Animal Nutrition 6 (2020) 467–479

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary supplementation of 25-hydroxycholecalciferol increases tibial mass by suppression bone resorption in meat ducks

Huaiyong Zhang [a], Qiufeng Zeng [a], Shiping Bai [a], Jianping Wang [a], Xuemei Ding [a], Yue Xuan [a], Zhuowei Su [a], Gergory S. Fraley [b], Bing Yao [c], Keying Zhang [a, *]

[a] Institute of Animal Nutrition, Key Laboratory for Animal Disease-Resistance Nutrition of China, Ministry of Education, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
[b] Biology Department, Hope College, Holland, 49423, USA
[c] DSM (China) Ltd., PuDong New Area, Shanghai, 201203, China

ARTICLE INFO

Article history:
Received 9 December 2019
Received in revised form
22 April 2020
Accepted 12 May 2020
Available online 20 September 2020

Keywords:
Vitamin D_3 metabolites
Vitamin
Bone turnover
Tibial mass
Meat duck

ABSTRACT

Leg problems often result from the rapid weight gain and poor bone quality in modern ducks, leading to a high risk of fractures and continuous pain. We hypothesized that improving bone quality in combination with delaying weight gain via a low nutrient density (LND) diet probably reverses these skeletal abnormalities. Studies indicated that 25-hydroxycholecalciferol (25-OH-D_3), a vitamin D_3 metabolite, is effective in treating bone-related disorders. Therefore, Exp. 1 evaluated the effects of 25-OH-D_3 on tibial mass of meat ducks. Male meat ducklings were fed a standard nutrient density diet (containing a regular vitamin regimen) without or with 25-OH-D_3 at 0.069 mg/kg for 35 d. The results showed that 25-OH-D_3 supplementation improved the mineral content, microarchitecture and mechanical properties of tibias, and this companied by a decreased serum bone resorption marker and a concomitant decrement in osteoclast-specific marker genes expression. Subsequently, Exp. 2 was conducted to examine the impacts of 25-OH-D_3 incorporating an LND diet on tibial quality of ducks under 2 different vitamin regimens (regular and high). Ducklings were allocated to a 2 × 2 factorial arrangement with 2 kinds of vitamin premixes and without or with 25-OH-D_3 at 0.069 mg/kg in LND diets. The high premix had higher levels of all vitamins except biotin than the regular premix. The results demonstrated that high vitamin diets exhibited more significant effects than regular vitamin diets on inhibiting bone turnover and increasing minerals deposition. Tibial mineral content, microarchitecture, and strength of birds under the regular vitamin regimen were increased by 25-OH-D_3 supplementation; However, these positive effects were not observed in ducks under the high vitamin regimen. To conclude, 25-OH-D_3 supplementation improves tibial mass by suppressing osteoclast-mediated bone resorption in meat ducks, and this positive impact only was observed in regular but not high vitamin regimen when birds fed an LND diet.

1. Introduction

Increases in growth rate and breast muscle mass results from genetic progress and intensive nutrition had been an increased incidence of locomotion (gait) problems (Paxton et al., 2013). Estimates of the prevalence of gait problems of commercial meat ducks have been reported on 14% and 21% (Jones and Dawkins, 2010). Altered gait in livestock is an important welfare issue, causing a reduction in mobility, that may be associated with pain and a reduction in normal behaviors (Bradshaw et al., 2002).

The increased incidence of leg problems with recent years may be due in part to the change that has taken place in the rapid rate of growth and body weight (BW) in meat-type birds (Williams et al.,

* Corresponding author.
E-mail address: zkeying@sicau.edu.cn (K. Zhang).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2020.05.006

2004), because heavier weight causes the cranial shift in the body's center of mass and increasing the risk of fractures (Corr et al., 2003; Paxton et al., 2013). Accelerating weight gain through increasing dietary nutrient density led to a higher incidence of gait abnormality, whereas feed withdrawal or reducing dietary nutrient density has been shown to alleviate the gait abnormality of broilers (Brickett et al., 2007). Our previous study also showed that a low nutrient density (LND) diet decreased the incidence of tibial dyschondroplasia (TD) and score by decelerating weight gain, improved the microarchitecture of bone trabecula, and promoted a down-regulation of bone turnover in meat ducks (Zhang et al., 2018).

Poor bone quality is certainly one of the main causes of skeletal disorders and increases the risk of fractures in domestic birds (Bradshaw et al., 2002). A lot of therapeutic strategies are used to improve bone mass and mechanical properties. According to some studies, the use of vitamin D and its metabolite fat soluble vitamins, regulating the absorption and metabolism of calcium (Ca) and phosphorus (P), has beneficial effects on bone mineralization and biomechanical properties (Jiang et al., 2015; Khan et al., 2010; Sun et al., 2013). Studies suggest supplementation of vitamin D_3 improved the walking ability and bone quality characteristics, and consequently decreased the leg diseases in broilers (Jiang et al., 2015; Sun et al., 2013), and a deficiency can lead to a higher incidence of leg problems of birds (Khan et al., 2010). It is well-established that dietary vitamin D_3 is absorbed in the small intestine and hydroxylated in the liver to form 25-hydroxycholecalciferol (25-OH-D_3), the major circulating form of the vitamin D_3 (Kulda, 2012). An evaluated trial of the relative bioavailability of 25-OH-D3 and vitamin D_3 found that 25-OH-D_3 was approximately twice as active as cholecalciferol (vitamin D_3) in promoting bone strength in broilers (Han et al., 2016). Supplementation with 25-OH-D_3 has been reported to significantly increase tibial mineralization of broilers (Ferket et al., 2009; Santiago et al., 2016; Wideman et al., 2015). All studies aforementioned focus on the therapeutic potential for 25-OH-D_3 in treating leg problems and its related complications, the mechanisms underlying the beneficial effects of vitamin D or its metabolites and bone mass are still limited to poultry. A candidate mechanism for the increase of bone mass by vitamin D treatment as well-known is stimulating the intestinal absorption of Ca and P (Christakos et al., 2014). Another possible mechanism is to regulate bone remodeling by directly targeting to serval bone cell types including osteoblast, osteocyte and osteoclast (Nakamichi et al., 2018; van Driel and van Leeuwen, 2017).

Another important effect described for 25-OH-D_3 was the dependence on the dietary basal vitamin level. Ren et al. (2017) reported that the positive impacts of maternal canthaxanthin and 25-OH-D_3 supplementation on growth performance and serum P of ducklings only were observed in a low but not a high vitamin regimen which has higher levels of all vitamins except nicotinic acid than the low vitamin recommendations, different responses of dietary 25-OH-D_3 to distinct vitamin regimen probably due to different doses of vitamin D or interaction between vitamins (Bonjour et al., 2018). There are various recommendations of vitamin premix in the duck industry, and the variations in each vitamin content of each other is very wide, especially National Nutrition Council (1994) and China Agricultural Industry Standards (NY/T 2122-2012). Accordingly, this is reasonable to assume that the biological effect of 25-OH-D_3 on tibial mass may lie on the dietary basal vitamin regimen.

Therefore, our goals for this study were to first evaluate if the 25-OH-D_3 is efficient in increasing the tibial mass of meat ducks by affecting osteoblast and/or osteoclast activity. Second, considering that the beneficial effect of LND diet and the dependence of 25-OH-D_3 on the dietary basal vitamin level, the present study also aims to evaluate the effect of 25-OH-D_3 under different vitamin regimens on the tibial quality of meat ducks fed an LND diet.

2. Materials and methods

The experiments were carried out in accordance with the guidelines on the Sichuan Agricultural University Experimental Animal Committee.

2.1. Birds, diets and experimental design

One-day-old male Cherry Valley meat-type ducks were obtained from a commercial hatchery, and reared in individual cages (2.2 m × 1.2 m × 0.9 m) situated in a temperature- and humidity-controlled room. They were fed ad libitum pellet-type experimental diets, including the standard nutrient density positive control (PC) diets and LND diets. The PC diet was designed to satisfy the recommendations (NY/T 2122-2012) excepting vitamins. In the PC diet, the contents of Ca and non-phytate phosphorus (NPP) were 0.9%, 0.42% in starter diets (d 1 to 14) and 0.85%, 0.4% in grower diets (d 15 to 35), respectively. The LND diet contained 0.7% Ca, 0.35% NPP, 10.25 MJ/kg apparent metabolism energy (AME), and the grower diet of LND diets had a constant ratios of crude protein (CP) and essential nutrients, such as limiting amino acids relative to AME compared to PC diet (Table 1). The diets were designed to differ in their 25-OH-D_3 and vitamin regimens. For this purpose, various amounts of 25-OH-D_3 (Exp. 1) and/or vitamin regimens

Table 1
Composition and nutrient levels in the basal diets (dry matter basis).[1]

Item	Starting diet (d 1 to14)	Grower diet (d 15 to 35)	
	PC diet	PC diet	LND diet
Ingredients, %			
Maize	58.05	62.1	56.61
Soya oil	3	3	0
Soybean meal	34.8	23.48	0
DDGS	0	3	4
Wheat bran	0	2	26.6
Rapeseed meal	0	2.85	8.61
L-Lysine	0.08	0	0.2
DL-Methionine	0.145	0.13	0.1
L-Threonine	0.03	0	0.07
L-Tryptophan	0	0	0.12
Choline chloride	0.2	0.2	0.2
Sodium chloride	0.3	0.3	0.3
Mineral premix [2]	0.2	0.2	0.2
Vitamin premix [3]	0.03	0.03	0.03
Limestone	1.06	1.04	1
Dicalcium phosphate	1.75	1.6	1
Bentonite	0.355	0.07	0.96
Total	100	100	100
Calculated nutrient analysis, %			
AME, MJ/kg	12.14	12.14	10.25
CP	20.05	17.50	13.28
Dig Lys	1.02	0.76	0.53
Dig Met	0.42	0.38	0.27
Calcium	0.90	0.85	0.71
Total-phytate P	0.65	0.65	0.76
Non-phytic acid P	0.42	0.40	0.35

DDGS = distillers dried grains with soluble; AME = apparent metabolizable energy; CP = crude protein; Dig = digestibility.

[1] PC diet, standard nutrient density positive control diet; LND diet, low nutrient density diet.

[2] Provided per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 90 mg; Mn ($MnSO_4 \cdot H_2O$), 70 mg; Se ($NaSeO_3$), 0.3 mg; I (KI), 0.4 mg.

[3] Provided per kilogram of diet: regular or high vitamin regimens as shown in Table 2.

(Exp. 2) were added to the basal ingredients. Vitamin regimens included regular and high, which was formulated based on the recommendations of NRC (1994) and China Agricultural Industry Standards (NY/T 2122-2012), respectively (Table 2). All vitamins used in this study were provided by DSM Ltd. (China).

During the experiments, BW and feed intake were recorded weekly, BW gain and feed conversion as the feed-to-BW gain ratio (F:G) was calculated on a per-cage basis. The mortality due to leg deformations, which is defined as the percentage of mortality and culled ducks due to leg deformations and fractures, also was recorded during feeding period on a pen basis.

2.1.1. Experiment 1

The aim of this trial was to compare the effects of 25-OH-D_3 on tibia mass of meat ducks fed the PC diet containing the regular vitamin regimen. With this aim, 256 male meat ducklings at 1 d old were allocated to 2 dietary treatments with 8 replicates (16 birds each). They were fed PC diets containing either no (control group) or 25-OH-D_3 (0.069 mg/kg) for 35 d.

