$ *100
CI_{CrCd}	%	$[(Ext_{CrCd}D- Int_{CrCd}D)/ Ext_{CrCd}D]$ *100
L	mm	Bone length
F	N	Bending force
I	mm^4	Moment of inertia
σ	MPa	Ultimate Strength
S	N/mm	Stiffness
E	MPa	Elastic Modulus

Results

Morphometric Measurements

The analysis of morphometric parameters revealed significant differences in specific measurements among the groups (Table 3). The external mediolateral diameter ($Ext_{ML}D$) was significantly greater in the nano zinc oxide (NZn) group (2.89 ± 0.03 mm) compared to the control (C) group (2.78 ± 0.02 mm) and the zinc oxide (Zn) group (2.77 ± 0.03 mm) (P= 0.003). However, no significant differences were observed in the internal mediolateral diameter ($Int_{ML}D$) or the external and internal craniocaudal diameters ($Ext_{CrCd}D$ and $Int_{CrCd}D$) among the groups (P > 0.05).

In gender comparison between groups, statistically significant differences were detected in the Cgroup for both outer and inner diameter values in the medio-lateral (ML) direction (P=0.040, P=0.006, respectively). However, no significant differences were observed in the Zn and NZn groups.

In the cranio-caudal (CrCd) direction, statistical analysis revealed significant differences in the outer diameter measurements of the Zn group (P=0.009). Significant differences were identified in both C (P=0.001) and NZn (P=0.007) groups regarding inner diameter values.

Analysis of cortical index values demonstrated a statistically significant difference (P=0.015) exclusively in the C group in the ML direction, while significant gender differences were observed in all groups in the CrCd direction (P=0.000, P=0.002, P=0.017, respectively) (Table 4).

The biomechanical analysis highlighted some group-related trends, although many differences were not statistically significant (Table 5). The breaking force (F) was

Table 3. Morphometric values between groups.

	C (n:40)	Zn (n:39)	NZn (n:39)	P
$Ext_{ML}D$ (mm)*	2.78±0.02[b]	2.77±0.03[b]	2.89±0.03[a]	
	(2.73-2.83)	(2.71-2.83)	(2.84-2.95)	**0.003**
$Int_{ML}D$ (mm)*	1.83±0.03	1.85±0.03	1.86±0.03	
	(1.77-1.88)	(1.80-1.91)	(1.80-1.93)	0.665
$Ext_{CrCd}D$ (mm)*	2.73±0.02	2.72±0.03	2.80±0.03	
	(2.68-2.77)	(2.66-2.78)	(2.732.86)	0.141
$Int_{CrCd}D$ (mm)*	1.85±0.03	1.83±0.03	1.87±0.03	
	(1.79-1.90)	(1.78-1.89)	(1.81-1.93)	0.642
L (mm)*	51.99±0.25	52.14±0.19	52.29±0.29	
	(51.48-52.51)	(51.76-52.52)	(51.70-52.88)	0.701
CI_{ML}*	34.09±1.01	32.82±1.12	35.47±1.17	
	(32.05-36.15)	(30.56-35.08)	(33.11-37.84)	0.240
CI_{CrCd}*	32.03±1.17	32.36±1.03	32.32±1.21	
	(29.66-34.40)	(30.28-34.44)	(30.32-35.20)	0.900

[a,b] :Means within a row that do not share a common superscript differ significantly ($P < 0.05$). *Abbreviations in the table are as explained in Table 2.

Table 4. Morphometric values in the female and male.

