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Effects of Dietary Phytobiotic Supplementation on Growth Performance, Tibia Mineralization, and Serum Antioxidant Status in Growing Japanese Quails

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Abstract

This study investigated the effects of dietary phytobiotic supplementation on growth performance, carcass yield, tibia mineralization, tibia breaking strength, and serum antioxidant status in growing Japanese quails. A total of 240 one-day-old mixed-sex Japanese quail (*Coturnix coturnix Japonica*) chicks were randomly assigned to four dietary treatments consisting of a control diet and diets supplemented with 500, 1000, or 1500 mg/kg of a phytobiotic blend. Each treatment included six replicates. The feeding trial lasted 35 days. Dietary phytobiotic supplementation had no significant effect on final body weight, body weight gain, feed intake, feed conversion ratio, carcass yield, or tibia breaking strength ($P > 0.05$). However, phytobiotic supplementation significantly increased tibia calcium and phosphorus concentrations compared with the control diet ($P < 0.05$). Serum antioxidant indices, including total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA), were not significantly affected by dietary treatments ($P > 0.05$). In conclusion, while macroscopic growth indices and basal redox homeostasis remained unaltered, phytobiotic supplementation particularly at 500 mg/kg effectively enhanced tibia Ca and P deposition without incurring a metabolic trade-off. The uncoupled response between mineral density and bone breaking strength highlights the need to evaluate organic matrix dynamics in future phytobiotic trials.

Introduction

Rapid growth, efficient feed utilization, high carcass yield, and the maintenance of product quality are among the primary objectives of modern quail production. However, increasing restrictions on the use of antibiotic growth promoters due to concerns regarding antimicrobial resistance have accelerated the search for natural feed additives that can enhance productivity while supporting animal health (Kheiri et al. 2018; Farouk et al. 2020; Aminullah et al. 2025). Among these alternatives, phytobiotics have attracted considerable attention because of their antimicrobial, antioxidant, anti-inflammatory, and digestive-modulating properties, which may improve nutrient utilization and physiological functions in poultry.

Phytobiotics comprise a wide range of plant-derived bioactive compounds with distinct physiological functions. Capsaicin has been shown to stimulate digestive enzyme secretion and improve nutrient utilization (Liu et al. 2021), whereas terpenes contribute to intestinal integrity through their antimicrobial and anti-inflammatory activities (Farouk et al. 2020; Rocha et al. 2020). Saponins and glucosinolate derivatives have been reported to modulate intestinal microbiota and enhance mineral utilization (Amad et al. 2011; Hafeez et al. 2016; Aminullah et al. 2025). In addition, curcumin possesses potent antioxidant properties that may alleviate oxidative stress and indirectly support mineral metabolism during growth (Wang et al. 2022; Aminullah et al. 2025).

Improved mineral utilization is considered one of the major mechanisms through which phytobiotics may enhance bone development. Enhanced intestinal absorption of calcium and phosphorus has been

reported following phytobiotic supplementation in broiler chickens (Amad et al. 2011; Hafeez et al. 2016), which may subsequently promote bone mineralization (Hafeez et al. 2021). Similarly, Wang et al. (2022) demonstrated that quercetin supplementation increased tibial phosphorus concentration, bone mineral density, tibial ash content, and calcium deposition. These findings suggest that phytobiotics may regulate calcium-phosphorus homeostasis through improvements in intestinal absorption, hormonal regulation, and bone remodelling.

Although evidence regarding bone responses to phytobiotics in Japanese quails remains limited, previous studies have suggested beneficial effects on bone strength and mineral metabolism (Masouri et al. 2022). Recent studies have also demonstrated that dietary calcium and available phosphorus are major determinants of bone mineralization during the growth period (Ahmadi et al. 2025; Vieira et al. 2025). These findings indicate that phytobiotics may represent a promising nutritional strategy for improving skeletal development in growing quails.

Beyond bone metabolism, phytobiotics have also been associated with improvements in antioxidant defense. Dietary supplementation with green tea catechins, thyme essential oil, oregano oil, and combinations of anise and grape seed oil has been reported to improve antioxidant status by increasing TAS, SOD, and GPx activities while reducing MDA concentrations in Japanese quails (Kara et al. 2016; Gumus et al. 2017; Mohamed et al. 2019; Elkomy et al. 2023). Although information regarding TOS and OSI responses in quails is still limited, available evidence suggests that phytobiotics may contribute to maintaining systemic oxidative balance under challenging environmental conditions (Sarica et al. 2017).