2.1.2. Experiment 2

The aim of this trial was to evaluate the effects of 25-OH-D_3 under different vitamin regimens on the tibial quality of meat ducks fed the LND diets. For this purpose, 512 ducks were assigned to one of 4 LND diets (8 replicate pens; 16 ducks/pen). The 4 experimental treatments consisted of a 2 × 2 factorial arrangement of 2 dietary inclusion vitamin regimens (regular and high) and 2 concentrations of 25-OH-D_3 (0 or 0.069 mg/kg diet). Ducklings were all fed the PC diets until d 14, subsequently, they were subjected to corresponding LND diets until d 35.

2.2. Sampling

Eight ducks per treatment (1 bird per pen) were randomly selected and sacrificed by cervical dislocation on d 35. Left tibias (the proximal end) were dissected and fixed at 10% buffered formalin (pH 7.4) for tibial histology analysis. After fasting for 12 h, other 8 ducks per treatment, with BW close to the pen average, were selected and subjected to blood collection through the jugular vein. Then, the animals were sacrificed by cervical dislocation, and left tibias (the proximal end) were dissected and immediately flash-frozen in liquid N_2 for mRNA analyses. The right tibias were taken without soft tissues, and both the length and width (50% of length) were measured immediately. These tibias were kept frozen for later analysis of mineral content and biomechanical properties.

Table 2
Composition of the vitamin premixes for meat ducks.[1]

Item	d 1 to 14		d 15 to 35	
	Regular [2]	High [3]	Regular [2]	High [3]
Vitamin A, IU	2,500	4,000	2,500	3,000
Vitamin D_3, IU	400	2,000	400	2,000
Vitamin E, IU	10	20	10	20
Vitamin K_3, mg	0.5	2	0.5	2
Vitamin B_1, mg	1.8	2	1.8	1.5
Vitamin B_2, mg	4	10	4	10
Vitamin B_6, mg	2.5	4	2.5	3
Vitamin B_{12}, mg	0.01	0.02	0.01	0.02
Niacin, mg	55	50	55	–
Pantothenic acid, mg	11	20	11	10
Biotin, mg	0.15	0.15	0.15	0.15
Folic acid, mg	0.55	1	0.55	1

[1] Supplied in per kilogram of diet.
[2] The vitamin levels recommended by the National Research Council (NRC, 1994).
[3] The vitamin levels recommended by the China Agricultural Industry Standards (NY/T 2122-2012).

2.3. Evaluation of leg abnormalities

At the end of the experiments, 3 birds from each pen were randomly selected and sacrificed by cervical dislocation. Tibias were removed from both legs, and the proximal end was sectioned to grade for TD using the TD scoring system described by Edwards and Veltmann (1983). Tibial dyschondroplasia lesions were scored as 0, 1, 2 or 3 (0 = normal bone or no lesion, 1 = slight lesion, 2 = medium lesion and, 3 = most severe lesion).

2.4. Serum biochemical analysis

Serum Ca and P concentrations were determined with Biochemistry Analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH, USA). Serum parathyroid hormone (PTH) and 25-OH-D concentrations were assayed using commercial ELISA kit (ALPCO Diagnostics, NH, USA) according to the manufacturers' recommendations. In addition, Serum procollagen type I N-terminal propeptide (P1NP), alkaline phosphatase (ALP) (both bone formation markers), C-terminal cross-linked telopeptide of type I collagen (CTx) and tartrate-resistant acid phosphatase (TRAP) (both bone resorption markers) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All samples were tested in triplicate within each assay.

2.5. Tibia-breaking strength analysis

Breaking force test was performed to evaluate tibial strength using the texture analyzer (TA. XT Plus; Stable Microsystems), employing exponent lite express software (Stable Micro Systems). The tibia was rested on 2 points with a gap of 13 mm and pressure was applied with pressure sensitive load cell (50 kg) at the center of the 2 points, and the blade perpendicularly hit the tibia at its midpoint at a test speed of 5 mm/s for a distance of 30 mm.

2.6. Measurement of mineralization

Tibial fat-free weight and tibial density were determined according to our previous methods (Zhang et al., 2017). Specifically, bone volume (cm^3) was measured based on quantum of water overflowing from a fully filled container, and subsequently these tibias were air-dried for 24 h at room temperature, extracted by refluxing diethyl ether in a Soxhlet apparatus for 16 h, oven-dried at 108 °C for 24 h for fat-free bone weight (g) determination, thus the tibial density (g/cm^3) was calculated as tibial fat-free weight (g)/tibial volume (cm^3). For ash, Ca and P analysis, dry-defatted tibia was ashed in a muffle furnace at 550 °C for 24 h, and the ash was measured on the basis of the percentage of dry-defatted weight. Tibial Ca and P were determined through ethylenediaminetetraacetic acid (EDTA) titration and ammonium metavanadate colorimetry, respectively, and values were also presented basing on dry fat-free weight.

2.7. Bone histological analysis

Bone histological analysis was performed as previously described (Vidal et al., 2012). Briefly, the fixed tibia samples were decalcified with 10% EDTA (pH 7.4) for d 21, dehydrated conventionally, and embedded in paraffin wax. The entire blocks containing the samples were cut into sagittal sections, after roughing the microtome approximately 120 times of 10-μm thickness. After that, slides of 4-μm thickness were obtained for the toluidine blue

staining, in which the mineralized bone as violet blue and the osteoid as pale blue. The bone slides were visualized in a microscope equipped (Nikon Corporation, Tokyo, Japan). Histomorphometry were performed in 3 randomly selected non-overlapping microscopic fields in each section by Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). Undergoing gray scale transform, the manual grid was used to divide the samples in several fields with the same dimensions, allowing an easier quantification and analysis of the histomorphometry parameters. Some measurements such as total area (T.Ar), calcified bone area (B.Ar), bone perimeter (B.Pm) can be obtained directly from images, and several key parameters, including the trabecular bone area (Tb.Ar), trabecular number (Tb.N), thickness (Tb.Th) and spacing (Tb.Sp), are derived indirectly using the following equations:

$$\text{Tb.Ar} = \text{B.Ar/T.Ar} \times 100\ ,$$

$$\text{Tb.Th} = 2 \times \text{B.Ar/B.Pm}\ ,$$

$$\text{Tb.N} = (\text{B.Ar/T.Ar})/\text{Tb.Th}\ ,$$

$$\text{Tb.Sp} = (1/\text{Tb.N}) - \text{Tb.Th}\ .$$

The selected area was similar in all samples and the same parameters were measured in order to standardize the analysis. At least 10 sections from each sample were analyzed.

2.8. Tartrate-resistant acid phosphatase staining for osteoclast counts

To quantity the osteoclast, the tibial slides for staining with the TRAP detecting kit (Sigma–Aldrich, St. Louis, MO, USA). The numbers of osteoclast number per millimeter bone surface (N.Oc/BS) with multinucleated cells were identified as TRAP-positive and were determined in 5 consecutive microscopic fields (400× magnification). At least 5 serial vertical sections were evaluated for each animal per analysis. The slides were counted by 2 examiners, who was blinded to group status.

2.9. Gene expression assays

Real-time (RT) PCR was conducted to determine the mRNA levels of osteocyte-, osteoblast- and osteoclast-specific marker genes. Total RNA of the tibia proximal end was extracted with a standard Trizol protocol. The cDNA was synthesized from 200 ng of total RNA by a PrimeScript RT Reagent Kit (Takara). RT-PCR was performed on ABI 7500 RT-PCR detection system (Applied Biosystems), using Fast SYBR Green Master Mix (Thermo Fisher Scientific). Target cDNA was amplified by 40 cycles (one cycle: 95 °C for 5 s, 60 °C for 34 s) and a final melting curve analysis. All reactions were run in duplicate. Primers were designed using Primer 3 as shown in Table 3. The standard curve method was used to estimated reaction efficiency (slope) and genes expression. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and β-actin were selected as the reference genes, and a normalization factor was obtained by calculating the geometric mean of the values of the selected reference genes, which was subsequently used to normalize the relative amounts of RNA of interests (Vandesompele et al., 2002).

2.10. Statistical analysis

Data represent the mean ± standard deviation. The statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC, USA).

Table 3
The primers for real-time-PCR.

Gene	Gene ID	Primer	Sequence (5′-3′)	Size, bp
Phex	XM_005010837.3	Reverse	tgccaactatctggtgtgga	99
		Forward	ccgtagatcacccgagaaaa	
Dmp1	XM_005012780.3	Reverse	aaccttggtcaccttcatgc	92
		Forward	tcggcaaagtcctgctctat	
Sclerostin	XM_005026106.3	Reverse	ggaagggtggcaagtgttta	115
		Forward	tgcctggttcattgtgttgt	
Cathepsin K	XM_021277116.1	Reverse	actgctggtcctgtttgtcc	98
		Forward	gcttgcggtacgttttcttc	
V-ATPase	XM_021267166.1	Reverse	tccgtgtctggttcatcaaa	111
		Forward	caggacaccagacttcagca	
OPG	XM_005017709.3	Reverse	gcctaactggctgaacttgc	106
		Forward	gaaggtctgctcttgcgaac	
RANKL	XM_021276016.1	Reverse	gccttttgcccatctcatta	100
		Forward	taagtttgcctggcctttgt	
β-actin	NM_001310408.1	Reverse	ccagccatctttcttgggta	105
		Forward	gtgttggcgtacaggtcctt	
GAPDH	XM_005016745.3	Reverse	tttttaaccgtggctccttg	94
		Forward	actgggcatggaagaacatc	

Phex = phosphate regulating endopeptidase homolog x-linked; *Dmp1* = dentin matrix protein 1; *V-ATPase* = vacuolar-type H^+-ATPase; *OPG* = osteoprotegerin; *RANKL* = receptor activator of nuclear Factor κB ligand; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

Statistical power of 0.80 (80%) was obtained in this study when the minimally detectable effect size was 1.0 and the significance level was 0.05. Mortality and TD scores were $\sqrt{n+1}$ and arcsine transformed before analysis to satisfy the analysis of variance (ANOVA) requirements for normality and homogeneity of variance, respectively. Unpaired Student's *t*-test was used to compare the effect of 25-OH-D_3 on bone mass in the PC diets with regular vitamin regimen. Two-way ANOVA followed by Tukey's test (SAS 9.2) were used to analyze the effect of dietary vitamin regimens, 25-OH-D_3, and their interaction on bone mass in LND diets. Statistical significance was detected at $P < 0.05$.

3. Results

3.1. Experiment 1: tibial quality responses to 25-OH-D_3 in PC diets contained regular vitamin regimen

3.1.1. Performance

The results of growth performance showed that supplementation of 25-OH-D_3 (0.069 mg/kg diet) increased ($P < 0.05$) the BW (d 14) and BW gain (d 1 to 14), but did not notably change feed intake and F:G, as compared to the control group (without 25-OH-D_3 supplementation) (Fig. 1).

