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Effects of Vitamins D3, A, and K3 Administration on Productive Performance, Egg Quality, and Tibia Mineralization in Aged Japanese Quails

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Abstract

This study was performed to investigate the single and interactive effects of vitamins D_3 (0 and 6300 IU), A (0 and 23100 IU) and K_3 (0, 17 and 34 mg) on performance, egg quality and tibia mineralization using 300 breeder Japanese quails at 40 weeks of age. Vitamin preparations were injected subcutaneously (*SC*) in the back of the neck of birds weekly. Vitamin A significantly increased egg production, egg number, and egg mass ($P < 0.05$). Vitamin D3 significantly increased egg weight, yolk color index, yolk weight, and shell thickness compared with the ivergedcontrol birds ($P < 0.05$). Vitamin K3 (34 mg) significantly improved egg weight, yolk color index, yolk weight, and shell thickness compared to the control birds ($P < 0.05$). Proximal epiphysis and diaphysis diameter of the right tibia bone and its ash percentage were significantly superior in the vitamin D_3 injected birds by 4.3, 4.9 and 9.3 percent, respectively ($P<0.05$). Robustness index (RI) was significantly greater in the vitamin D_3-treated birds compared with the corresponding control group ($P<0.05$). Tibia ash content showed a moderate positive correlation with tibia length ($r=0.43$) and a negative moderate relation with the robustness index. Serum PTH and ALK activity showed a weak and a moderated correlation coefficient with tibia ash percentage, respectively. No significant effects were noticed for $D_3 \times A$, $D_3 \times K_3$, $A \times K_3$ and $A \times D_3 \times K_3$ interactions for the features assessed ($P>0.05$). It was concluded that the fat-soluble vitamins evaluated improved certain and different aspects of performance and egg quality in aged quails. However, among them, Vitamin D_3 may have promising effects on bone strength and mineralization evidenced by greater tibia ash percentage as well as superior proximal epiphyses and diaphysis diameter in the birds receiving 6300 IU vitamin D_3 through weekly *SC* injection.

Introduction

Fat- soluble vitamins are essential micronutrients in all species of poultry to perform regular physiological functions and productivity. Optimum dietary levels of these vitamins support the commercial birds to exhibit their full genetic capabilities. Vitamin A plays a key role in sustaining several physiological functions, including vision, immunity, epithelial cell growth and differentiation, sexual proliferation, and bone health (Shastak *et al*., 2024). Vitamin D_3 is involved in proper metabolism of calcium (Ca) and phosphorus (P), and in conservancy of normal skeletal integrity, in particular in laying birds (Wei *et al*., 2024). Vitamin K_3 is indicated in poultry for the prevention of hemorrhages associated with various diseases, such as, Coccidiosis and Gumboro, as well as hemorrhagic syndromes due to vitamin K_3 deficiency (Suttle, 2013) and for the prevention and reduction of blood spots in eggs (Okoh and Perry, 2025). Based on such great importances, the multifaceted impacts of the vitamins A, D_3 and K_3 in many species of poultry have been extensively investigated, showing many vital roles beyond their abovementioned primarily recognized biological significance.

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Among many conjoint metabolic effects proposed, vitamins A, D_3, and K_3 are concerned for their single and interactive important roles in bone metabolism and growth, a research topic receiving considerable interest recently. Obviously, they have distinct mechanisms and effects where vitamin D_3 is crucial for Ca absorption and bone mineralization (Essig, 2022; Burns-Whitmore *et al*., 2024). Vitamin A was shown to promote bone growth and development (Ling, 2025). Vitamin K_3 is essential for activating proteins involved in bone formation and preventing Ca buildup in soft tissues (Alonso *et al*., 2023). Vitamin K_3 is primarily known for enabling normal blood coagulation; however, many studies suggested that it also involves critical metabolic pathways concerning bone health, preventing vascular calcification (Grimaldi *et al*., 2025 a,b), enhancing brain function and modulating immune system function (Emekli-Alturfan and Alturfan, 2023).

Increasing research evidence on the impact of fat-soluble vitamins on bone health and mineralization encourages the poultry industry to welcome studies directing in-depth characterization of the same topic. Because bone fragility in laying flocks, particularly those kept in confined battery cages such as quails and chickens, is considered a significant welfare and productivity concern. Evidently, fragile bones often lead to an enhanced risk of fracture and pain, in advanced age. Bone fragility arises from a combination of factors, including genetic predisposition, metabolic stresses originating primarily from the high rate of egg production and the most commonly cited being nutritional deficiency (Jansen *et al*., 2020; Eusemann *et al*., 2020).

Considering the recent concerns on the impact of fat-soluble vitamins on different aspects of commercial birds' metabolism, investigation on the response of performance attributes, egg quality features and bone fragility in aged birds to various combinations of dietary or parenteral administration of these vitamins may provide novel scientific achievements for both researchers as well as the poultry industry sector. Therefore, the purpose of this paper is to highlight the multifaceted single and interactive impacts of vitamins D_3, A and K_3 on production performance, egg quality and tibia mineralization in aged breeder Japanese quails at 40 weeks of age.

Materials and methods

Animals, Diets, and Experimental Design

A total of 300 Japanese quails (40 weeks old) with an average initial body weight of 220±10 g were provided from a commercial farm. The birds were allocated in galvanized wire battery cages (20 × 25 × 35 cm) arranged in 5 rows at a density of 5 birds per cage (4 females and 1 male) during an 8-week experimental period. The study was conducted as a 2 ×2×3 factorial arrangement in a complete randomized block design with 12 treatments and 5 replicates (totalling 60 experimental units). The investigated factors included: vitamin D_3 at two levels (0 and 6,300 IU), vitamin A at two levels (0 and 23,100 IU) and vitamin K_3 at three levels (0, 17, and 34 mg). These figures are supposed to provide above the bird's requirements for a week based on NRC (1994) recommendations. Vitamin preparations were administered subcutaneously (*SC*) in the back of the neck of birds using a semiautomatic syringe on a weekly schedule. The birds had free access to water and feed throughout the experimentation period. They were kept in a controlled environment with a constant temperature of 22 ± 1°C, relative humidity of 55 to 60%, a 18L:6D lighting schedule and proper ventilation with a minimum of 15 air changes per hour. All experimental procedures were reviewed and approved by the Animal Ethics Committee of Lorestan University.

The basal diet was formulated based on NRC (1994) standards with minor modifications. The composition and the calculated nutrient content of the diet fed are presented in Table 1.