Parametre	Gender	C (n:40)	Zn (n:39)	NZn (n:39)
$Ext_{ML}D$ (mm)*	**Female**	2.77±0.03	2.80±0.04	2.88±0.03
		(2.71-2.84)	(2.71-2.89)	(2.82-2.95)
	Male	2.78±0.03	2.74±0.04	2.92±0.06
		(2.72-2.85)	(2.66-2.82)	(2.80-3.04)
	P	**0.040**	0.318	0.531
Int $_{ML}$D (mm)*	**Female**	1.76±0.03	1.82±0.04	1.85±0.04
		(1.69-1.83)	(1.75-1.90)	(1.77-1.94)
	Male	1.91±0.04	1.90±0.04	1.88±0.05
		(1.83-1.98)	(1.82-1.99)	(1.78-1.98)
	P	**0.006**	0.170	0.685
$EXT_{CrCd}D$ (mm)*	**Female**	2.75±0.03	2.79±0.04	2.79±0.04
		(2.69-2.81)	(2.71-2.87)	(2.71-2.87)
	Male	2.70±0.03	2.63±0.04	2.80±0.07
		(2.62-2.78)	(2.55-2.72)	(2.65-2.96)
	P	0.268	**0.009**	0.862
$Int_{CrCd}D$ (mm)*	**Female**	1.76±0.03	1.81±0.04	1.82±0.03
		(1.69-1.84)	(1.73-1.88)	(1.75-1.89)
	Male	1.95±0.03	1.87±0.04	1.99±0.05
		(1.88-2.01)	(1.78-1.96)	(1.89-2.10)
	P	**0.001**	0.239	**0.007**
L (mm)*	**Female**	52.39±0.33	52.38±0.23	52.69±1.92
		(51.69-53.08)	(51.90-52.85)	(52.03-53.34)
	Male	51.52±0.37	51.84±0.31	51.41±0.55
		(50.75-2.29)	(51.19-52.50)	(50.19-52.63)
	P	0.088	0.164	**0.040**
CI_{ML} (%)*	**Female**	36.28±6.53	34.62±7.03	35.58±7.56
		(20.33-50.52)	(18.60-45.95)	(23.24-50.87)
	Male	31.43±5.30	30.49±6.35	35.24±6.95
		(21.15-39.63)	(20.00-42.55)	(20.71-45.43)
	P	**0.015**	0.066	0.895
CI_{CrCd} (%)*	**Female**	35.66±7.08	35.06±5.95	34.65±6.43
		(20.88-47.02)	(24.15-47.23)	(22.14-46.18)
	Male	27.58±5.09	28.86±5.32	28.52±8.33
		(18.18-35.46)	(22.22-43.46)	(10.50-42.15)
	P	**0.000**	**0.002**	**0.017**

*Abbreviations in the table are as explained in Table 2.

Table 5. Biomechanic values between groups.

	C (n:40)	Zn (n:39)	NZn (n:39)	P
F (N)*	49.87±1.68 (46.48-53.26)	48.00±1.53 (44.91-51.10)	50.30±1.78 (46.69-53.91)	0.587
I (mm⁴)*	2.20±0.09 (2.03-2.38)	2.21±0.11 (1.99-2.44)	2.54±0.13 (2.27-2.82)	0.055
S (N/mm)*	73.93±2.24 (69.40±78.47)	73.35±2.12 (69.05-77.65)	78.63±2.49 (73.59-83.66)	0.209
σ (MPa)*	158.94±5.14 (148.55-169.33)	153.25±4.44 (144.27-162.23)	144.78±4.64 (135.40-154.17)	0.110
E (MPa)*	5769.74±167.71 (5429.526107.96)	5799±.93±191.02 (5413.22-6186.64)	5463.64±200.02 (5058.73-5868.56)	0.376

*Abbreviations in the table are as explained in Table 2.

marginally higher in the C and NZn groups than the Zn group; however, these differences were not statistically significant (p = 0.587). The moment of inertia (I) tended to be higher in the NZn group (2.54 ± 0.13 mm^4) compared to the Zn (2.21 ± 0.11 mm^4) and Zn groups (2.20 ± 0.09 mm^4), approaching significance (p = 0.055). The ultimate strength was highest in the C group (158.94 ± 5.14 N) compared to the NZn (144.78 ± 4.64 N) and Zn (153.25 ± 4.44 N) groups, though these differences were not statistically significant (p = 0.110). Similarly, stiffness and elastic modulus values did not show significant differences between the groups (p = 0.209 and p = 0.376, respectively).

Sex-based differences were most evident in the C and Zn groups (Table 6). Female quails in the Zn group exhibited significantly higher breaking force (p = 0.001), moment of inertia (p = 0.003) and stiffness (p = 0.002) compared to males. A similar trend was observed in the C group, where females displayed higher breaking force (p = 0.001), moment of inertia (p = 0.038) and stiffness (p = 0.020) than males. In contrast, in the NZn group, sex-related differences were less pronounced, and no significant variations were detected in most parameters (p > 0.05).

Figure 2 presents correlation matrices constructed using R statistical software, depicting the relationships between variables for females (A) and males (B). The correlation coefficients, ranging from -1 to +1, are visualized through circles where the magnitude and direction of correlations are indicated by the size and color intensity of the circles. Analysis revealed predominantly weak to moderate correlations among variables in both groups.