3.1.2. Leg abnormalities and tibial growth

As shown in Fig. 2, the mortality due to leg deformations of the 25-OH-D_3-fed birds was slightly lower than that of the control birds (Fig. 2A), and 25-OH-D_3 treatment significantly decreased ($P < 0.05$) the TD score, an indicator for leg abnormalities, and increased ($P < 0.05$) the tibial fat-free weight when compared to the control group (Fig. 2B and E), but tibial length and width were not affected by the 25-OH-D_3 diets (Fig. 2C and D).

3.1.3. Bone mass

The tibial mass was assessed through mineral content, bone histological analysis, and the osteocyte-specific marker genes expression in this study. As shown in Fig. 3, the birds fed the 25-OH-D_3 diets exhibited markedly higher ($P < 0.05$) ash level, Ca content, density, and strength of tibia, as well as a slightly increase in tibial P content, when compared with the control ducks (Fig. 3A to E). The outcomes of bone histomorphometry analysis of the

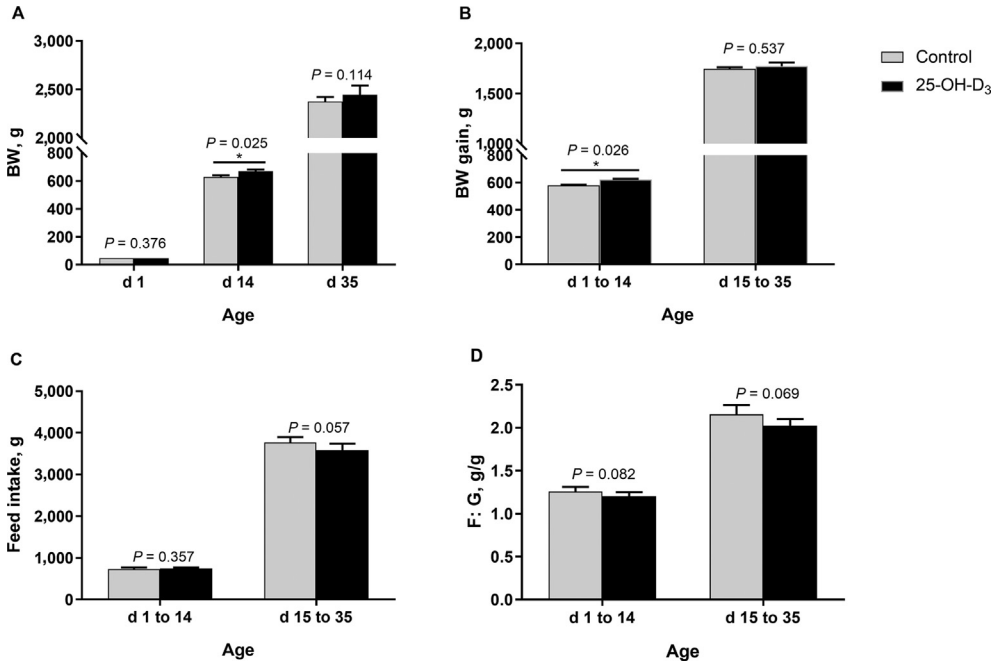

Fig. 1. Effect of 25-hydroxycholecalciferol (25-OH-D_3) supplementation on growth performance in the standard nutrient density positive control (PC) diets with a regular vitamin regimen: (A) Body weight (BW), (B) BW gain, (C) feed intake, and (D) the ratio of feed intake to BW gain (F:G). Values are means ± standard deviation represented by vertical bars ($n = 8$). * Mean values are significantly different (Student's t-test, $P < 0.05$).

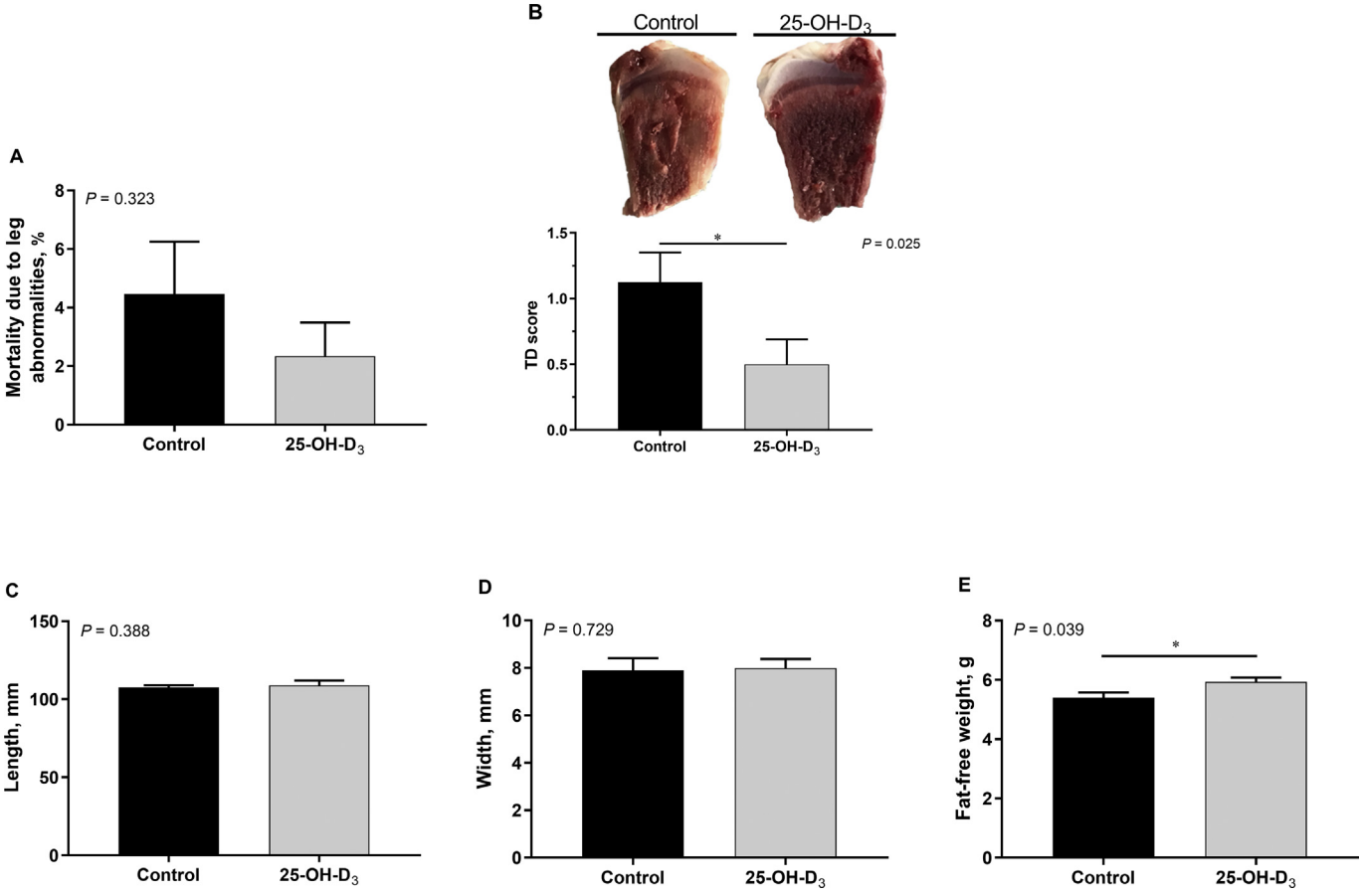

Fig. 2. Effect of 25-hydroxycholecalciferol (25-OH-D_3) supplementation on leg weakness and tibial growth in the standard nutrient density positive control (PC) diets with a regular vitamin regimen: (A) mortality due to leg abnormalities, (B) tibial dyschondroplasia (TD) score, and tibial growth including (C) length, (D) width, and (E) fat-free weight. Values are means ± standard deviation represented by vertical bars ($n = 8$). * Mean values are significantly different (Student's t-test, $P < 0.05$).

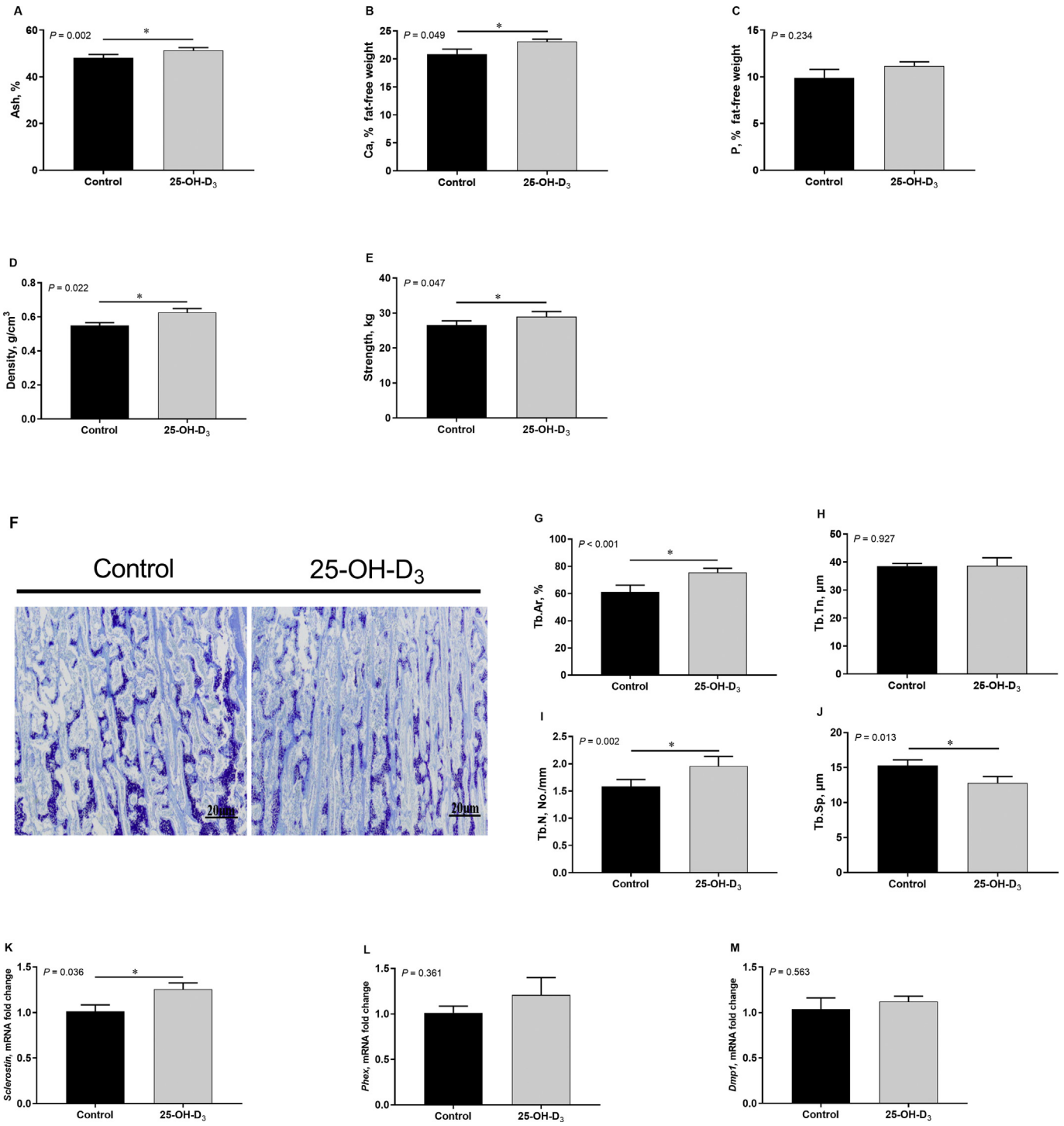

Fig. 3. Effect of 25-hydroxycholecalciferol (25-OH-D_3) supplementation on tibial mineralization and microstructure in the standard nutrient density positive control (PC) diets with a regular vitamin regimen. Tibial mineralization including (A) ash, (B) calcium (Ca), (C) phosphorus (P) and (D) density, as well as (E) tibia-breaking strength; (F) toluidine blue stained and the morphometric analysis for (G) the trabecular bone area (Tb.Ar), (H) trabecular thickness (Tb.Th), (I) number (Tb.N), and (J) spacing (Tb.Sp) determined by histomorphometry. Real-time-PCR analysis of osteocyte marker genes including (K) phosphate regulating endopeptidase homolog x-linked (*Phex*), (L) sclerostin, and (M) dentin matrix protein 1 (*Dmp1*) in the tibia proximal end. Values are means ± standard deviation represented by vertical bars ($n = 8$). * Mean values are significantly different (Student's *t*-test, $P < 0.05$).