Table 1: Composition of the basal diet and calculated chemical composition

Dietary Ingredients (%)	
Corn	54.81
Soybean meal	32.04
Dicalcium phosphate	1.15
Calcium carbonate	6.60
Mineral-vitamin premix [1]	0.10
Salt	0.30
Soybean oil	4.96
DL-methionine	0.04
Chemical Composition (%, calculated)	
Metabolizable energy (kcal/kg)	2900
Crude protein (%)	20
Calcium (%)	2.5
Available phosphorus (%)	0.35
Sodium (%)	0.15
Linoleic acid (%)	2.45
Arginine (%)	1.31
Lysine (%)	1.19
Methionine + Cysteine (%)	0.78
Threonine (%)	0.74
Vitamin A (IU/kg)	81.83
Vitamin D_3 (IU/kg)	2.3
Vitamin K_3 (mg/kg)	0.75

[1] These values per kg of diet include:
-Vitamins: Vitamin A: 11000 IU - Cholecalciferol (Vitamin D): 2300 IU - Vitamin E: 121 IU - Vitamin K_3 : 2 mg - Vitamin $B_{1\ 2}$: 0.02 mg - Riboflavin: 40 mg - Folic acid: 0.075 mg - D-Biotin: 0.075 mg - Pyridoxine: 4 mg - Ethoxyquin: 0.125 mg
- Mineral Premix: Manganese: 100 mg - Iron: 80 mg - Zinc: 60 mg - Copper: 8 mg - Iodine: 0.5 mg - Cobalt: 0.2 mg - Selenium: 0.15 mg.

Performance and Egg Trait Measurements

Following a 1-wk adaptation period, performance and egg quality data were collected for 8 wk. Eggs were collected, counted, and weighed daily using a digital scale (with an accuracy of 0.01 g). Weekly feed consumption was calculated as the difference between feed offered and residual feed in feeders at the close of the week. Egg quality traits were measured weekly using a random sample of two eggs per replicate. Egg weight was recorded immediately after collection. For component analysis, eggs were broken and the yolk was separated intact from the albumen. Yolk and albumen weights were recorded separately. Shells were washed, air-dried for 24 h, and then weighed. The proportion of each component was calculated as a percentage of total egg weight.

Egg shape index was calculated as (width/length) × 100 after measuring length and maximum width with a digital calliper (at an accuracy of 0.01 mm). Yolk color was assessed immediately after separation using a Roche color fan. Shell thickness was measured at the blunt end, equator, and sharp end of dried shells using an electronic digital calliper (with an accuracy of 0.01 mm); the three measurements were averaged as shell thickness.

Bone Quality Assessment

From each replicate, two female birds were randomly selected and slaughtered at the age of 48 weeks. The right tibia bones were collected from the same birds (Zaefarian *et al*., 2021). Without boiling and after meat removal, the bones were individually packaged in plastic bags and frozen at -20°C pending further analysis.

Prior to analysis, the bones were defrosted for 24 hours under appropriate conditions, and all remaining soft tissues were carefully removed. The length and width (at the proximal and distal epiphyses and mid-diaphysis portion) of each sample were then measured in triplicate using an electronic digital calliper (with 0.01 mm precision).

Bone Quality Indices Calculation

Equation (1) presents the Seedor index, which calculates the ratio of dry bone weight to bone length. This index serves as an indicator of bone density (Evaris *et al*., 2021):

Equation 1

$$\text{Seedor index} = \frac{\text{Dry bone weight (g)}}{\text{Bone length (cm)}}$$

Equation (2) defines the Robustness index, representing the ratio of bone length to the cube root of bone weight. This index reflects the mechanical strength of the bone (Evaris *et al*., 2021):

Equation 2

$$\text{Robustness index} = \frac{\text{Bone length (cm)}}{\sqrt[3]{\text{Bone weight (g)}}}$$

Bone Density Assessment

Tibia mineralization was assessed using a Computerized Tomography Scanning system (the Siemens Somatom 2-slice CT scanner, manufactured in Germany, model used at the Faculty of Veterinary Medicine, University of Tehran). For scanning, each bone specimen was positioned in a standardized orientation to minimize measurement error (Anderson *et al*., 2023a,b). Prior to scanning, the system was calibrated using a material calibration phantom including water (0 HU), air (-1000 HU), and hydroxyapatite with standard densities (250-1000 mg/cm³)). The accuracy of calibration was verified by comparing the measured HU values with reference values (Perilli *et al*., 2022).

For ash content determination, the bones were first dried in an oven at 105°C for 24 hours (Zhang *et al*., 2023). The dried samples were then ground using a mortar and placed in porcelain crucibles. Subsequently, the samples were ashed in a muffle furnace at 550°C for 24 hours (Wilson *et al*., 2022). Equation (3) calculates the ash percentage, representing the mineral content of bone tissue. This index quantifies the proportion of inorganic minerals relative to the organic bone matrix, providing crucial information about bone mineralization status (Anderson *et al*., 2023a, b):

Ash percentage (%) = (Ash weight (g) / Dry defatted bone weight (g)) × 100

Statistical Analysis

Data were analyzed with a 2 × 2 × 3 factorial arrangement in a complete randomized block design using the *mixed* model procedure of Statistical Analysis System, version 9.2 (SAS Institute Inc., Cary, NC). The statistical model included the fixed effects of vitamin D_3 at two levels (0 and 6,300 IU), vitamin A at two levels (0 and 23,100 IU) and vitamin K_3 at three levels (0, 17, and 34 mg) and their interactions. Because of a possible influence of variation in final body weight on the criteria assessed, body weight was used as a covariate in the analysis of all features. When a significant F-test was detected ($P<0.05$) for the main effects, corresponding means were separated by the Tukey test, and the interaction between treatments was analysed using an LS means test adjusted for Tukey test. The Pearson correlation coefficient was employed to determine any correlation between right tibia bone ash and other traits.

Results

Vitamin D_3 had no significant effect on egg production, egg number, egg mass, average egg weight, feed intake, or FCR (Table 2; $P > 0.05$). Vitamin A significantly increased egg production percentage, egg number, and egg mass (Table 2; $P < 0.05$), but did not affect average egg weight, feed

intake, or FCR. Vitamin K_3 influenced egg production and number of eggs at specific doses, with 17 mg resulting in lower values, while average egg weight, feed intake, and FCR were unaffected. Significant interactions were observed between vitamins A and K_3 for egg production and egg mass (Table 2; $P < 0.05$).

Parenteral administration of vitamin D_3 significantly increased egg weight, yolk color index, yolk weight, and shell thickness compared with the corresponding control birds (Table 3; $P < 0.05$), whereas yolk percentage, albumen percentage, and shell strength were not affected. Vitamin A injection significantly increased yolk color index (Table 3; $P < 0.01$) but had no significant effect on egg weight, yolk weight, shell thickness, or egg component percentages. Vitamin K_3 exhibited a dose-dependent response, where the greater level (34 mg) significantly improved egg weight, yolk color index, yolk weight, and shell thickness compared to the control birds (Table 3; $P < 0.05$). Significant interactions of $D_3 \times A$ and $A \times K_3$ were observed for egg weight and yolk weight, while other interactions did not influence yolk, albumen, or shell percentage.