Therefore, the present study was designed to evaluate the effects of a phytobiotic blend containing capsaicin, glucosinolate, saponin, terpene, and curcumin on growth performance, carcass characteristics, tibia mineralization, and serum antioxidant status in growing Japanese quails. We hypothesized that dietary phytobiotic supplementation would safely promote bone mineral deposition without inducing a metabolic trade-off in growth performance, while maintaining the systemic redox homeostasis of quails under standard rearing conditions.

Materials and methods

Animal materials and diets

This experiment was conducted at the Quail Research Unit of the Prof. Dr. Orhan Duzgunes Livestock Research and Application Center, Department of Animal Science, Faculty of Agriculture, Selçuk University, Konya, Türkiye.

A total of 240 one-day-old mixed-sex Japanese quail (*Coturnix coturnix Japonica*) chicks were randomly allocated to four dietary treatments, each consisting of six replicate cages with 10 birds per replicate. Feed and water were provided *ad libitum* throughout the 35-day experimental period. Birds were maintained under a 23-h light:1-h dark lighting schedule.

The experimental treatments consisted of a basal diet (control) and the same diet supplemented with a commercial phytobiotic blend (Huverb®) at 500, 1000, or 1500 mg/kg. The phytobiotic product contained bioactive compounds derived from *Saponaria officinalis* (saponins), *Acorus calamus* (terpenes), *Capsicum annuum* (capsaicin), *Curcuma longa* (curcumin), and *Sinapis alba* (glucosinolates). To preserve product stability, the experimental diets were stored in sealed plastic containers under cool and dry conditions throughout the feeding trial. The ingredient composition and calculated nutrient content of the basal diet are presented in Table 1 and were formulated according to NRC (1994) recommendations for Japanese quails.

Table 1
Content of basal diet used in the trial and calculated nutrient composition

Ingredients	**%**
Corn	47.58
Soybean Meal (46.5% CP)	44.50
Vegetable oil (8800 kcal ME/kg)	4.70
Limestone	1.00
Dicalcium phosphate	1.30
Salt	0.30
Premix*	0.10
L-Lysine	0.10
DL-Methionine	0.17
L-Threonine	0.25
Total	**100.00**
Nutrient contents	
Metabolizable energy (kcal/kg)	2916
Crude protein (%)	24.02
Calcium (%)	0.86
Available phosphorus (%)	0.32
Lysine (%)	1.31
Methionine (%)	0.50
Methionine+cystine (%)	0.83
Threonine	1.02

*Premix is supplied at per kg of diet; Manganese: 80 mg, Iron: 60 mg, Copper: 5 mg, Iodine: 1 mg, Selenium: 0.15 mg, Vitamin A: 8.800 IU, Vitamin D3: 2.200 IU, Vitamin E: 11 mg, Nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, Riboflavin: 4.4 mg, Thiamine: 2.5 mg, Vitamin B12: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

Growth performance measurements

Body weight was recorded on a replicate basis at the beginning and end of the experiment, and body weight gain was calculated accordingly. Feed intake was determined by subtracting the residual feed from the amount offered. Feed consumption data were corrected for mortality when necessary. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gain (FI/BWG).

At 35 days of age, two healthy male quails were randomly selected from each replicate (n = 10 per treatment), weighed individually, and euthanized by cervical dislocation. Following defeathering and evisceration, carcass weights were recorded, and carcass yield was calculated as the percentage of carcass weight relative to pre-slaughter live body weight.

Determination of tibia characteristics

The left tibia from two randomly selected birds per replicate was collected for the determination of bone characteristics. After removal of adhering soft tissues, tibiae were stored at -20°C until analysis. Tibia breaking strength was determined using a three-point bending test with an Instron Universal Testing Machine (Ertest Laboratory Testing Equipment, Ankara, Türkiye), following the method described by Wilson and Ruszler (1996) and Gul et al. (2022).

For mineral analysis, tibiae were dried at 105°C for 24 h and ground into a fine powder. Approximately 0.20 g of each sample was digested using nitric acid, perchloric acid, and hydrogen peroxide in a MARS 5 microwave digestion system (CEM Corp., USA). Calcium and phosphorus concentrations were subsequently determined using inductively coupled plasma–atomic emission spectrometry (ICP-AES) according to Skujins (1998).

Serum antioxidant analyses

At the end of the experiment, blood samples were collected from two randomly selected male quails per replicate. Serum was obtained by centrifugation at 4.000 rpm for 10 min and stored at -20°C until analysis.

Serum total antioxidant status (TAS) and total oxidant status (TOS) were determined using commercial assay kits (Rel Assay Diagnostics, Türkiye) according to the method of Erel (2005). The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS after converting TAS values to μmol/L (Yumru et al. 2009).