tibia proximal end revealed that birds consuming the 25-OH-D_3 diets displayed a significant increase ($P < 0.05$) in Tb.Ar and Tb.N, and a pronounced decrease in Tb.Sp as compared to those fed the control diets (Fig. 3F to J). Furthermore, with regard to the osteocyte-specific marker genes expression, the 25-OH-D_3 diets increased ($P < 0.05$) the mRNA abundances of sclerostin as compared to the control diets (Fig. 3K). The expression of phosphate regulating endopeptidase homolog x-linked (*Phex*) and dentin matrix protein 1 (*Dmp1*) appears to be similar in the control and the 25-OH-D_3-fed birds (Fig. 3L and M). Collectively, these results suggest that 25-OH-D_3 supplementation enhances tibial microstructure and bone mass.

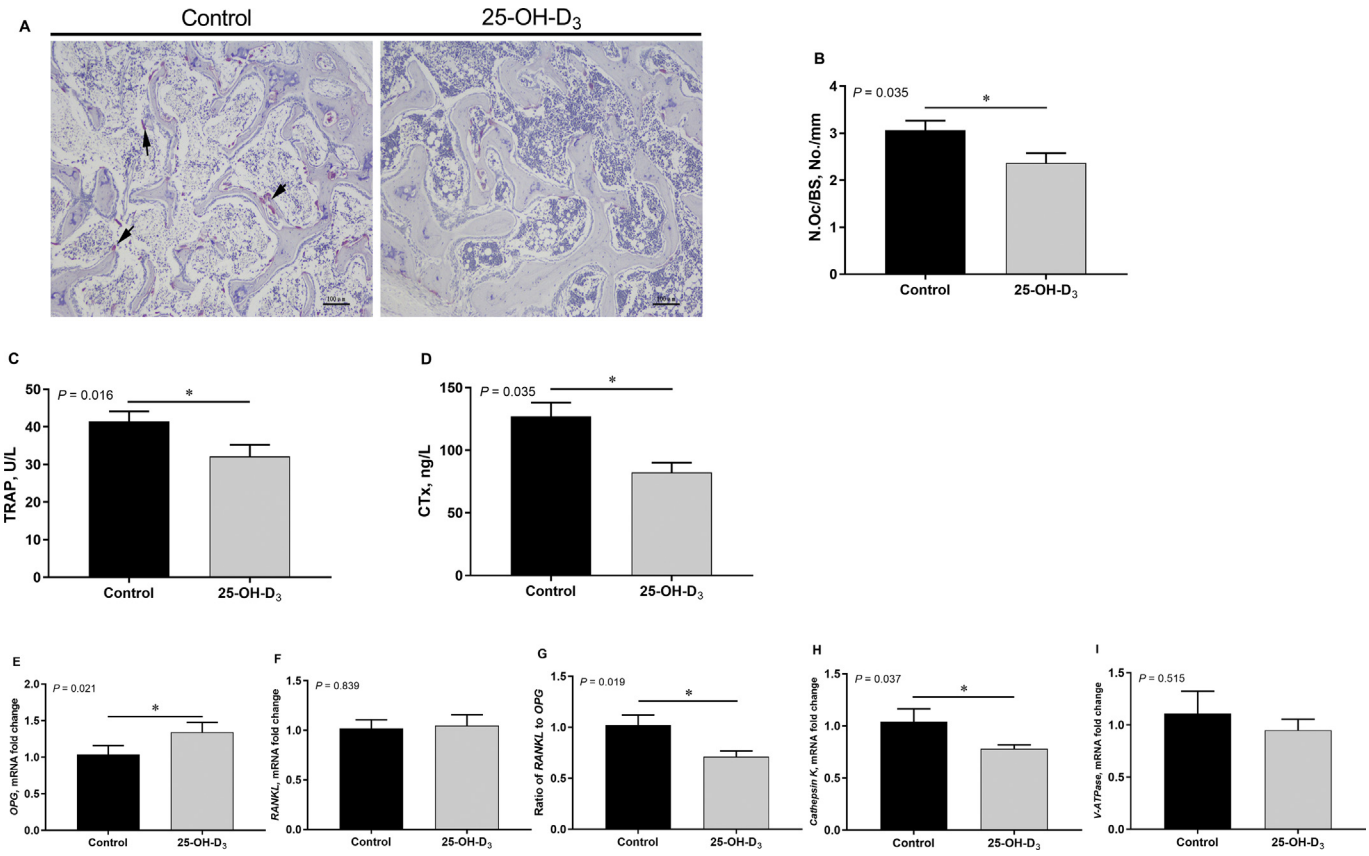

Fig. 4. Effect of 25-hydroxycholecalciferol (25-OH-D_3) supplementation on bone resorption in the standard nutrient density positive control (PC) diets with a regular vitamin regimen. (A) Tartrate-resistant acid phosphatase (TRAP) staining of tibia sections. (B) Osteoclast number per bone surface (N.Oc/BS) in tibia determined by histomorphometry. Serum (C) TRAP activity and (D) C-terminal cross-linked telopeptide of type I collagen (CTx) concentrations. Real-time-PCR analysis for mRNA expression of osteoclastogenesis related factors including (E) osteoprotegerin (*OPG*), (F) receptor activator of nuclear factor κB ligand (*RANKL*), (G) the ratio of *RANKL* to *OPG*, (H) cathepsin K, and (I) vacuolar-type H^+-ATPase (*V-ATPase*) in the tibia proximal end. Values are means ± standard deviation represented by vertical bars ($n = 8$). * Mean values are significantly different (Student's *t*-test, $P < 0.05$).

3.1.4. Bone resorption

We explored the role of 25-OH-D_3 in bone turnover by first investigating whether 25-OH-D_3 altered the statue of bone resorption. Thus, the osteoclast number and activity were evaluated histologically and biochemically in the present study, and these data showed that there was an apparent decrease in TRAP-positive cells from 25-OH-D_3-fed birds (Fig. 4A), and supplementation of 25-OH-D_3 reduced the N.Oc/BS by approximately 25% when compared to the control diets (Fig. 4B). The circulating concentrations of TRAP and CTx that reflect bone resorption were also decreased by the 25-OH-D_3 diets as compared to the control diets (Fig. 4C and D). We then examined the effects of 25-OH-D_3 on mRNA expression of osteoclastogenesis-related factors in bone. Here, the diets contained 25-OH-D_3 increased ($P < 0.05$) the expression level of osteoprotegerin (*OPG*) mRNA, thereby decreasing ($P < 0.05$) the receptor activator of nuclear factor κB ligand (*RANKL*)-to-*OPG* ratio when compared to control diets (Fig. 4E to G). In addition, the ducks receiving 25-OH-D_3 presented a significant decrease ($P < 0.05$) mRNA expression level of cathepsin K and a similar vacuolar H^+-ATPases *(V-ATPases)* mRNA abundance than those fed the control diets (Fig. 4H and I). Together, these results suggest that 25-OH-D_3 treatment suppressed osteoclast differentiation and activity.

3.1.5. Bone formation

Effects of 25-OH-D_3 treatment on bone formation were assessed by serum biochemical parameters (Fig. 5A and B). No obvious differences in the serum ALP activity and P1NP level as the indicators of bone formation were observed in the presence and absence of 25-OH-D_3 diets, suggesting that 25-OH-D_3 is unlikely to be a positive regulator of bone formation.

3.1.6. Serum Ca, P, and calciotropic hormones

As shown in Fig. 5, 25-OH-D_3 treatment significantly increased ($P < 0.05$) serum 25-OH-D and Ca levels when compared to the control group (Fig. 5C and D), whereas serum P levels were not altered by the 25-OH-D_3 manipulation (Fig. 5E). Also, serum PTH concentrations were comparable between the control and 25-OH-D_3-treated ducks (Fig. 5F), which indicates that the dosage of 25-OH-D_3 employed in this study did not induce hypercalcemia.

3.2. Experiment 2: tibial quality responses to 25-OH-D_3 and vitamin regimens in LND diets

3.2.1. Performance

Data on the growth performance of meat ducks are presented in Table 4. We found a significant increase ($P < 0.05$) in terms of BW on d 35 and BW gain from d 15 to 35 in the high vitamin diets as compared to regular vitamin diets. Likewise, supplementation of 25-OH-D_3 markedly increased ($P < 0.05$) the BW on d 14 and BW gain from d 1 to 14, as well as decreased F:G (d 1 to 14) in regular but not high vitamin diets (Table 4).