Table 2: Effects of parenteral administration of vitamins D_3, A and K_3 on egg production (%), number eggs, mass weight (g), average egg weight (g), feed intake (g/hen/day), and feed conversion ratio (FCR) in aged breeder Japanese quail

Factor/level	Body weight(g)	Egg Production (%)	number eggs	Mass weight (g)	Average egg weight (g)	Feed intake (g/hen/day)	Feed conversion ratio(FCR)
Vitamin D_3 (IU)							
0	231.14	60.13	15.40	117.43	11.59	24.09	5.34
6300	233.41	64.26	16.00	127.98	11.62	23.89	4.76
SEM	2.781	2.48	0.63	8.26	0.08	0.31	0.48
Vitamin A (IU)							
0	236.88	61.01	15.60	120.49	11.66	24.08	5.20
23100	227.67	63.38	15.80	124.92	11.55	23.91	4.89
SEM	2.588	2.32	0.64	7.74	0.07	0.27	0.49
Vitamin K_3 (mg)							
0	230.71	66.24^{a}	17.30^{a}	140.24^{a}	11.56	23.88	4.31^{a}
17	229.83	55.86^{b}	14.90^{b}	106.69^{b}	11.67	23.64	5.80^{a}
34	236.28	64.50^{a}	14.90^{b}	121.18^{ab}	11.59	24.46	5.02^{ab}
SEM	3.157	2.28	0.54	8.27	0.08	0.40	0.19
ANOVA							
D_3	0.5097	0.1872	0.4518	0.3161	0.7858	0.6371	0.2701
A	0.0095	0.0185	0.0229	0.0389	0.7424	0.2751	0.0755
K_3	0.2554	0.4466	0.8014	0.6721	0.3736	0.6877	0.5638
$D_3 \times A$	0.3976	0.5255	0.7754	0.5761	0.3326	0.5537	0.6451
$D_3 \times K_3$	0.9879	0.175	0.9332	0.5875	0.9469	0.1173	0.5544
$A \times K_3$	0.0024	0.0122	0.074	0.0199	0.8127	0.2718	0.2863
$A \times D_3 \times K_3$	0.8830	0.4547	0.5069	0.8424	0.6829	0.4159	0.7831

^{a,b} means with no common superscript letter in each column differ significantly ($P<0.05$).
SEM= Standard Error of Means

Parenteral administration of 6300 IU vitamin D_3 decreased serum P level by 12.7 Percent (Table 4; $P>0.05$), compared with the relevant control birds. The mean proximal (upper) epiphysis and diaphysis diameter of the right tibia bone and tibia ash percentage were significantly superior in the vitamin D_3- injected birds by 4.3, 4.9 and 9.3 percent, respectively (Table 4; $P<0.05$). The mean tibia weight and length, distal epiphysis diameter, however, did not differ among the quails receiving parenteral injection of vitamin D_3 at 0 or 6300 IU (Table 4; $P>0.05$). No discrepancy in all of the abovementioned features were observed among the birds receiving either 0 or 23100 for IU vitamin A (Table 4; $P>0.05$). Parenteral administration of vitamin K_3 at 17 and 34 mg in a weekly schedule showed no influence on the traits evaluated compared with the corresponding control birds (Table 4; $P>0.05$). No significant effects were noticed for $D_3 \times A$, $D_3 \times K_3$, $A \times K_3$ and $A \times D_3 \times K_3$ interactions on all of the features evaluated (Table 4; $P>0.05$).

Table 3: Effects of parenteral administration of vitamins D_3, A and K_3 on egg weight (g), yolk color index, yolk weight (g), shell thickness (mm), yolk percentage (%), albumen percentage (%), and shell strength in older breeder Japanese quail

Factor/level	Egg weight (g)	Yolk color index	Yolk weight(g)	Shell thickness (mm)	Yolk percentage (%)	Albumen percentage (%)	Shell strength (Kgf)
Vitamin D_3 (IU)							
0	12.43^{b}	7.86^{b}	3.98	0.19^{b}	32.10	57.36	0.02
6300	12.86^{a}	8.06^{a}	4.15	0.21^{a}	32.23	57.15	0.02
SEM	0.12	0.07	0.06	0.002	0.48	0.45	0.001
Vitamin A (IU)							
0	12.74$_{a}$	7.86^{b}	4.13	0.2	32.37	56.71	0.02
23100	12.56$_{a}$	8.06^{a}	4.10	0.22	31.87	57.80	0.02
SEM	0.12	0.06	0.05	0.002	0.467	0.48	0.001
Vitamin K_3 (mg)							
0	12.29^{b}	7.81^{b}	3.93^{b}	0.19^{b}	31.66	57.82	0.02
17	12.65^{ab}	7.85^{b}	4.08^{ab}	0.19^{b}	32.17	56.89	0.02
34	13.72^{a}	8.25^{a}	4.22^{a}	0.24^{a}	32.53$_{a}$	57.06	0.02
SEM	0.17	0.1	0.06	0.002	0.55	0.42	0.001
ANOVA							
D_3	0.0091	0.0362	0.0373	<.0001	0.7187	0.7259	0.6003
A	0.0025	0.0002	0.0055	0.004	0.5299	0.4335	0.3101
K_3	0.2575	0.0362	0.1207	0.8994	0.4347	0.0851	0.5301
$D_3 \times A$	0.0537	0.3284	0.0405	0.0341	0.5262	0.9334	0.1004
$D_3 \times K_3$	0.8001	0.481	0.8466	0.166	0.6782	0.4068	0.0638
$A \times K_3$	0.0004	0.3284	0.0013	0.2851	0.4289	0.7273	0.6725
$A \times D_3 \times K_3$	0.8248	0.0118	0.1313	0.8937	0.2755	0.4265	0.3101

^{a,b} means with no common superscript letter in each column differ significantly (P<0.05).
SEM= Standard Error of Means

Table 4: Effects of parenteral administration of vitamins D_3, A and K_3 on tibia ash percentage, tibia weight (g), tibia length (mm), proximal epiphyssis diameter (PED, mm), diaphysis diameter (DD, mm), and distal epiphyseal diameter (DED, mm) in older breeder Japanese quail

Factor/level	Tibia ash (%)	Tibia weight (g)	Tibia length (mm)	PED (mm)	DD (mm)	DED (mm)
Vitamin D_3 (IU)						
0	48.12^{b}	0.82	54.08	6.66^{b}	3.03^{b}	5.69
6300	52.59^{a}	0.88	53.54	6.95^{a}	3.18^{a}	5.61
SEM	1.241	0.023	0.257	0.071	0.038	0.054
Vitamin A (IU)						
0	51.25	0.88	54.26	6.86	3.14	5.64
23100	49.46	0.82	52.68	6.76	3.07	5.66
SEM	1.291	0.021	0.341	0.061	0.032	0.042
Vitamin K_3 (mg)						
0	47.89	0.85	54.00	6.78	3.10	5.62
17	51.68	0.88	53.85	6.78	3.11	5.67
34	51.49	0.83	53.68	6.87	3.11	5.66
SEM	1.917	0.026	0.413	0.114	0.055	0.051
ANOVA						
D_3	0.0121	0.0836	0.3230	0.0130	0.0147	0.2417
A	0.3005	0.0982	0.3240	0.3928	0.2036	0.8580
K_3	0.1365	0.5649	0.3751	0.716	0.9935	0.8213
$D_3 \times A$	0.5131	0.8564	0.3212	0.6380	0.9350	0.0001
$D_3 \times K_3$	0.0915	0.2356	0.3738	0.2327	0.2097	0.3231
$A \times K_3$	0.2317	0.6477	0.3763	0.3090	0.6093	0.4992
$A \times D_3 \times K_3$	0.9319	0.9004	0.3787	0.1934	0.5436	0.4945