Discussion

Quails are not only raised for egg or meat production but are also used as experimental animals and valuable birds for research purposes. The use of Japanese quails in biomedical research is increasingly widespread (Minvielle, 2004) and is widely used in biological and genetic studies. Bones contain significant amounts of zinc necessary for normal bone development. Increased dietary zinc levels have been reported to improve bone strength (Bahtiyarca et al., 2007). Mechanical tests and bone geometry are used to reveal the external and internal strength of bone tissue. External properties include strength, deformation values and geometric properties, while elastic modulus and strength values are used to evaluate internal properties. In particular, elastic modulus and strength data (internal properties) are evaluated when drug applications affecting bone tissue are applied (Turner and Burr, 1993). Since dietary zinc supplementation affects bone strength (Bahtiyarca et al., 2007), it is necessary to compare the internal properties of bone tissue to investigate the effect of nanozinc in the study.

In bone biomechanics, structural properties of bones contribute significantly to bone strength. Changes in cortical bone, such as density and porosity, directly affect the mechanical assessment of bone (Iolascon et al., 2013; Kralich and Zemel, 2020). Parameters such as F (fracture force) and I (moment of inertia) are directly related to cortical thickness. The geometrical properties of bones, especially the area moment of inertia, determine their behavior under mechanical loading. Cortical components play an important role in bone strength, and the moment of inertia values characterize the resistance of bones to deformation (Muszyński et al., 2018). In our study, cortical index assessments revealed that higher cortical thickness observed in female individuals leads to higher values of moment of inertia (I) and fracture force (F). The stiffness (S) parameter reflects the total deformation characteristic of the bone under load. Due to the positive correlation between the cortical index and the F and S parameters, S values were found to be significantly higher in female individuals. Moment of inertia (I) characterizes the resistance of the bone to deformation under mechanical loading. The high cortical thickness observed in female individuals under the applied load in the craniocaudal (CrCd) direction also causes an increase in the resistance values in this direction. The microstructural properties of the bone tissue affect the elastic modulus and strength parameters. The thickness and structure of the cortical component significantly affect these parameters (Muszyński et al., 2018). In addition, the increase in resistance (I) leads to a decrease in the elasticity property per unit area (mm^2) of the bone. In our study, bone geometry and mechanical properties showed significant differences between female and male individuals. Cortical thickness and

Table 6. Biomechanic values in the female and male.

	F (N)*		P	I (mm⁴)*		P	S (N/mm)*		P	σ (MPa)*		P	E (MPa)*		P
	Female	Male		Female	Male		Female	Male		Female	Male		Female	Male	
C (n:40)	54.53±2.27 (49.80-59.25)	44.17±1.75 (40.48-47.86)	**0.001**	2.36±0.12 (2.12-2.60)	2.00±0.12 (1.76-2.25)	**0.038**	78.95±3.31 (72.06-85.84)	67.80±2.24 (63.06-72.53)	**0.020**	163.64±7.56 (147.91-179.36)	153.19±6.68 (139.09-167.29)	0.318	5728.49±245.61 (5217.73-6239.26)	5817.93±228.15 (5336.58-6299.28)	0.545
Zn (n:39)	52.35±1.77 (48.68-56.03)	42.37±1.98 (38.17-46.56)	**0.001**	2.50±0.16 (2.16-2.83)	1.85±0.10 (1.63-2.07)	**0.003**	78.80±2.64 (73.30-84.30)	66.30±2.68 (60.61-71.99)	**0.002**	153.11±6.70 (139.18-167.03)	153.44±5.57 (141.63-165.25)	0.971	5537.62±260.98 (4994.87-6080.36)	6139.39±265.25 (5577.08-6701.71)	0.120
Nzn (n:39)	52.46±2.08 (48.19-56.72)	45.46±3.12 (38.59-52.32)	0.069	2.56±0.14 (2.27-2.85)	2.51±0.31 (1.83-3.19)	0.875	81.84±3.06 (75.56-88.13)	71.39±3.60 (63.47-79.31)	0.051	147.48±4.90 (137.41-157.56)	138.72±10.43 (115.75-161.68)	0.390	5513.20±172.75 (5157.10-5868.29)	5352.15±537.43 (4169.27-6535.02)	0.715

*Abbreviations in the table are as explained in Table 2.