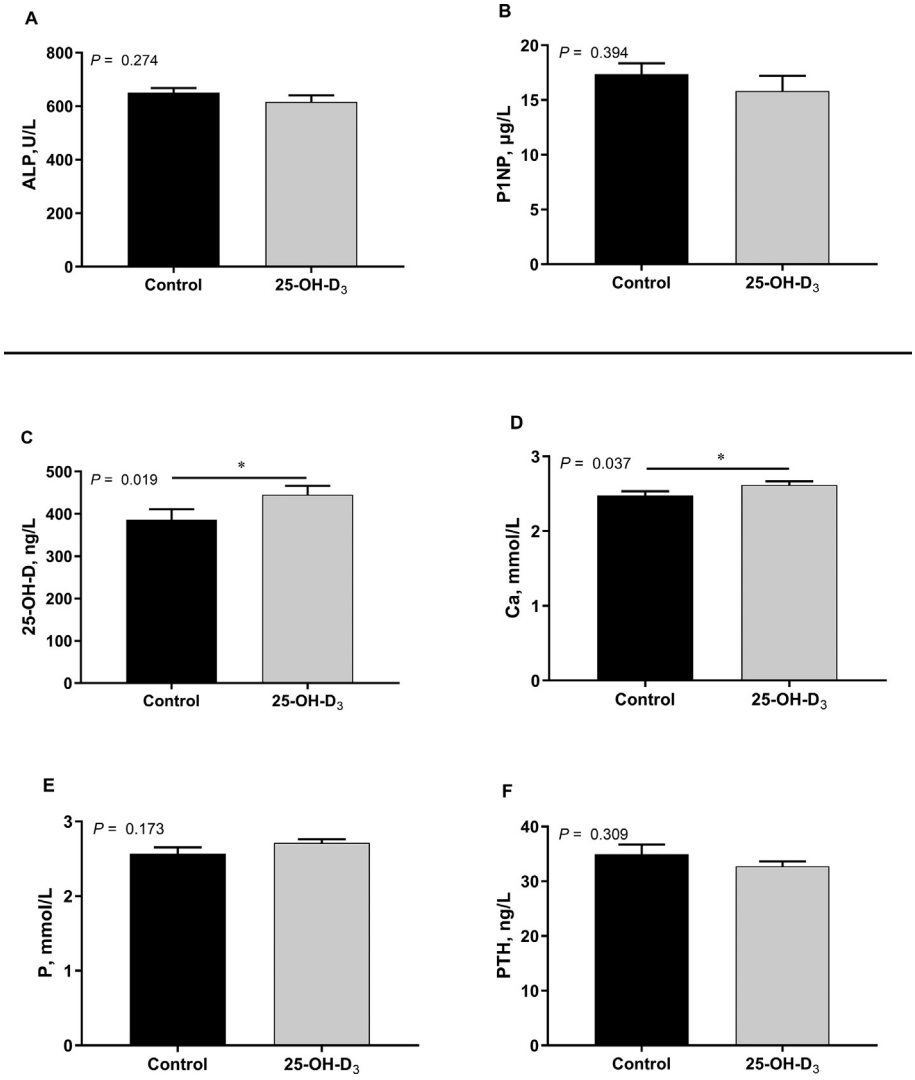

Fig. 5. Effect of 25-hydroxycholecalciferol (25-OH-D_3) supplementation on serum biochemistry in the standard nutrient density positive control (PC) diets with regular vitamin regimen. Serum bone formation markers, (A) alkaline phosphatase (ALP) and (B) procollagen type I N-terminal propeptide (P1NP) were determined, and serum levels of (C) 25-OH-D, (D) calcium (Ca), (E) phosphorus (P), and (F) parathyroid hormone (PTH). Values are means ± standard deviation represented by vertical bars ($n = 8$). * Mean values are significantly different (Student's t-test, $P < 0.05$).

3.2.2. Tibial characters

For leg abnormalities, there was no substantial effect ($P > 0.05$) of dietary treatments on the mortality due to leg abnormalities of meat ducks during trial period (Fig. 6A); nevertheless, supplementation of 25-OH-D_3 significantly decreased the TD score in regular but not high vitamin LND diets (Fig. 6B). With regard to tibial growth, no differences were found among all groups neither in length nor width (Fig. 6C and D). In addition, tibial fat-free weight was significantly increased ($P < 0.05$) by 25-OH-D_3 in regular vitamin diets (Fig. 6E).

Take account of tibial mass, under the circumstances of the absence of 25-OH-D_3, birds receiving high vitamin diets had significantly higher ($P < 0.05$) tibia strength, mineral depositions of Ca and P, and density than ducks receiving the regular vitamin diets. Dietary supplementation of 25-OH-D_3 significantly increased ($P < 0.05$) tibial mineral content, density and strength in regular but not high vitamin diets (Fig. 7). Taken together, these results suggest that a diet with high vitamin regimens probably is beneficial to tibial mass, and addition of 25-OH-D_3 to regular vitamin diet could significantly increase the mineral content of the tibia.

3.2.3. Bone metabolism and serum biochemistry

Analyzing bone resorption by histomorphometry and serum biochemical parameters revealed that a significant increase ($P < 0.05$) in TRAP-positive cells was observed in regular vitamin group when compared to high vitamin group (Fig. 8A). Dietary 25-OH-D_3 decreased ($P < 0.05$) TRAP-positive cells, serum TRAP activity and CTx level in regular but not high vitamin group (Fig. 8A to C). In addition, ducks consuming the high diets presented a significantly lower ($P < 0.05$) TRAP activity and CTx level in serum than those fed the regular vitamin diets (Fig. 8B and C). As far as bone formation is concerned, the values of ALP were higher in regular diets when compared with those in high diets, but supplementation of 25-OH-D_3 did not change the serum ALP activity within each dietary vitamin regimen (Fig. 8D). Similarly, the addition of 25-OH-D_3 and vitamin regimens did not result in any detectable difference in serum P1NP among all groups (Fig. 8E).

Furthermore, the serum 25-OH-D was higher in the high vitamin diets than that in the regular vitamin diets (Fig. 8F). Supplementation of 25-OH-D_3 notably increased ($P < 0.05$) serum 25-OH-D in regular and high vitamin groups, Ca and P levels in the

Table 4
Growth performance of meat ducks in response to 25-hydroxycholecalciferol (25-OH-D_3) administration in low nutrient density (LND) diets with 2 vitamin regimens.[1]

Item		Body weight, g/duck			Gain, g/duck		Feed intake, g/duck		Feed:Gain, g: g	
		d 1	d 14	d 35	d 1 to 14	d 15 to 35	d 1 to 14	d 15 to 35	d 1 to 14	d 15 to 35
Vitamin regimen	25-OH-D_3									
Regular	−	48.48	628.41^{b}	2,056.86^{b}	579.93^{b}	1,427.08^{c}	734.09	3,468.39	1.27^{a}	2.46
	+	48.54	669.95^{a}	2,111.3^{ab}	621.41^{a}	1,437.11^{bc}	742.62	3,530.03	1.19^{b}	2.46
High	−	48.46	650.21^{ab}	2,181.89^{a}	601.75^{ab}	1,529.78^{ab}	742.46	3,720.54	1.23^{ab}	2.43
	+	48.55	674.93^{a}	2,213.35^{a}	626.38^{a}	1,535.68^{a}	753.08	3,569.74	1.20^{b}	2.33
SEM		0.039	10.2	39.01	10.44	40.96	16.66	70.45	0.02	0.06
Vitamin regimen										
Regular		48.51	649.18	2,084.08^{b}	600.67	1,432.09^{b}	738.35	3,499.21	1.23	2.46
High		48.5	662.86	2,198.02^{a}	614.36	1,532.73^{a}	747.73	3,645.14	1.22	2.38
SEM		0.029	7.17	28.08	7.41	28.96	11.96	49.81	0.01	0.04
25-OH-D_3										
−		48.47	639.31^{b}	2,119.37	590.84^{b}	1,478.42	738.27	3,594.46	1.25^{a}	2.44
+		48.54	672.73^{a}	2,162.72	624.19^{a}	1,486.39	747.81	3,549.88	1.20^{b}	2.39
SEM		0.028	7.41	27.13	7.17	29.98	11.57	51.57	0.02	0.04
P-value										
Vitamin regimen		0.854	0.208	0.008	0.207	0.022	0.583	0.052	0.598	0.228
25-OH-D_3		0.177	0.004	0.289	0.004	0.850	0.578	0.539	0.023	0.397
Interaction		0.658	0.425	0.774	0.442	0.961	0.951	0.150	0.329	0.414

[a, b] Within a column, mean values with unlike letters were significantly different (two-way ANOVA, $P < 0.05$, Tukey's post hoc test).
[1] Ducklings were all fed a normal nutrient density starter diet until d 14, subsequently, they were subjected to an LND diet until d 35.

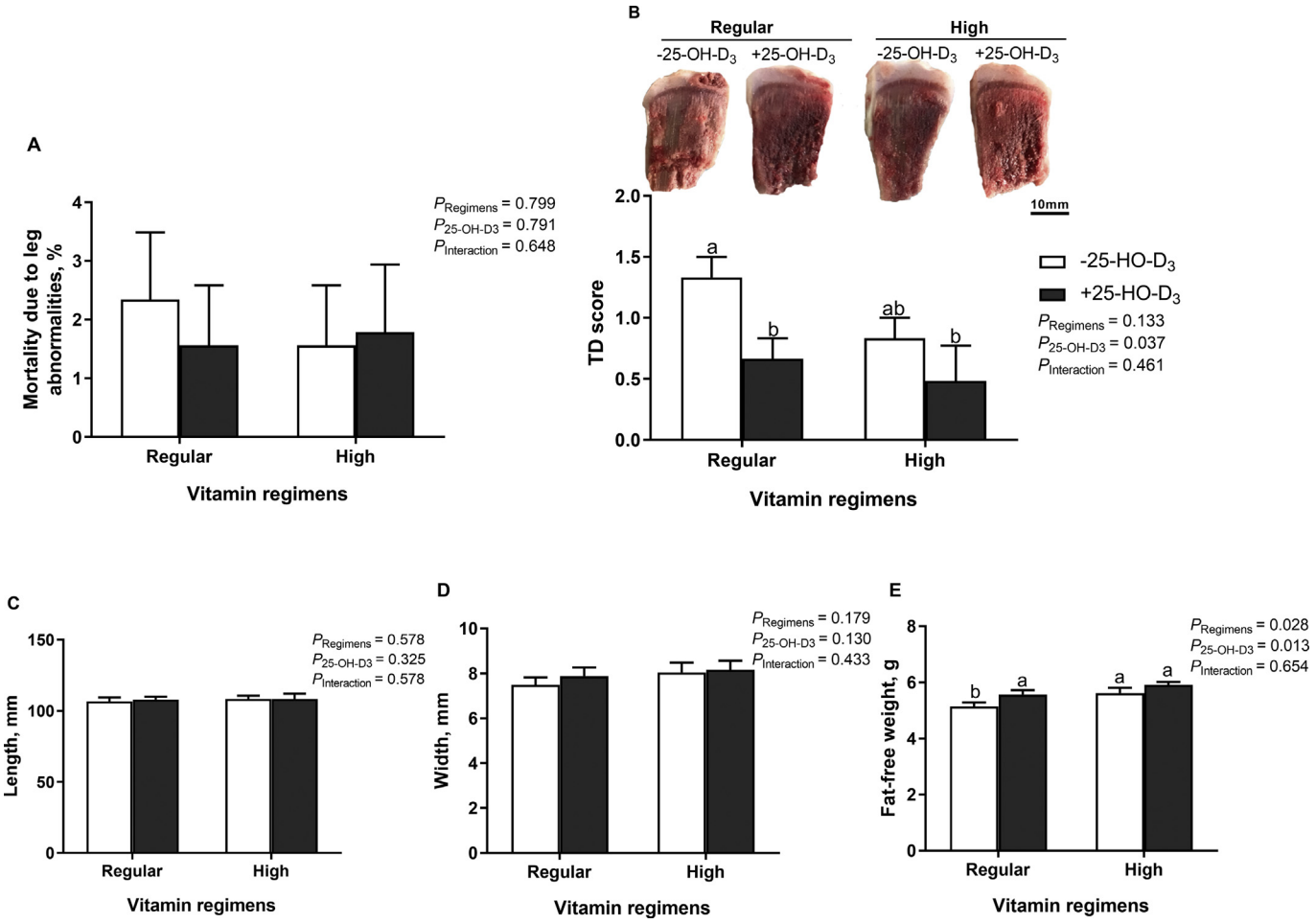

Fig. 6. Leg weakness and tibial growth responses to 25-hydroxycholecalciferol (25-OH-D_3) and vitamin regimen in a low nutrient density (LND) diet for meat ducks: (A) mortality due to leg abnormalities, (B) tibial dyschondroplasia (TD) score. Tibial growth was determined through (C) tibial length, (D) width, and (E) fat-free weight. Values are means ± standard deviation represented by vertical bars ($n = 8$). [a, b] Different letters on bars means a significant difference (two-way ANOVA, $P < 0.05$, Tukey's post hoc test).

regular but not high vitamin group (Fig. 8F to H). For serum PTH, no influence of vitamin regimens or 25-OH-D_3 in feed was observed among all groups (Fig. 8I). Overall, these results suggest that the actions of 25-OH-D_3 on reducing bone resorption, and enhancing serum Ca and P levels depend on the dietary basal vitamin level of LND diets.