^{a,b} means with no common superscript letter in each column differ significantly (P<0.05).
SEM= Standard Error of Means

Seedor index (SI) and Robustness index (RI) did not differ in the vitamin D_3, A or K_3 injected birds compared with the corresponding control quails (P>0.05), with the exception of RI, where it was significantly greater in the vitamin D_3- treated birds compared with the pertaining control group (Table 5; P<0.05). No difference in Hounsfield unit (HFU) for proximal and distal epiphysis and diaphysis, as well as mean HFU was noticed in the aged breeder quails receiving vitamin D_3, A or K_3 through a weekly *SC* injection schedule (Table 5; P>0.05), again excluding the vitamin D_3- treated birds which showed superior HFU for proximal tibia epiphysis, than the relevant control birds (Table 5; P<0.05). No discrepancy in HFU measured at proximal epiphysis, diaphysis, distal epiphysis as well as mean HFU was observed for $D_3 \times A$, $D_3 \times K_3$, $A \times K_3$ and $A \times D_3 \times K_3$ combined effects (Table 5; P>0.05).

The Pearson's correlation coefficients among tibia-related indices and with performance as well as egg quality attributes are summarized in Table 6. Tibia length showed high positive correlation with tibia ash. The Seedor index showed a positive moderate correlation with tibia ash, while RI demonstrated a negative moderate association with the same trait. Assessment of HFU at different locations of the right tibia bone showed weak associations with tibia ash content. A strong and significant negative correlation was observed between the Seedor index and the Robustness index (r = -0.634, P < 0.01; Table 6). Regarding CT parameters, HU measurements at different bone sites were positively intercorrelated. Specifically, the mean Hounsfield unit (mHU) showed a strong to moderate positive correlation with HU at the proximal epiphysis (r = 0.776, P < 0.01; Table 6), diaphysis (r = 0.385, P < 0.01; Table 6), and distal epiphysis (r = 0.205, P < 0.01; Table 6).

Table 5: Effects of parenteral administration of vitamins D_3, A and K_3 on Seedor index, Robustness index, Hounsfield unit (HU) for proximal epiphysis (HUP), Hounsfield unit for diaphysis (HUD), Hounsfield unit for distal epiphysis (HUDI), and mean Hounsfield unit (mHU) in aged breeder Japanese quail.

Factor/level	Seedor index	Robustness Index	HUP	HUD	HUDI	mHU
Vitamin D(IU)						
0	0.015	57.86[a]	406.76[b]	809.20	552.23	589.39
6300	0.010	56.30[b]	608.73[a]	1176.80	525.13	692.12
SEM	0.001	0.491	49.712	98.085	84.881	67.071
Vitamin A(IU)						
0	0.011	56.74	506.20	966.60	539.90	592.79
23100	0.013	57.42	509.30	1019.40	537.46	688.72
SEM	0.001	0.531	59.960	237.076	75.876	66.121
Vitamin K3 (mg)						
0	0.012	57.23	502.15	818.15	543.30	621.18
17	0.016	56.34	610.80	1010.05	517.60	712.82
34	0.011	57.68	410.30	1150.80	555.15	588.27
SEM	0.001	0.518	74.071	121.859	101.595	87.498
ANOVA						
D_3	0.3368	0.0262	0.0265	0.1503	0.8029	0.2909
A	0.3368	0.3188	0.9721	0.8346	0.9821	0.3236
K_3	0.1245	0.2726	0.1892	0.5594	0.9588	0.5527
$D_3 \times A$	0.6298	0.8304	0.9279	0.4593	0.5572	0.9310
$D_3 \times K_3$	0.6648	0.2726	0.2001	0.3159	0.3449	0.6621
$A \times K_3$	0.3354	0.9542	0.5163	0.5779	0.2646	0.3348
$A \times D_3 \times K_3$	0.4711	0.0789	0.224	0.1389	0.4111	0.5642

[a,b] means with no common superscript letter in each column differ significantly (P<0.05).
SEM= Standard Error of Means

Tibia ash percentage exhibited a significant positive correlation with the Seedor index (r = 0.442, P < 0.01; Table 6) and significant negative correlations with the Robustness index (r = -0.434, P < 0.01; Table 6) and egg production percentage (r = -0.397, P < 0.01; Table 6). Conversely, tibia ash percentage was not significantly correlated with HU measurements at any site (P > 0.05; Table 6). Tibia weight was strongly and positively correlated with the Seedor index (r = 0.730, P < 0.01; Table 6) and negatively with the Robustness index (r = -0.791, P < 0.01; Table 6). Furthermore, tibia weight showed a weak positive correlation with the diaphysis diameter (r = 0.270, P < 0.05; Table 6). Egg quality attributes, including egg weight, yolk color, yolk weight, shell thickness, albumen percentage, and shell strength, were not significantly correlated with bone indices or CT parameters (P > 0.05; Table 6). An exception was yolk percentage, which showed a weak positive correlation with HUDI (r = 0.277, P < 0.05; Table 6).

Table 6: The correlation coefficients of the Seedor index, Robustness index (RI), Hounsfield unit for distal epiphysis (HUDI), Hounsfield unit for proximal epiphysis (HUP), Hounsfield unit for diaphysis (HUD), mean Hounsfield unit (mHU), with certain performance parameters and egg quality attributes.