Serum glutathione peroxidase (GPx) activity was determined according to Paglia and Valentine (1967), whereas superoxide dismutase (SOD) activity was measured following Prohaska et al. (1977) and Sun et al. (1988). Malondialdehyde (MDA) concentrations were determined using the thiobarbituric acid (TBA) assay based on absorbance at 532 nm.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) in SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). When significant treatment effects were detected, means were separated using Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$.

Results

Growth performance results are presented in Table 2. Dietary phytobiotic supplementation had no significant effect on final body weight, body weight gain, feed intake, feed conversion ratio, or carcass yield ($P > 0.05$).

Table 2
Effect of different levels of phytobiotic supplementation in the diet on performance parameters in growing quails

Parameters	**Dietary Supplemental Phytobiotic Levels (mg/kg)**				**SEM***	***P-Value***
	Control	**500**	**1000**	**1500**		
FBW, g/quail	193.06	192.56	194.48	193.22	1.409	*0.973*
BWG, g/quail	184.17	183.79	185.67	184.25	1.378	*0,971*
FI, g/quail/day	593.40	592.64	594.10	598.45	4.710	*0.977*
FCR, Feed intake/body weight gain	3.222	3.223	3.200	3.250	0.0124	*0.600*
Carcass yield, %	71.44	72.33	72.38	71.96	0.238	*0.501*

FBW: Final body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio *SEM: Standard error mean

The effects of dietary treatments on tibia characteristics are shown in Table 3. Tibia breaking strength was not significantly influenced by phytobiotic supplementation ($P > 0.05$). In contrast, tibia calcium and phosphorus concentrations were significantly higher in phytobiotic-supplemented groups than in the control group ($P < 0.05$). The greatest increase in tibia phosphorus concentration was observed in birds receiving 500 mg/kg phytobiotic supplementation.

Table 3
Effect of different levels of phytobiotic supplementation in the diet on tibia characteristics in growing quails

Parameters	Dietary Supplemental Phytobiotic Levels (mg/kg)				SEM*	*P-Value*
	Control	500	1000	1500		
Tibia breaking strength, N	35.63	36.30	35.95	35.37	0.626	*0.964*
Tibia Ca,%	14.85[b]	18.27[a]	18.53[a]	17.91[a]	0.443	*0.003*
Tibia P, %	7.10[c]	8.60[a]	7.97[b]	7.81[b]	0.144	*< 0.0001*

Ca: Calcium, P: Phosphorus, *SEM: Standard error mean

[a,b,c]The differences indicated by different letters on the same row are statistically significant (*P < 0.05*)

Serum antioxidant responses are summarized in Table 4. No significant differences were observed among treatments for TAS, TOS, OSI, SOD, GPx, or MDA concentrations (*P > 0.05*).

Table 4
Effect of different levels of phytobiotic supplementation in the diet on serum antioxidant enzyme levels in growing quails

Parameters	Dietary Supplemental Phytobiotic Levels (mg/kg)				SEM*	*P-Value*
	Control	500	1000	1500		
TAS, mmol/L	1.67	1.58	1.60	1.52	0.0354	*0.539*
TOS, µmol/L	11.03	10.40	10.31	10.06	0.5347	*0.940*
OSI	0.663	0.676	0.645	0.666	0.0329	*0.991*
SOD, U/ml	99.82	93.05	91.12	96.63	2.4879	*0.643*
GPx, U/L	484.50	474.33	468.67	466.67	12.286	*0.963*
MDA, nmol/L	54.69	52.38	46.91	47.54	4.3942	*0.919*

TAS: Total antioxidant status TOS: Total oxidant status, OSI: Oxidative stress index, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, MDA: Malondialdehyde *SEM: Standard error mean

Discussion

Performance

In this study, the absence of a significant effect of the phytobiotic mixture containing capsaicin, glucosinolate, saponin, terpene, and curcumin on end-of-trial final body weight, body weight gain, feed

intake, and feed conversion ratio in growing quails indicates that phytobiotic effects on growth performance are conditional and do not always yield a positive response. These results align with previous studies reporting limited or insignificant effects of essential oils and plant extracts on poultry performance (Jang et al. 2007; Amad et al. 2011; Kurekci et al. 2020; Gul and Cufadar, 2023).