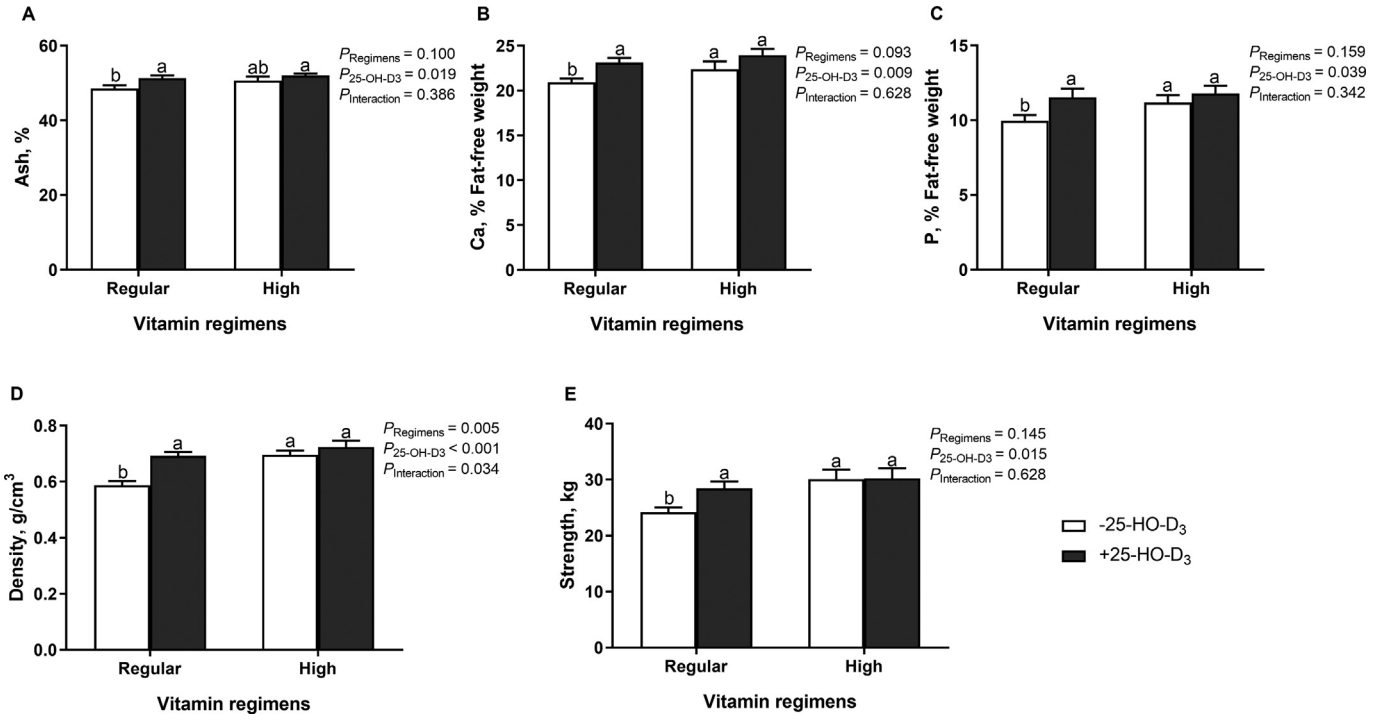

Fig. 7. Tibial mineralization responses to 25-hydroxycholecalciferol (25-OH-D_3) and vitamin regimen in a low nutrient density (LND) diet for meat ducks: (A) tibial ash, (B) calcium (Ca), (C) phosphorus (P) and (D) density, as well as (E) tibia-breaking strength. Values are means ± standard deviation represented by vertical bars ($n = 8$). [a, b] Different letters on bars means a significant difference (two-way ANOVA, $P < 0.05$, Tukey's post hoc test).

4. Discussion

For many years, selection for growth rate has been associated with an increase in leg disorders due to this weight redistribution, which results in considerable attention being given to the morphological characteristics and composition of leg bones. Many factors can affect bone properties, including genetics, sex, growth rate, bone deformities, nutrition, infection, and stocking density, etc. (Bradshaw et al., 2002). Specifically, differences between sexes have been reported in bone growth and composition of broilers by Yalçin et al. (2001). For this reason, the male ducks only were selected as research subjects to exclude gender implications. Herein we investigated the repercussions of 25-OH-D_3 supplementation in the tibial mass of meat ducks. Our data showed that 25-OH-D_3 improved the tibial mass of meat ducks by suppressing osteoclast-mediated bone resorption that corroborates the lower number of osteoclasts, decreased serum TRAP activity and CTx level, as well as the downregulation osteoclast-specific marker genes expression in trabecular bone. Furthermore, 25-OH-D_3 supplementation increased tibial mass which was characteristic of higher minerals deposition and strength in regular but not high vitamin regimen when birds fed the LND diets, showing that the effects of 25-OH-D_3 on the tibial mass of meat ducks depended on the dietary basal vitamin level of the LND diets.

Findings from previous experiments indicated that supplementation of vitamin D_3 at 2,000 IU/kg increased the BW of broilers (Gomezverduzco et al., 2013). The positive effect of supplementing 25-OH-D_3 was also reflected in an increased BW gain and feed conversion ratio (Santiago et al., 2016). The present study similarly demonstrated that 25-OH-D_3 administration significantly increased BW and BW gain on d 14. Moreover, a beneficial impact of 25-OH-D_3 on leg problems of meat ducks was also noticed in the present study, which was confirmed by the lower mortality due to leg abnormalities and TD score in the 25-OH-D_3 group. These results were in agreement with the study conducted by Khan et al. (2010) who reported that a feeding level of 1,500 or 3,500 IU/kg of vitamin D_3 notably decreased mortality and TD score in broilers.

Vitamin D_3 exerts a variety of actions on bone health of broilers. These actions were presented by the study of Świątkiewicz et al. (2017), which showed that the supplementation of vitamin D at about 3,000 IU/kg, much higher than National Nutrition Council (1994) recommendations, is optimal for mineral digestibility and bone quality of broilers. In the present study, supplementing 25-OH-D_3 at 0.069 mg/kg (the equivalent of vitamin D_3 at 2,760 IU/kg) to PC diets that containing regular vitamin regimen was found to significantly increase tibial minerals deposition and density for the 35-d-old meat ducks. These findings were in accordance with previous studies conducted in broilers (Santiago et al., 2016; Sun et al., 2013; Wideman et al., 2015). Morphometric analyses further demonstrated that dietary 25-OH-D_3 administration significantly increased the Tb.Ar and Tb.N, and decreased the Tb.Sp of tibias in our current trial. Reports have also consistently showed that treated with eldecalcitol, an active vitamin D analog, increased bone volume by increasing Tb.N and Tb.Th, as well as decreasing Tb.Sp in tibias of mice (Nakamichi et al., 2017). It is well established that bone density and trabecular architecture are strongly related with yield strength of bone (Ito et al., 2002). Thus, enhancing trabecular microstructure and increasing the mineral content due to supplementation of 25-OH-D_3 contributed to an increase in bone biomechanical strength in this study. Taken together, these findings suggested that supplementation with 25-OH-D_3 improved tibial mass and strength. These conclusions were further bolstered by the upregulation of mRNA expression of *Phex*, sclerostin, and *Dmp1* in 25-OH-D_3-treated ducks. In general, osteocytes are the most cells in bone matrix that account for 90% to 95% of all bone associated cells. In addition to

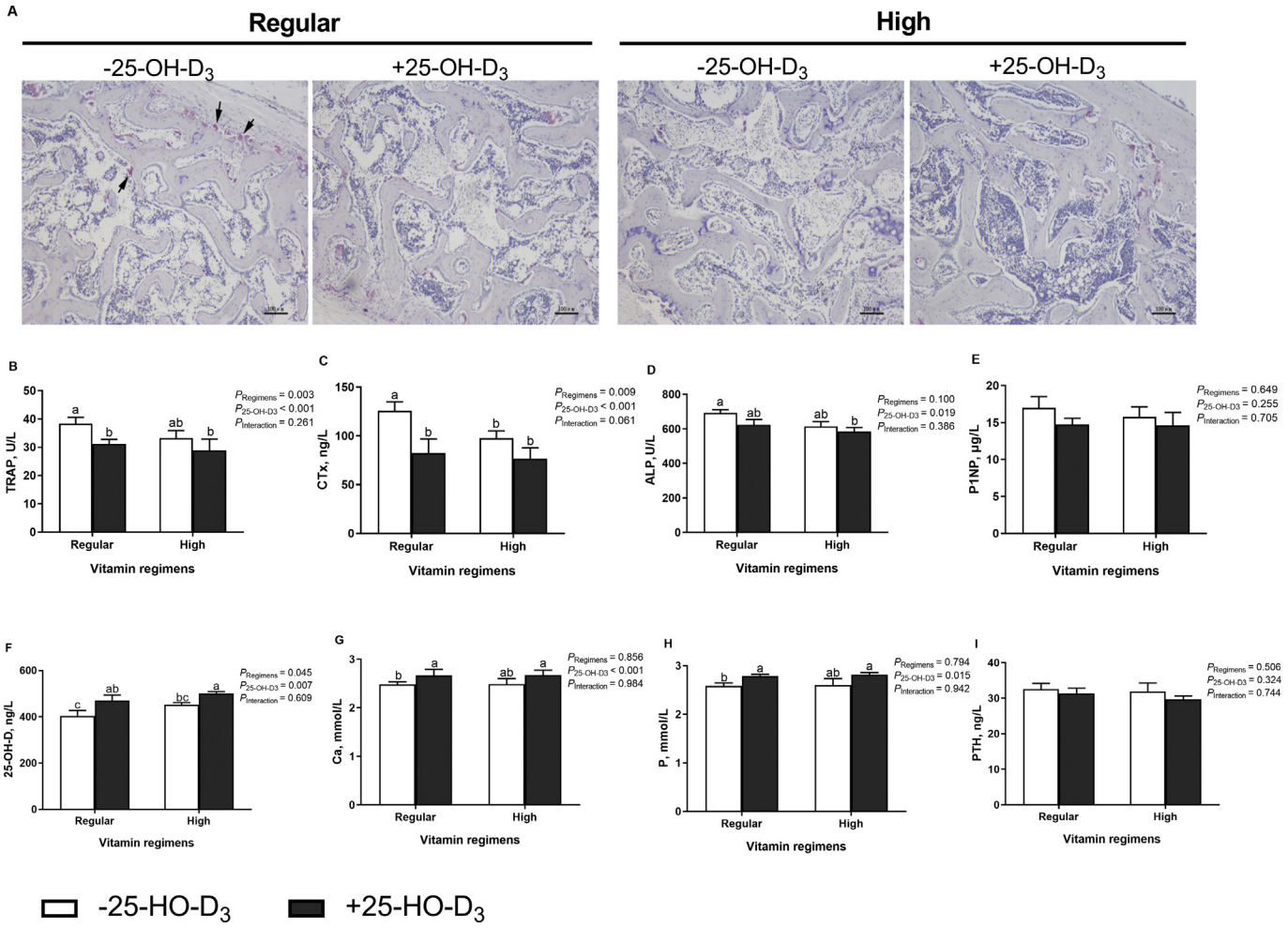

Fig. 8. Bone metabolism responses to 25-hydroxycholecalciferol (25-OH-D_3) and vitamin regimen in a low nutrient density (LND) diet for meat ducks. (A) Tibia was stained using tartrate-resistant acid phosphatase (TRAP), and serum level of bone resorption markers such as (B) TRAP activity and (C) C-terminal cross-linked telopeptide of type I collagen (CTx) were measured, as well as serum level of bone formation markers including (D) alkaline phosphatase (ALP) and (E) procollagen type I N-terminal propeptide (P1NP) were also determined. Serum levels of (F) 25-OH-D, (G) calcium (Ca), (H) phosphorus (P), and (I) parathyroid hormone (PTH). Values are means ± standard deviation represented by vertical bars ($n = 8$). $^{a-c}$ Different letters on bars means a significant difference (two-way ANOVA, $P < 0.05$, Tukey's post hoc test).

regulating bone mass, osteocytes may determine bone quality (Milovanovic et al., 2013). An upregulation of osteocyte-specific genes implied that the increase of osteocyte number is probably associated with an enhanced bone mass.