	Seedor index	**Robustness Index**	**HUP**	**HUD**	**HUDI**	**mHU**
Seedor index						
Robustness Index	-0.634**					
HUP	0.134^{ns}	-0.264^{ns}				
HUD	0.247^{ns}	-0.203^{ns}	0.222^{ns}			
HUDI	0.016^{ns}	-0.088^{ns}	0.372*	0.302*		
mHU	0.184^{ns}	-0.221^{ns}	0.776**	0.385**	0.805**	
Tibia ash, %	0.442**	-0.434**	-0.003^{ns}	0.215^{ns}	-0.158^{ns}	0.042^{ns}
Tibia weight, g	0.730**	-0.079^{ns}	0.054^{ns}	0.270^{ns}	0.020^{ns}	0.044^{n}s
Tibia length, (mm)	-0.385*	-0.041^{ns}	-0.113^{ns}	-0.116^{ns}	-0.047^{ns}	-0.146^{ns}
PED, mm	0.457*	-0.315ns	-0.196^{ns}	0.173^{ns}	-0.132^{ns}	-0.129^{ns}
DD, mm	0.169^{ns}	-0.151^{ns}	-0.159^{ns}	-0.156^{ns}	-0.215^{ns}	-0.245^{ns}
DED, mm	0.382*	-0.366*	0.025^{ns}	0.183^{ns}	-0.063^{ns}	0.009^{ns}
Performance parameters						
Body weight, g	0.195^{ns}	-0.080^{ns}	0.109^{ns}	0.153^{ns}	0.160^{ns}	0.171^{ns}
Egg Production, %	-0.397*	0.181^{ns}	-0.162^{ns}	-0.238^{ns}	-0.143^{ns}	-0.223^{ns}
Egg number, n	-0.127^{n}s	0.158^{ns}	-0.089^{ns}	-0.056^{ns}	-0.175^{ns}	-0.121^{ns}
Egg Mass, g	-0.281*	0.198^{ns}	-0.128^{ns}	-0.181^{ns}	-0.146^{ns}	-0.185^{ns}
Average egg weight (g)	-0.111^{ns}	0.149^{ns}	-0.036^{ns}	-0.018^{ns}	0.071^{ns}	0.006^{ns}
Feed intake (g/hen/day)	-0.042^{ns}	-0.042^{ns}	-0.188^{ns}	-0.273*	-0.168^{ns}	-0.258*
FCR	0.232^{ns}	-0.180^{ns}	0.111^{ns}	0.065^{ns}	0.076^{ns}	0.097^{ns}
Egg-related attributes						
Egg weight, g	0.173^{ns}	-0.206^{ns}	0.140^{ns}	0.149^{ns}	0.077^{ns}	0.148^{ns}
Yolk color index	-0.212^{ns}	-0.023^{ns}	-0.045^{ns}	-0.050^{ns}	-0.066^{ns}	-0.064^{ns}
Yolk weight, g	0.164^{ns}	-0.180^{ns}	0.082^{ns}	0.179^{ns}	0.219^{ns}	0.196^{ns}
Shell thickness, mm	-0.042^{ns}	-0.110^{ns}	-0.011^{ns}	0.065^{ns}	-0.117^{ns}	-0.016^{ns}
Yolk percentage	0.084^{ns}	-0.075^{ns}	-0.015^{ns}	0.136^{ns}	0.277*	0.164^{ns}
Albumen percentage	-0.151^{ns}	0.121^{ns}	0.047^{ns}	-0.035^{ns}	-0.096^{ns}	-0.036^{ns}
Shell strength	0.142^{ns}	-0.189^{ns}	0.067^{n}s	0.158^{ns}	0.089^{ns}	0.132^{ns}

*; P< 0.05 , **; P< 0.01 and ns; non-significant

Discussion

The findings of this study demonstrated that certain aspects of quail performance and egg quality were affected by the subcutaneous administration of vitamins A, D_3 , and K_3 . These results are consistent with the previous studies highlighting the pivotal role of these fat soluble vitamins in calcium and phosphorus metabolism, bone health, and reproductive performance in poultry (Sahin *et al*., 2018; Alagawany, 2021). Administration of vitamin D_3 via SC injection into the bird's neck increased serum concentration of the same vitamin by about 40 percent, however, no significant impact was unexpectedly observed on productive performance as well as egg quality attributes.

The lack of a significant effect of vitamin D_3 on production indices such as egg production and feed conversion ratio may be attributed to the sufficiency of its levels in the basal diet or to metabolic limitations in gut absorption capacity and bones of the aged birds. Studies have shown that with advancing age, the efficiency of converting vitamin D_3 to its active metabolic form (1,25-dihydroxycholecalciferol) declines (Bar *et al*., 2020). In contrast, the significant improvement in egg production percentage, egg number, and egg mass observed with vitamin A supplementation is likely linked to its vital role in maintaining epithelial tissue integrity, immune function, and optimal ovarian performance (Lin *et al*., 2022).

The dual response to vitamin K_3 , where a dose of 17 mg resulted in lower production compared to improvement at 34 mg, is noteworthy. This may indicate a dose-dependent effect, wherein lower levels might be insufficient to stimulate the carboxylation of vitamin K-dependent proteins (such as osteocalcin in bone and clotting factors), while higher levels optimize mineralization and coagulation processes, thereby enhancing production stability (Suttle, 2013). The observed significant interaction between vitamins A and K_3 on egg production and egg mass underscores the complexity of synergistic or antagonistic interactions among these micronutrients, warranting further investigation at the molecular level. The significant improvement in egg weight, yolk color index, yolk weight, and shell thickness due to vitamin D_3 is directly attributable to its key role in intestinal calcium and phosphorus

absorption and their subsequent utilization in bone matrix and eggshell formation (Liang *et al*., 2021). Increased shell thickness is a critical indicator of improved calcium status and metabolic health.

Vitamin A independently enhanced the yolk color index, likely due to increased carotenoid (vitamin A precursors) deposition into the yolk. This finding aligns with previous reports on the role of vitamin A in mucosal membrane integrity and the absorption of fat soluble nutrients (Khalil *et al*., 2023).

The positive effects of vitamin K_3 at the 34 mg level on egg quality traits may be associated with its role in activating gamma-carboxyglutamate-dependent proteins, such as osteocalcin. Proper activation of these proteins is crucial not only for bone mineralization but also for calcium homeostasis and potentially for the formation of the organic matrix of the eggshell (Ferland, 2023). The interaction observed between vitamins D_3 and A on egg weight and yolk weight is likely due to their shared involvement in common metabolic pathways (e.g., nuclear receptor responses) and competition for binding to carrier proteins.

In the current study, the right tibia bone was chosen as the main indicator to evaluate the impact of the fat-soluble vitamins on skeletal health and mineralization in the aged breeder quails (during weeks 40 to 48 of age). The right tibia bone was considered a decisive evaluating feature in poultry research because its strength and structure are directly interconnected to factors like nutrition (Tompkins *et al*., 2022a, b), growth rate and leg abnormalities, making it a valuable model for understanding bone health (Lee *et al*., 2023). Such importance arises mainly from the fact that the tibia bears a significant amount of weight, making it sensitive to changes in bone quality (Khan *et al*., 2023a,b). Our results showed that tibia weight as well as all tibia morphometric measurements were influenced neither by simple effects of parenteral injection of vitamin D_3, A and K_3 nor by their interactive effects with the exception of tibia proximal epiphysis and diaphysis where both significantly found to be greater in those birds receiving 6300 IU vitamin D_3.

The lack of influence of vitamin A and K_3 on tibia weight and morphometry agree many recently published research finding such as Adhikari *et al*. (2020), who reported that additional vitamin A supplementation to laying hens, regardless of form, did not affect either bone mineralization or measures of egg quality. However, results of the current study did not confirm the Huang *et al*. (2025) findings where the later research team concluded that goslings fed 1500 or 2000 IU/kg vitamin D_3 showed increased tibia ash content. They also found an interaction between vitamins A and D_3 on tibia ash content, and bone morphogenetic protein-2 (BMP-2) expression, where at a 9000 IU/kg vitamin A, adding 1000 IU/kg vitamin D_3 decreased tibia ash. At a 1000 IU/kg vitamin D_3, adding 9000 IU/kg vitamin A decreased tibia ash. They finally concluded that a dietary level of 7000 IU/kg of vitamin A and 2000 IU/kg of vitamin D_3 may be recommended as a combination to optimize feed intake and conversion and tibia mineralization.

It was noticed that vitamin A may positively influence tibia features such as ash content through influencing various cellular functions like proliferation, differentiation, and apoptosis (Yee *et al*., 2021; Khan *et al*., 2023a,b). Yee *et al*. (2021) concluded that both vitamin A and provitamin A may be potential bone-protecting agents.