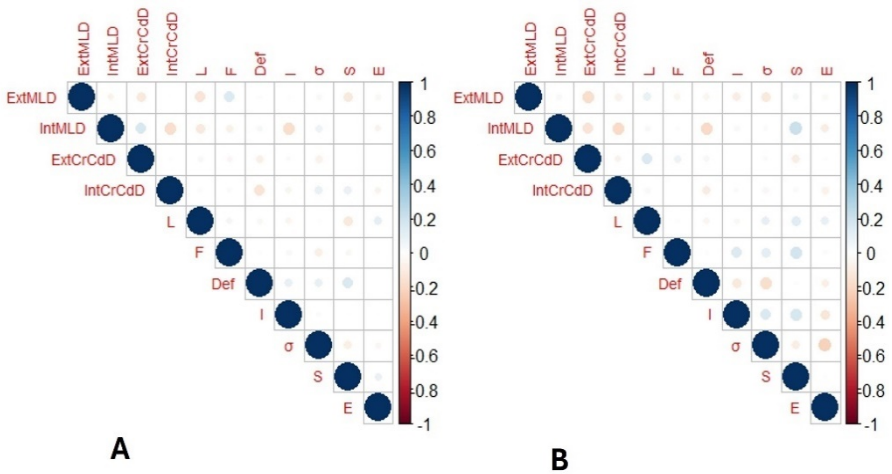

Figure 2. A Comparative Analysis of Correlation Patterns the Male Groups (A), Female Groups (B). Red Colours: negative correlation, Blue Colours: positive correlation. Abbreviations in Figure are as explained in Table 2.

moment of inertia values varied depending on gender, affecting the mechanical properties of the bone (Ciosek et al., 2021). Therefore, although no statistically significant difference was observed between the sexes, it was found that the elasticity parameters showed lower values in female individuals with high resistance values.

In addition, poultry has three types of bone tissue: compact, spongy and medulla bone. The Medulla bone meets the additional calcium (Ca) requirement for eggshell formation. The primary purpose of this bone type is to prevent skeletal defects that may occur due to decreased calcium supply during growth in laying hens (Korver et al., 2004). Since the materials used in our study were 35 days old and had not yet started laying eggs, it is thought that the amount of calcium required for eggshell formation was not used from the bone tissue. This situation led to higher values in female animals.

Although no statistically significant difference was observed between the groups, it was determined that F, I and S parameters showed higher values in the NZn group with high cortical thickness, like gender dimorphism. On the other hand, it was found that the elastic modulus and strength parameters reflecting the mechanical properties of the bone at the tissue level showed lower values in this group.

In conclusion, while nano zinc oxide supplementation appears to enhance periosteal growth, its limited impact on biomechanical properties underscores the complexity of zinc's role in bone development. The contrast between morphometric and biomechanical outcomes highlights the influence of factors such as zinc form, species, and experimental design. Future studies should investigate the long-term effects of nano zinc, histological changes in bone microstructure, and sex-specific metabolic pathways to elucidate its mechanisms. Additionally, comparative studies across poultry species could clarify whether the observed effects are unique to quails or broadly applicable.

Although the effects of nano zinc oxide supplementation on bone development were examined in detail in this study, there are some limitations. First, the lack of histological analyses limited the understanding of microstructural changes in bone tissue. Finally, since the results were evaluated only on Japanese quails, the generalizability of the findings to other species is limited. Addressing these shortcomings may provide an important roadmap for future studies.

Conclusion

Nano zinc oxide (NZn) supplementation increased the outer mediolateral diameter (ExtMLD) of the tibiotarsus bone in Japanese quails and promoted periosteal development, but did not show a significant effect on biomechanical resistance (fracture strength, elastic modulus). The high fracture resistance observed in female individuals in the control and Zn groups may be attributed to the interactions between sex hormones and mineral metabolism. Further studies are required to elucidate the bone remodeling mechanisms and bioavailability of nanoparticles. These findings emphasize the morphometric adaptation potential of nanomineral use in poultry feeding but reveal that the biomechanical effect is limited.

Ethical Permission

This study was carried out with the permission of Aydın Adnan Menderes University, Animal Experiments Local Ethics Committee, number 64583101/2023/46.

Conflict Of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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This research received no external funding.

Author Contributions

Conceptualization; investigation; writing – original draft; formal analysis. FTY

Project administration; writing – review and editing: İGY

Supervision; methodology; validation; visualization; software; data curation: FSK

Sımılarıty Rate

We declare that the similarity rate of the article is 12% as stated in the report uploaded to the system.

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