In spite of the anti-rachitic activity of vitamin D in vivo, the impacts of vitamin D on osteoblast are still less uniform. Some studies showed a stimulation of osteoblast differentiation and mineralization after vitamin D treatment (Turner et al., 2014; van der Meijden et al., 2014), but also an inhibition of osteoblast differentiation in mice (Nakamichi et al., 2017) and in cultures of osteoblast lineage cells (Chen et al., 2012). This discrepancy could be partly explained by the stage differentiation of osteoblast, Ca condition, or the dose of vitamin D used in these studies (Lieben and Carmeliet, 2013; van der Meijden et al., 2014). In the present study, however, circulating bone formation markers, P1NP and ALP, were did not discernibly altered by supplementation of 25-OH-D_3, which suggested that the positive effect of 25-OH-D_3 on bone mass of meat ducks is unlikely mediated by increasing bone formation.

In addition, vitamin D has also been reported to be a moderator of osteoclastic number and activity. TRAP, a histochemical and biochemical marker for osteoclast, allows for the examination of osteoclastic differentiation and function (Halleen et al., 2000). Our current results revealed that 25-OH-D_3 decreased the number of TRAP-positive cells, consistent with previous studies, which reported that calcitriol, the active form of vitamin D, strongly inhibited the proliferation of osteoclasts (Li et al., 2017). In another study, vitamin D strongly stimulated the formation of osteoclasts and induced bone resorption (Sato et al., 2007). These controversial data may be due to the different concentrations and methods of vitamin D administration (Zarei et al., 2016). The effect of vitamin D on osteoclastic activity has largely been established to mediated via osteoblast, which mainly regulates the secretion of the RANKL and OPG (Baldock et al., 2006). Receptor activator of nuclear factor κB ligand binds to receptor activator of nuclear factor kappa-B (RANK) that expressed on the surface of osteoclast to induce osteoclast differentiation, whereas OPG acts as a decoy receptor by blocking the interaction of RANKL with its functional receptor RANK (Yamamoto et al., 2006). The ration of RANKL to OPG is therefore an important index of osteoclastogenic stimulation and plays a key role in the regulation of bone metabolism. In this study, dietary 25-OH-D_3 increased the mRNA expression of *OPG* but did not significantly change the *RANKL* mRNA level, thereby decreasing the ratio of *RANKL* to *OPG*, and subsequently reduced the osteoclast numbers in trabecular bone. Such metabolic effects of active 25-OH-D_3 were in line with a previous

report that vitamin D decreased the osteoclast numbers by inducing the expression of *OPG* mRNA and decreasing the *RANKL*-to-*OPG* ratio in mice (Nakamichi et al., 2017). Besides that, some important enzymes such as V-ATPases and cathepsin K are secreted by mature osteoclasts to dissolve the organic and inorganic components of bone in the process of bone resorption (Fujisaki et al., 2007; Riihonen et al., 2007). Downregulation of cathepsin K mRNA expression by the 25-OH-D_3 administration in our study indicated that 25-OH-D_3 inhibited bone resorption in meat ducks. Decreased circulating bone resorption markers, TRAP and CTx, also further confirmed the inhibitory effect of 25-OH-D_3 on tibia of meat ducks. Collectively, these results demonstrate that treating with 25-OH-D_3 suppressed bone resorption by decreasing osteoclastic numbers and activities in meat ducks.

Moreover, studies indicated that the biochemical actions of 25-OH-D_3 are highly dependent on the dietary vitamin regimen in ducks (Ren et al., 2016; 2017). In agreement with our results, 25-OH-D_3 treatment significantly increased tibial mineral content, density and strength in the regular but not high vitamin diets. These observations suggest that the actions of 25-OH-D_3 on improving bone mass depend on dietary basal vitamin level in LND diets. Also, the increase of tibial mass due to supplying 25-OH-D_3 in the regular vitamin diets was accompanied by the decreased concentrations in serum TRAP and CTx, indicating that the positive effects of 25-OH-D_3 on tibial mass were mainly because of the suppression of bone resorption in the LND diets with regular vitamin regimen. This differential response of dietary 25-OH-D_3 to distinct vitamin regimens on tibial mass appears to be a dosage dependent or interaction between dietary vitamins. Specifically, the high premix had higher levels of all vitamins except biotin than regular premix in the present study.

Regarding to the effect of vitamin regimens on tibial mass. Our previous study found that supplementation of 25-OH-D_3 in a high vitamin diet significantly promoted the minerals deposition of sterna compared to a low vitamin diet for 49-d-old meat ducks (Zhang et al., 2019). Consistent with this finding, feeding the high vitamin diets in this study improved the tibial fat-free weight, mineral deposition and strength compared to the regular vitamin diets, suggesting that the high vitamin diets had a positive effect on tibial mass. The increase of bone mass probably is a result of decreased bone turnover rate (Takeda et al., 2015), evidenced by the lower levels of both bone formation markers (ALP and P1NP) and bone resorption markers (TRAP and CTx) on the high diets than those on the regular diets.

There are 2 limitations in our study. The first is the bone histological assessment. Although the skills for the histomorphometry can be applied in other reports (Vidal et al., 2012), however, the bias from sampling, sectioning, acquisition images and data are inevitable. Therefore, we admit the possibility that some of our conclusions may include overestimation or underestimation of roles of 25-OH-D_3 in tibial mass. The second is experiments using the LND diet. Due to birds possess the ability to regulate feed intakes based on the dilution of nutrient density, it was expected that birds receiving the LND diets could consume more foods than birds fed the PC diets (Kamran et al., 2008). The current study is failing to examine the effect of the nutrient density on feed intake of meat ducks. A possible explanation for this discrepancy might be there is a certain range of the nutrient density of duck to regulate feed intake based on the dilution of dietary nutrient density. Further studies would be essential to elucidate these possibilities.

5. Conclusions

This study provides further evidence proving the beneficial functions of 25-OH-D_3 on the tibial mass of meat ducks. The supplementation of 25-OH-D_3 to regular vitamin regimen increased the tibial mass of meat ducks in both PC and LND diets by suppression of bone resorption, and this positive impact only was observed in regular but not high vitamin regimen when birds fed LND diets. In addition, the LND diets with high vitamin regimen made a favorable effect on the tibial mass of meat duck via decreasing bone turnover rate.

Author contributions

Huaiyong Zhang, Qiufeng Zeng, Bing Yao and Keying Zhang: conceptualization; Huaiyong Zhang, Qiufeng Zeng, Shiping Bai and Bing Yao: validation; Keying Zhang, Jianping Wang and Gergory S. Fraley: formal analysis; Shiping Bai, Jianping Wang and Xuemei Ding: investigation; Huaiyong Zhang, Qiufeng Zeng, Xuemei Ding Yue Xuan and Zhuowei Su: data curation; Huaiyong Zhang, Gergory S. Fraley, Shiping Bai and Keying Zhang: writing — original draft preparation; Huaiyong Zhang, Qiufeng Zeng and Shiping Bai: writing — writing and editing; Gergory S. Fraley, Jianping Wang and Keying Zhang: visualization; Huaiyong Zhang, Xuemei Ding and Qiufeng Zeng: supervision; Keying Zhang: project administration and funding acquisition.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This work was supported by the Modern Agri-industry Technology Research System (CARS-42-10) and the 111 Project.

References

Baldock PA, Thomas GP, Hodge JM, Baker SU, Dressel U, O'Loughlin PD, Nicholson GC, Briffa KH, Eisman JA, Gardiner EM. Vitamin D action and regulation of bone remodeling: suppression of osteoclastogenesis by the mature osteoblast. J Bone Miner Res 2006;21(10):1618–26. https://doi.org/10.1359/jbmr.060714.

Bonjour JP, Dontot-Payen F, Rouy E, Walrand S, Rousseau B. Evolution of serum 25OHD in response to vitamin D3-fortified yogurts consumed by healthy menopausal women: a 6-month randomized controlled trial assessing the interactions between doses, baseline vitamin D status, and seasonality. J Am Coll Nutr 2018;37(1):34–43. https://doi.org/10.1080/07315724.2017.1355761.

Bradshaw RH, Kirkden RD, Broom DM. A Review of the aetiology and pathology of leg weakness in broilers in relation to welfare. Avian Poultry Biol Rev 2002;13(2):45–103.

Brickett KE, Dahiya JP, Classen HL, Annett CB, Gomis S. The impact of nutrient density, feed form, and photoperiod on the walking ability and skeletal quality of broiler chickens. Poultry Sci 2007;86(10):2117–25. https://doi.org/10.1093/ps/86.10.2117.

China Agricultura Industry Standards. Nutrient requirement of meat-type ducks (NY/T 2122-2012). Beijing: China Agricultural Industry Standards; 2012.

Chen YC, Ninomiya T, Hosoya A, Hiraga T, Miyazawa H, Nakamura H. 1alpha,25-Dihydroxyvitamin D3 inhibits osteoblastic differentiation of mouse periodontal fibroblasts. Arch Oral Biol 2012;57(5):453–9. https://doi.org/10.1016/j.archoralbio.2011.10.005.

Christakos S, Lieben L, Masuyama R, Carmeliet G. Vitamin D endocrine system and the intestine. BoneKEy Rep 2014;3:496. https://doi.org/10.1038/bonekey.2013.230.

Corr S, Gentle M, McCorquodale C, Bennett D. The effect of morphology on walking ability in the modern broiler: a gait analysis study. Anim Welf 2003;12(2):159–71.

Edwards Jr HM, Veltmann Jr JR. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J Nutr 1983;113(8):1568–75. https://doi.org/10.1093/jn/113.8.1568.