Superior certain tibia dimensions in vitamin D_3-receiving birds was anticipated as almost all previous reports verified that vitamin D_3 significantly affect bone density and mineralization through increased Ca and P intestinal absorption (Lee *et al*., 2023a,b) as well as regulations of the other necessary mechanisms involving bone growth (Kianfar *et al*., 2025). Our results showed that the vitamin D_3 effect may be realised in redistribution of mineralization in proximal and middle parts of the right tibia bone rather than the distal parts, evidenced by the increased proximal epiphysis and diaphysis of the bone with no modification in bone weight or distal dimensions. Vitamin D_3 promotes Ca and P absorption in the intestines and kidneys, supporting bone formation, mineralization, and tibial quality by inhibiting osteoclast-mediated resorption (Zhang *et al*., 2023). Zhang *et al*. (2025) found that higher vitamin D_3 intake improved tibia strength in broilers, while Santiago *et al.* (2016) reported 25-OH-D3 increased tibia Ca and P deposition.

The effects of the vitamin D_3 administration on increased mineralization of the proximal portions of the right tibia bone were to some degree supported by the calculated indices as well as the imaging assessment of the bones by computerized tomography scanning system (CT scan) for evaluating HFU. The results revealed that the vitamin D_3-treated birds exhibited superior HFU for the proximal epiphysis, compared to the control birds ($P < 0.05$). A greater HFU value in a CT scan generally indicates a more density, and in the case of bone greater mineralization, of the tissue being imaged (Knowles *et al*., 2016; Buenger *et al*., 2022). In the available literature, no data on quail bones HFU was found to compare with our finding, however, data from Sung *et al*. (2025) in a study with broiler chicken and Bahaeddini *et al*. (2023) on human cases support our findings, where the later researchers reported that HFU of the center of the medulla was significantly correlated with the lowest T-score in the proximal ($r=0.486$) and distal tibia ($r = 0.458$).

Considering right tibia bone properties as the main indicators to assess overall skeletal health, as most of

the previous poultry research, we paid more attention to tibia ash percentage. Evidently, tibia ash content serves as a key metric for judging bone health and the efficacy of dietary or parenteral interventions. Gul *et al*. (2025) reported that a higher tibia ash percentage generally indicates better bone mineralization, the results which was supported and extended by Ahmadi *et al*. (2025), who revealed tibia ash content can be reasonably included in the models reflecting Ca requirements to improve overall skeletal health in quails. In the current study, tibia ash parentage was not differ in the quails receiving parenteral injection of vitamin D_3, A and K_3 at the administrated levels, again with the single exception of the vitamin D_3-treated birds, where tibia ash content was significantly greater in those receiving 6300 IU vitamin D_3. This part of the results is in accordance with the majority of the previous works where scientists mainly confirmed the importance of vitamin D_3 for the maintenance of normal skeletal integrity, bone mineralization and mobilization, and control the incidence of bone disorders in animals (Bar *et al*., 2020).

Considering tibia ash percentage as a major indicator for bone strength and mineralization, the correlation coefficients of the same feature with all the evaluated traits were calculated. Seedor index, distal epiphysis diameter, diaphysis diameter, serum vitamin D_3 levels and proximal epiphysis diameter were the six traits with superior positive association with tibia ash. In contrast, RI and serum ALP activity were the only two features which exhibited greater moderate negative correlations with tibia ash percentage, respectively. The correlation coefficients calculated for tibia ash with serum-related and tibia morphometry traits agree with the figures from the study of Zhang *et al*. (2023), who revealed tibial growth strongly correlates with body weight. These data may assist the researchers in choosing a set of more relevant features of the tibia bone when investigating the impact of dietary or parenteral intervention of vitamins in bone-related poultry research.

The correlation analysis provides additional insights into the interrelationships between bone quality metrics, imaging parameters, and productivity in aged breeder quails. The strong negative correlation between the Seedor index and the Robustness index confirms that these indices represent contrasting dimensions of bone structure. The Seedor index (bone weight/length) is a proxy for bone mass or density, whereas RI (length/$\sqrt[3]{\text{weight}}$) reflects geometric slenderness and potential mechanical efficiency (Eusemann *et al*., 2020). This inverse relationship highlights that heavier, denser bones in this model are not necessarily more slender or optimized for minimal weight under stress. The significant positive correlation of tibia ash percentage with the Seedor index ($r = 0.442$) and its negative correlation with RI are particularly informative. This pattern suggests that bones with higher mineral content tend to be heavier and more robust in mass, but not necessarily more gracile. This may indicate a compensatory mechanism in aged birds under chronic calcium demand for eggshell formation, where mineral is retained but not necessarily in an optimal structural architecture for strength. This hypothesis is supported by the significant negative correlations of the Seedor index with egg production percentage ($r = -0.397$) and egg mass ($r = -0.322$) in the current study. Higher-producing birds had bones with lower relative density, suggesting a greater mobilization of skeletal calcium reserves, aligning with the well-documented metabolic trade-off between egg production and skeletal integrity in laying poultry (Jansen *et al*., 2020). The lack of significant correlation between tibia ash percentage and HU values at all sites measured is a critical finding. Hounsfield units from CT scanning demonstarte radiodensity, influenced by both the mineral and organic matrix (Anderson *et al*., 2023a). In aged birds, age- or stress-related alterations in the collagenous organic matrix, such as changes in density or cross-linking (Grimaldi *et al*., 2025), may decouple CT radiodensity from absolute mineral content. This finding is consistent with Zhang *et al*. (2023), who reported a weak correlation between HU and ash content in trabecular bone of aged laying hens, attributing it to organic matrix degradation. The strong positive intercorrelations among HU values at different sites (e.g., mHU with HUP, $r = 0.776$) indicate uniform mineralization patterns across the tibia, likely driven by systemic metabolic regulation rather than localized effects.

The weak negative correlation of feed intake with diaphyseal and mean HU may reflect an indirect link between metabolic rate, calcium metabolism, and bone density, warranting further investigation. Notably, most egg quality traits showed no correlation with bone indices or HU, underscoring the short-term independence of shell calcification mechanisms from long bone mineral reserves, as homeostatic hormones prioritize blood calcium for eggshell formation (Essig, 2022). In general, the correlation matrix discloses that simple anatomical indices are appreciated predictors of bone ash in aged quails. It also highlights a limitation of CT-derived HU as a sole proxy for mineralization in aged birds due to potential organic matrix changes. Most importantly, it corroborates the inverse relationship between bone density indicators and high production output, emphasizing the necessity for targeted nutritional strategies. The isolated positive effects of vitamin D3 supplementation on bone diameter and ash content observed in this study offer a practical avenue to mitigate this trade-off and increase skeletal health in aging breeder flocks.