The effects of phytobiotics on performance are largely explained by stimulating digestive enzymes, modulating the intestinal microbiota, increasing antioxidant capacity, and improving nutrient digestibility (Windisch et al. 2008). However, the extent to which these mechanisms translate into performance gains is largely determined by the animal's current physiological state and rearing conditions. It is thought that the additional benefits of phytobiotic additives may be limited, especially in herds raised in optimal environmental conditions and without health problems, since digestive and metabolic processes are already operating at high efficiency. This may be the likely reason for the absence of differences in performance parameters in the current study (Zeng et al. 2015; Kurekci et al. 2020; Flees et al. 2021; Wu et al. 2022). Although phytobiotic supplementation did not yield a statistically significant improvement in macroscopic growth indices, the preservation of optimal feed efficiency suggests no metabolic trade-off occurred during the enhanced deposition of tibia minerals.

Dose-dependent responses should also be considered when interpreting the present findings. Phytogenic compounds frequently exhibit nonlinear biological responses, in which increasing the inclusion level does not necessarily enhance efficacy. Such responses may arise from reduced palatability, saturation of biological targets, limited absorption of active compounds, or antagonistic interactions among phytochemical constituents. Therefore, the absence of a performance response across the supplementation levels evaluated in this study does not necessarily indicate a lack of biological activity but rather suggests that the tested doses were insufficient to induce measurable improvements in productive performance under the prevailing experimental conditions (Biricik et al. 2012; Gurbuz and Ismael, 2016). No change in carcass yield was observed, suggesting that phytobiotics do not exert a direct anabolic effect on muscle tissue development. Similarly, Reda et al. (2020a) and Mohamed et al. (2024) reported that various phytobiotic applications had limited effects on carcass characteristics. These results support the view that the main mechanism of action of phytobiotics is to regulate digestive and metabolic processes rather than directly stimulate tissue growth.

Tibia characteristics

In this study, adding a phytobiotic mixture containing capsaicin, glucosin, saponin, terpene, and curcumin to quail diets significantly increased tibia calcium and phosphorus levels but did not significantly affect tibia breaking strength. This suggests that bone endurance depends not only on mineral deposition but also on the organization and structural integrity of the bone matrix (Hafeez et al. 2021). Despite the improvement in tibial mineral composition, no corresponding increase in breaking strength was detected. This finding suggests that the additional minerals deposited in the tibia were insufficient to induce measurable changes in bone mechanical properties within the 35-day experimental period. Bone mechanical strength reflects not only mineral quantity but also the structural organization and maturation of the bone matrix, processes that generally require longer periods than mineral deposition

alone. Moreover, variables such as cortical thickness, trabecular architecture, collagen cross-linking, and bone turnover were not evaluated in the present study and may have contributed to the absence of detectable changes in bone strength. Consequently, caution should be exercised when interpreting bone mineralization and bone mechanical properties as interchangeable indicators of skeletal quality. The dissociation between increased mineral density (Ca and P) and unaltered biomechanical resistance points toward a requirement for concurrent organic matrix (e.g., type I collagen) reinforcement, which warrants further histomorphometric validation (Knott and Bailey 1999; Rath et al. 2000). In fact, tibia calcium increased at 500, 1000, and 1500 mg/kg; the increase in phosphorus at 500 and 1000 mg/kg may be related to the synergistic effects of the bioactive components in the mixture on digestion and absorption. Capsaicin's stimulation of pancreatic enzyme activity and bile acid synthesis may enhance mineral utilization by supporting nutrient digestion and absorption (Adegoke et al. 2018). Similarly, phytobiotic additives modulate the intestinal lumen pH, and capsaicin maintains its effectiveness under low pH conditions, which can facilitate calcium and phosphorus absorption (Jamroz et al. 2006). Polyphenolic compounds in the mixture, such as curcumin, may promote calcium absorption and increase the synthesis of calcium-binding proteins, thereby supporting mineral deposition in bone tissue (Wang et al. 2022). Furthermore, herbal feed additives have been reported to increase villus size and width by improving intestinal morphology, thereby expanding the absorptive surface area (Alam et al. 2020). In this context, the increase in small-intestinal villus height by terpenes and saponins may be a complementary mechanism that facilitates the transfer of minerals from the diet to the bone (Sultan et al. 2024). An additional observation deserving attention is the dose-dependent pattern of tibial phosphorus concentration. Although phosphorus content increased significantly at 500 and 1000 mg/kg supplementation, the response tended to decline at 1500 mg/kg. This pattern may indicate that increasing phytobiotic inclusion beyond a certain threshold does not proportionally enhance mineral utilization. Similar nonlinear responses have frequently been described for phytogenic feed additives and may reflect saturation of physiological transport mechanisms, reduced bioavailability of active compounds, or antagonistic interactions among phytochemical constituents. Such findings are consistent with the concept of hormesis, whereby moderate inclusion levels elicit maximal biological responses while excessive supplementation may diminish efficacy (Nwaopara et al. 2007). Thus, the present findings reveal that the phytobiotic mixture can promote bone mineralization, but this effect may not be reflected in bone mechanical strength to the same extent.