Ferket PR, Oviedo-Rondon EO, Mente PL, Bohorquez DV, Santos Jr AA, Grimes JL, Richards JD, Dibner JJ, Felts V. Organic trace minerals and 25-hydroxycholecalciferol affect performance characteristics, leg abnormalities,

and biomechanical properties of leg bones of turkeys. Poultry Sci 2009;88(1):118–31. https://doi.org/10.3382/ps.2008-00200.
Fujisaki K, Tanabe N, Suzuki N, Kawato T, Takeichi O, Tsuzukibashi O, Makimura M, Ito K, Maeno M. Receptor activator of NF-kappaB ligand induces the expression of carbonic anhydrase II, cathepsin K, and matrix metalloproteinase-9 in osteoclast precursor RAW264.7 cells. Life Sci 2007;80(14):1311–8. https://doi.org/10.1016/j.lfs.2006.12.037.
Gomezverduzco G, Moraleslopez R, Avilagozalez E. Use of 25-hydroxycholecalciferol in diets of broiler chickens: effects on growth performance, immunity and bone calcification. J Poult Sci 2013;50(1):60–4.
Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Vaananen HK. Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. J Bone Miner Res 2000;15(7):1337–45. https://doi.org/10.1359/jbmr.2000.15.7.1337.
Han JC, Chen GH, Wang JG, Zhang JL, Qu HX, Zhang CM, Yan YF, Cheng YH. Evaluation of relative bioavailability of 25-hydroxycholecalciferol to cholecalciferol for broiler chickens. Asian-Australas J Anim Sci 2016;29(8):1145–51. https://doi.org/10.5713/ajas.15.0553.
Ito M, Nishida A, Koga A, Ikeda S, Shiraishi A, Uetani M, Hayashi K, Nakamura T. Contribution of trabecular and cortical components to the mechanical properties of bone and their regulating parameters. Bone 2002;31(3):351–8.
Jiang S, Jiang Z, Yang K, Chen F, Zheng C, Wang L. Dietary vitamin D3 requirement of Chinese yellow-feathered broilers. Poultry Sci 2015;94(9):2210–20. https://doi.org/10.3382/ps/pev163.
Jones TA, Dawkins MS. Environment and management factors affecting Pekin duck production and welfare on commercial farms in the UK. Br Poultry Sci 2010;51(1):12–21. https://doi.org/10.1080/00071660903421159.
Kamran Z, Sarwar M, Nisa M, Nadeem MA, Mahmood S, Babar ME, Ahmed S. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poultry Sci 2008;87(3):468–74. https://doi.org/10.3382/ps.2007-00180.
Khan SH, Shahid R, Mian AA, Sardar R, Anjum MA. Effect of the level of cholecalciferol supplementation of broiler diets on the performance and tibial dyschondroplasia. J Anim Physiol Anim Nutr 2010;94(5):584–93. https://doi.org/10.1111/j.1439-0396.2009.00943.x.
Kulda V. Vitamin D metabolism. Vnitr Lek 2012;58(5):400–4.
Li A, Cong Q, Xia X, Leong WF, Yeh J, Miao D, Mishina Y, Liu H, Li B. Pharmacologic calcitriol inhibits osteoclast lineage commitment via the BMP-Smad1 and IkappaB-NF-kappaB pathways. J Bone Miner Res 2017;32(7):1406–20. https://doi.org/10.1002/jbmr.3146.
Lieben L, Carmeliet G. The delicate balance between vitamin D, calcium and bone homeostasis: lessons learned from intestinal- and osteocyte-specific VDR null mice. J Steroid Biochem Mol Biol 2013;136:102–6. https://doi.org/10.1016/j.jsbmb.2012.09.019.
Milovanovic P, Zimmermann EA, Hahn M, Djonic D, Puschel K, Djuric M, Amling M, Busse B. Osteocytic canalicular networks: morphological implications for altered mechanosensitivity. ACS Nano 2013;7(9):7542–51. https://doi.org/10.1021/nn401360u.
Nakamichi Y, Udagawa N, Horibe K, Mizoguchi T, Yamamoto Y, Nakamura T, Hosoya A, Kato S, Suda T, Takahashi N. VDR in osteoblast-lineage cells primarily mediates vitamin D treatment-induced increase in bone mass by suppressing bone resorption. J Bone Miner Res 2017;32(6):1297–308. https://doi.org/10.1002/jbmr.3096.
Nakamichi Y, Udagawa N, Suda T, Takahashi N. Mechanisms involved in bone resorption regulated by vitamin D. J Steroid Biochem Mol Biol 2018;177:70–6. https://doi.org/10.1016/j.jsbmb.2017.11.005.
National Nutrition Council. Nutrient requirements of poultry. 9th rev. ed. Washington, DC: National Academies Press; 1994.
National Nutrition Council. Nutrient Requirements of Poultry. 9th rev. ed. Washington, DC: National Academies Press; 1994. In this issue.
Paxton H, Daley MA, Corr SA, Hutchinson JR. The gait dynamics of the modern broiler chicken: a cautionary tale of selective breeding. J Exp Biol 2013;216(Pt 17):3237–48. https://doi.org/10.1242/jeb.080309.
Ren Z, Jiang S, Zeng Q, Ding X, Bai S, Wang J, Luo Y, Su Z, Xuan Y, Yao B, Cisneros F, Zhang K. Effect of dietary canthaxanthin and 25-hydroxycholecalciferol supplementation on the performance of duck breeders under two different vitamin regimens. J Anim Sci Biotechnol 2016;7:2. https://doi.org/10.1186/s40104-016-0062-3.
Ren ZZ, Jiang SZ, Zeng QF, Ding XM, Bai SP, Wang JP, Luo YH, Su ZW, Xuan Y, Zhang KY. Effect of maternal canthaxanthin and 25-hydroxycholecalciferol supplementation on the performance of ducklings under two different vitamin regimens. J Anim Physiol Anim Nutr 2017;101(2):359–68. https://doi.org/10.1111/jpn.12453.
Riihonen R, Supuran CT, Parkkila S, Pastorekova S, Vaananen HK, Laitala-Leinonen T. Membrane-bound carbonic anhydrases in osteoclasts. Bone 2007;40(4):1021–31. https://doi.org/10.1016/j.bone.2006.11.028.
Santiago M, David S, Alexandra N, Eduardo A, Jimmy Q. Effect of 25-hydroxycholecalciferol (25-OH-D3) on productive performance and bone mineralization in broiler. Open J Anim Sci 2016;6(3):180–4.
Sato M, Nakamichi Y, Nakamura M, Sato N, Ninomiya T, Muto A, Nakamura H, Ozawa H, Iwasaki Y, Kobayashi E, Shimizu M, DeLuca HF, Takahashi N, Udagawa N. New 19-nor-(20S)-1alpha,25-dihydroxyvitamin D3 analogs strongly stimulate osteoclast formation both in vivo and in vitro. Bone 2007;40(2):293–304. https://doi.org/10.1016/j.bone.2006.09.007.
Sun ZW, Yan L, G YY, Zhao JP, Lin H, Guo YM. Increasing dietary vitamin D3 improves the walking ability and welfare status of broiler chickens reared at high stocking densities. Poultry Sci 2013;92(12):3071–9. https://doi.org/10.3382/ps.2013-03278.
Świątkiewicz S, Arczewskawłosek A, Bederskalojewska D, Jozefiak D. Efficacy of dietary vitamin D and its metabolites in poultry - review and implications of the recent studies. World's Poult Sci J 2017;73(1):57–68.
Takeda S, Smith SY, Tamura T, Saito H, Takahashi F, Samadfam R, Haile S, Doyle N, Endo K. Long-term treatment with eldecalcitol (1alpha, 25-dihydroxy-2beta-(3-hydroxypropyloxy) vitamin D3) suppresses bone turnover and leads to prevention of bone loss and bone fragility in ovariectomized rats. Calcif Tissue Int 2015;96(1):45–55. https://doi.org/10.1007/s00223-014-9937-5.
Turner AG, Hanrath MA, Morris HA, Atkins GJ, Anderson PH. The local production of 1,25(OH)2D3 promotes osteoblast and osteocyte maturation. J Steroid Biochem Mol Biol 2014;144(Pt A):114–8. https://doi.org/10.1016/j.jsbmb.2013.10.003.
van der Meijden K, Lips P, van Driel M, Heijboer AC, Schulten EA, den Heijer M, Bravenboer N. Primary human osteoblasts in response to 25-hydroxyvitamin D3, 1,25-dihydroxyvitamin D3 and 24R,25-dihydroxyvitamin D3. PloS One 2014;9(10):e110283. https://doi.org/10.1371/journal.pone.0110283.
van Driel M, van Leeuwen J. Vitamin D endocrinology of bone mineralization. Mol Cell Endocrinol 2017;453:46–51. https://doi.org/10.1016/j.mce.2017.06.008.
Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3(7). Research0034.
Vidal B, Pinto A, Galvao MJ, Santos AR, Rodrigues A, Cascao R, Abdulghani S, Caetano-Lopes J, Ferreira A, Fonseca JE, Canhao H. Bone histomorphometry revisited. Acta Reumatol Port 2012;37(4):294–300.
Wideman Jr RF, Blankenship J, Pevzner IY, Turner BJ. Efficacy of 25-OH Vitamin D3 prophylactic administration for reducing lameness in broilers grown on wire flooring. Poultry Sci 2015;94(8):1821–7. https://doi.org/10.3382/ps/pev160.
Williams B, Waddington D, Murray DH, Farquharson C. Bone strength during growth: influence of growth rate on cortical porosity and mineralization. Calcif Tissue Int 2004;74(3):236–45. https://doi.org/10.1007/s00223-002-2124-0.
Yalçin S, Ozkan S, Coşkuner E, Bilgen G, Delen Y, Kurtulmuş Y, et al. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Br Poult Sci 2001;42(2):184–90. https://doi.org/10.1080/00071660120048429.
Yamamoto Y, Udagawa N, Matsuura S, Nakamichi Y, Horiuchi H, Hosoya A, Nakamura M, Ozawa H, Takaoka K, Penninger JM, Noguchi T, Takahashi N. Osteoblasts provide a suitable microenvironment for the action of receptor activator of nuclear factor-kappaB ligand. Endocrinology 2006;147(7):3366–74. https://doi.org/10.1210/en.2006-0216.
Zarei A, Morovat A, Javaid K, Brown CP. Vitamin D receptor expression in human bone tissue and dose-dependent activation in resorbing osteoclasts. Bone Res 2016;4:16030. https://doi.org/10.1038/boneres.2016.30.
Zhang H, Liao H, Zeng Q, Wang J, Ding X, Bai S, Zhang K. Effects of commercial premix vitamin level on sternum growth, calcification and carcass traits in meat duck. J Anim Physiol Anim Nutr 2019;103(1):53–63. https://doi.org/10.1111/jpn.13001.
Zhang H, Zeng Q, Bai S, Wang J, Ding X, Xuan Y, Su Z, Zhang K. Effect of graded calcium supplementation in low-nutrient density feed on tibia composition and bone turnover in meat ducks. Br J Nutr 2018;120(11):1217–29. https://doi.org/10.1017/s0007114518002556.
Zhang HY, Liao H, Zeng QF, Wang JP, Ding XM, Bai SP, Zhang KY. A study on the sternum growth and mineralization kinetic of meat duck from 35 to 63 days of age. Poultry Sci 2017;96(11):4103–15. https://doi.org/10.3382/ps/pex223.