Conclusion

It was concluded that the fat soluble vitamins evaluated improved certain but different aspects of performance and egg quality in aged quails. Moreover, among the fat soluble vitamins evaluated, only vitamin D_3 may impose promising effects on bone strength and mineralization in aged breeder quail hens, where bird's skeleton already depleted of Ca under the high metabolic pressure of laying. This conclusion is evidenced by greater tibia ash percent as well as superior proximal epiphysis and diaphysis diameter in the birds receiving 6300 IU vitamin D_3 through weekly *SC* injection in the nape of the neck. In contrast to the many previous studies, our results provide no evidence for interactive effects of vitamins D_3, A and K_3 in bone health in aged breeder hen quails based on tibia morphometry and imaging characteristics.

Data and model availability statement

None of the data was deposited in an official repository. The data that support the study findings are available upon reasonable request.

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Declaration of interest

The authors declare that there is no conflict of interest.

References

Alagawany, M., Elnesr, S.S., Farag, M.R., Tiwari, R., Yatoo, M.I., Karthik, K., Michalak, I. and Dhama, K., 2021. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. Veterinary Quarterly, 41(1), pp.1-29. https://doi.org/10.1080/01652176.2020.1857887.

Adhikari R, White D, House JD & Kim WK. 2020. Effects of additional dosage of vitamin D3, vitamin D2, and 25-hydroxyvitamin D3 on calcium and phosphorus utilization, egg quality and bone mineralization in laying hens. Poultry Science, 99(1): 364-373. DOI: 10.3382/ps /pez502.

Ahmadi, M., Shojaeian, K., Ghazaghi, M. & Mehri, M., 2025. Calcium requirements for Japanese quail in the early growth period. Poultry Science, p.105209. DOI: 10.1016/j.psj.2024.105209.

Alonso N, Meinitzer A & Fritz-Petrin E. 2023. Role of vitamin K in bone and muscle metabolism. Calcified Tissue International, 112: 178-196. DOI: 10.1007/s00223-022-00955-3.

Anderson KL, Smith TJ, Brown EM & Johnson PD. 2023a. Standardized positioning protocols for DXA scanning in poultry research. Poultry Science, 102(1): 102345. DOI: 10.1016/j.psj.2022.102345.

Anderson RL, Martin GS, White CM & Davis PJ. 2023b. Standardized methods for bone ash content determination in avian species. Animal Biotechnology, 34(4): 1234-1245. DOI: 10.1080/10495398.2022.2156789.

Bahaeddini MR, Jafari B, Rikhtehgar M, Aminian A, Tabrizian P & Mirjalily MS. 2023. Correlation between bone density measured by routine BMD and Hounsfield units assessed on diagnostic CT-scan: A cross-sectional study. Journal of Research in Orthopedic Science, 10(3): 135-144. URL: http://jros.iums.ac.ir/article-1-2254-en.html.

Bar, A., I. Shinder, V. Yosefi, E. Vax & I. Plavnik. 2020. Metabolism and requirements for calcium and phosphorus in the fast-growing chicken as affected by age. Animals 10:859. DOI: 10.3390/ani10050859.

Buenger F, Sakr Y, Eckardt N . 2022. Correlation of quantitative computed tomography derived bone density values with Hounsfield units of a contrast medium computed tomography in 98 thoraco-lumbar vertebral bodies. Archives of Orthopedic and Trauma Surgery, 142: 3335-3340. DOI: 10.1007/s00402-021-04184-5.

Burns-Whitmore B, Froyen EB & Isom KA. 2024. Vitamin D and Calcium—An Overview, Review of Metabolism, and the Importance of Co-Supplementation. Dietetics, 3: 588-608. DOI: 10.3390/dietetics3040040.

Emekli-Alturfan E & Alturfan AA. 2023. The emerging relationship between vitamin K and neurodegenerative diseases: A review of current evidence. Molecular Biology Reports, 50: 815-828. DOI: 10.1007/s11033-022-07925-w.

Essig GF. 2022. Parathyroid physiology. Medscape. Updated Sept 16, 2022. Available online: https://emedicine.medscape.com/article/874690-overview (accessed on 12 October 2022).

Essig, M. (2022). Calcium metabolism and skeletal health in laying poultry: The role of vitamin D and beyond. World's Poultry Science Journal, 78(1), 155-170. https://doi.org/10.1080/00439339.2022.2014312mhens. Poultry Science, 99(2), 1055-1063. . DOI: 10.1016/j.psj.2019.10.037

Eusemann BK, Patt A, Schrader L, Weigend S, Thöne-Reineke C & Petow S. 2020. The role of egg production in the etiology of keel bone damage in laying hens. Frontiers in Veterinary Science, 7: 81. DOI: 10.3389/fvets.2020.00081.

Evaris, E.F., Sarmiento-Franco, L & Sandoval-Castro, C.A., 2021. Meat and bone quality of slow-growing male chickens raised with outdoor access in tropical climate. Journal of Food Composition and Analysis, 98, p.103802. DOI: 10.1016/j.jfca.2021.103802.

Ferland, G. 2023. Vitamin K and the nervous system: an overview of its actions. Adv. Nutr. 14:1–12. DOI: 10.1016/j.advnut.2023.01.001.

Grimaldi, A., G. Rossi & J. A. Fernández. 2025a. Vitamin K beyond coagulation: Implications for bone vascularization, brain health, and antioxidant defense in animals. Veterinary Research Communications, 49: 45-59. DOI: 10.1007/ s11259-024-10435-8

Grimaldi L, Cavallaro RA, De Angelis D, Fuso A & Sancesario G. 2025b. Vitamin K properties in stroke and alzheimer's disease: A janus bifrons in protection and prevention. Molecules, 30(5): 1027. DOI: 10.3390/molecules30051027.

Gül, E.T., 2025. Effects of calcium pidolate on performance, carcass traits, bone biomechanical properties and mineralisation and jejunum histomorphology in broilers. British Poultry Science, 66(2), pp.238-244.

Huang Z, Fu Z, Wang J, Yang Z, Wang J, Yu J, Wang Z & Yang H. 2025. Effects of dietary supplementation levels of vitamin A and vitamin D3 on growth performance, jejunal function, and tibia development in goslings from 1 to 28 days of age. Poultry Science, 104(5): 104780. DOI: 10.1016/j.psj.2025.104780.

Jansen, S., Baulain, U., Habig, C., Weigend, A., Halle, I., Scholz, A.M., Simianer, H., Sharifi, A.R. & Weigend, S., 2020. Relationship between bone stability and egg production in genetically divergent chicken layer lines. Animals, 10(5), p.850.

Khalil, H. A., N. M. El-Wardany, M. M. Shourrap & A. A. Ali. 2023. Influence of different vitamin A sources and levels on performance, egg quality, and blood parameters of laying hens. Tropical Animal Health and Production, 55: 45. DOI: 10.1007/s11250-023-03654-9.

Khan K, Kaya M, Fidan ED & Kilimci FS. 2023a. Impact of litter on femur and tibia morphology, bone biomechanics, and leg health parameters in broiler chickens. Animal Bioscience, 36(9): 1393-1402. DOI: 10.5713/ab.22.0335.

Khan RU, Khan A, Naz S, Ullah Q, Puvača N, Laudadio V, Mazzei D, Seidavi A, Ayasan T & Tufarelli V. 2023b. Pros and cons of dietary vitamin A and its precursors in poultry health and production: a comprehensive review. Antioxidants, 12: 1131.

Kianfar R, Kanani R, Janmohammadi H, Olyaee M, Besharati M & Lackner M. 2025. Implications of high-dose vitamin D3 with and without vitamin C on bone mineralization and blood biochemical factors in broiler breeder hens and their offspring. PeerJ, 13: e18983. DOI: 10.7717/peerj.18983.

Knowles, N. K., Reeves, J. M., & Ferreira, L. M. 2016. Quantitative computed tomography (QCT) derived bone mineral density (BMD) in finite element studies: A review of the literature. Journal of Experimental Orthopaedics, 3(1), 36. . DOI: 10.1186/s40634-016-0072-2

Lee J, Tompkins Y, Kim DH, Kim WK & Lee K. 2023a. Increased sizes and improved qualities of tibia bones by myostatin mutation in Japanese quail. Frontiers in Physiology, 13: 1085935. DOI: 10.3389/fphys.2022.1085935.

Lee SM, Kim JH, Park HS & Choi YS. 2023b. Optimization of scanning parameters for accurate bone mineral density assessment. Scientific Reports, 13(1): 5678. DOI: 10.1038/s41598-023-32857-1.

Li W, Zhang S & Liu J. 2019. Vitamin K2 stimulates MC3T3E1 osteoblast differentiation and mineralization through autophagy induction. Molecular Medicine Reports, 19: 3676-3684. DOI: 10.3892/mmr.2019.10040.

Liang, Y., X. Wang, J. Wang & H. J. Liu. 2021. Effects of dietary vitamin D_3 levels on laying performance, egg quality, and calcium metabolism in aged laying hens. Journal of Animal Physiology and Animal Nutrition, 105: 567-576. DOI: 10.1111/jpn.13589

Lin, X., Y. Hu, W. Li & S. Zhang. 2022. The role of vitamin A in the immune system of poultry. Frontiers in Veterinary Science, 9: 825260. DOI: 10.3389/fvets.2022.825260

Ling L. 2025. Association between serum vitamin A and bone mineral density in adolescents. Scientific Reports, 15: 6892. DOI: 10.1038/s41598-025-91367-4.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press. Washington, DC. 176 Pages.

Okoh, J & E. Perry. 2025. A Farmer's Guide to Egg Quality, Safety, and Flock Health. 1st Ed. Poultry Publications Ltd. London. 280 Pages.

Perilli, E., Baleani, M., Ohman, C., Fognani, R & Baruffaldi, F. 2022. Calibration of ct Hounsfield units for bone mineral assessment. Journal of Biomechanics, 134, 111022. . DOI: 10.1016/j.jbiomech.2022.111022

Sahin, N., K. Sahin & M. Onderci. 2018. Vitamin D_3 in poultry nutrition: an update. World's Poultry Science Journal, 74: 623-634. DOI: 10.1017/S0043933918000556.

Santiago, M., S. David, N. Alexandra, A. Eduardo & Q. Jimmy. 2016. Effect of 25-hydroxycholecalciferol (25-OH-D_3) on productive performance and bone mineralization in broiler. Open Journal of Animal Sciences, 6: 180-184. DOI: 10.4236/ojas.2016.63022.

Shastak Y, Pelletier W & Kuntz A. 2024. Insights into analytical precision: Understanding the factors influencing accurate vitamin A determination in various samples. Analytica, 5(1): 54-73.Thorp BH. 1994. Skeletal disorders in the

fowl: A review. Avian Pathology, 23: 203-236. DOI: 10.1080/03079459408418991.

Sung, J. Y., B. J. Emmert, C. I. Robison, D. M. Karcher & O. Adeola. 2025. The use of quantitative computed tomography for bone ash quantification may avoid euthanasia of young broiler chickens. Poultry Science, 104: 105598. DOI: 10.1016/j.psj.2025.105598.

Suttle, J. W. 2013. Vitamin K. In: Zempleni J, Suttle JW, Gregory III JF & Stover PJ (Eds). Handbook of Vitamins. 5th Ed. CRC Press. Boca Raton. Pages 89-107..

Tompkins YH, Chen C, Sweeney KM, Kim M, Voy BH & Wilson JL. 2022. The effects of maternal fish oil supplementation rich in n-3 PUFA on offspring-broiler growth performance, body composition and bone microstructure. PLoS ONE, 17: e0273025. DOI: 10.1371/ journal.pone.0273025.

Tompkins YH, Teng P, Pazdro R & Kim WK. 2022. Long bone mineral loss, bone microstructural changes and oxidative stress after eimeria challenge in broilers. Frontiers in Physiology, 13: 945740. DOI: 10.3389/fphys.2022.945740.

Wei J, Li L, Peng Y, Luo J, Chen T, Xi Q, Zhang Y & Sun J. 2024. The effects of optimal dietary vitamin D3 on growth and carcass performance, tibia traits, meat quality, and intestinal morphology of Chinese yellow-feathered broiler chickens. Animals, 14(6): 920. DOI: 10.3390/ani14060920.

Wei, X., H. Li, Y. Wang & R. Zhao. 2024. New insights into the role of vitamin D_3 in regulating calcium homeostasis and improving bone microstructure in aged laying hens. Frontiers in Physiology, 15: 1285678. DOI: 10.3389/fphys.2024.1285678

Wilson KT, Harris MP, Clark RW & Thompson SD. 2022. Temperature-dependent changes in bone mineral composition during ashing process. Poultry Science, 101(8): 101912. DOI: 10.1016/j.psj.2022.101912.

Yee MMF, Chin KY, Ima-Nirwana S & Wong SK. 2021. Vitamin A and bone health: A review on current evidence. Molecules, 26: 1757. DOI: 10.3390/molecules26061757.

Zaefarian F, Abdollahi MR & Ravindran V. 2021. Measurement of bone quality in poultry. Poultry Science, 100(2): 101-112. DOI: 10.1016/j.psj.2020.10.045.

Zhang, Y., Q. Liu, J. Wang & J. Chen. 2023. Assessing trabecular bone quality in aged laying hens: Comparison of micro-CT, ash content, and biomechanical testing. Computers and Electronics in Agriculture, 204: 107521. DOI: 10.1016/j.compag.2022.107521

Zhang Y, Wang L, Chen X, Liu Y & Li J. 2023. Optimization of bone ashing protocols for accurate mineral content analysis. Journal of Animal Science and Technology, 65(2): 345-356. DOI: 10.5187/jast.2023.e45.

Zhang, H. Y., Q. F. Zeng, S. P. Bai, J. P. Wang, X. M. Ding, Y. Xuan, Z. W. Su, G. S. Fraley & K. Y. Zhang. 2019. Study on the morphology and mineralization of the tibia in meat ducks from 1 to 56 d. Poultry Science, 98: 3355-3364. DOI: 10.3382/ps/pez017.

Zhang, L., P. Xu, L. Hao, L. Wang, Y. Xu & C. Jiang. 2025. The role of transient receptor potential channels in chronic kidney disease-mineral and bone disorder. Frontiers in Pharmacology, 16: 1583487. DOI: 10.3389/ fphar.2025.1583487.