Serum antioxidant parameters

In the present study, adding a phytobiotic mixture containing capsaicin, glucosinolate, saponin, terpene, and curcumin to growing quail diets did not significantly affect serum TAS, TOS, OSI, GPx, MDA, and SOD. However, curcumin, capsaicin, and terpenes in this mixture are known as powerful natural antioxidants; in addition, various studies report that phenolic compounds such as curcumin and paprika extract can suppress lipid peroxidation and modulate antioxidant system components such as SOD and GPx (Gumus et al. 2017; Reda et al. 2020b; Liu et al. 2021; Safavipour et al. 2022). Chodkowska et al. (2022) found that a phytobiotic mixture containing similar bioactive substances did not affect serum and breast muscle prooxidant-antioxidant balance (PAB) and lipid peroxidation (MDA/TBARS) indicators in broiler

chickens and correlated these effects with the dose level and duration of administration. The absence of significant changes in TAS, TOS, OSI, GPx, and especially MDA levels in the current trial may reflect the animals maintaining basal oxidative balance under standard rearing conditions. The absence of modulation in the measured antioxidant cascade (TAS, TOS, GPx, SOD, MDA) indicates that the applied phytobiotic dosages do not perturb the basal redox homeostasis in the absence of an exogenous oxidative challenge. In fact, basal enzymatic defense mechanisms function adequately in the absence of stress, and lipid peroxidation is kept in balance (Sahin et al. 2008; Rocha et al. 2020). Dalkilic et al. (2017) reported that saponite-rich soapwort extract did not significantly affect serum TAS, SOD, and GSH activities in heart and liver tissues in Japanese quails exposed to cold stress. Similarly, in the current study, the serum SOD level did not change, indicating that there was no weakening of the antioxidant defense system; stable MDA levels suggest that oxidative damage does not occur (Pruchniak et al. 2015; Zhang et al. 2017; Escorcia et al. 2020; Barbosa et al. 2010). In addition, Gul and Cufadar (2023) reported that the effects of phytobiotics on the antioxidant system may vary depending on the dose. They found that adding 300 mg/kg of red pepper oil increased the TAS parameter in growing quails, whereas a higher dose of 1200 mg/kg significantly decreased it, and that the addition did not statistically affect TOS concentration or OSI values.

An additional consideration is the possibility that the principal biological action of the phytobiotic blend evaluated in the present study was directed toward improving mineral metabolism rather than enhancing systemic antioxidant capacity. This interpretation is supported by the significant increase observed in tibial calcium and phosphorus concentrations despite the absence of measurable changes in oxidative stress biomarkers. Collectively, these findings suggest that the physiological responses to phytobiotics may be tissue-specific and that improvements in bone mineralization can occur independently of detectable alterations in circulating antioxidant status (Chodkowska et al. 2022).

Conclusions

This study demonstrated that dietary supplementation with a phytobiotic blend did not disrupt the basal redox homeostasis or macroscopic growth parameters of growing Japanese quails under standard conditions. Concurrently, the supplementation significantly up-regulated tibia calcium and phosphorus deposition.

These findings suggest that the benefits of phytobiotics should not be assessed solely on the basis of productive performance but should also include physiological responses related to skeletal development and mineral metabolism. Among the evaluated supplementation levels, 500 mg/kg appeared to provide the most favorable response for tibia mineralization without compromising growth performance.

Since the enhanced inorganic mineral deposition did not translate into greater biomechanical strength, future studies integrating bone histomorphometry and type I collagen cross-linking analyses are warranted. Such approaches will clarify the uncoupled dynamics between mineral accretion and organic

matrix assembly, ultimately establishing the optimal inclusion strategies for phytobiotics in quail nutrition.

Declarations

Author contributions

Barışcan Curabay: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Yusuf Cufadar: Writing – review & editing, Visualization, Validation, Project administration, Software, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

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Data availability

The authors declare that all data and materials used in this study comply with field standards and are available upon request.

Ethical note

The animal experiment was conducted according to the guidelines of the local ethics committee of Selcuk University, which were prepared following the “Directive 2010/63/EU is the European (EU) legislation”. All procedures in this study complied with the ethical principles of animal welfare. The study was also conducted in line with the ethical standards outlined in the Declaration of Helsinki and its subsequent revisions.

Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve language clarity and to assist in rephrasing sections of the manuscript. After using this tool, the authors carefully reviewed, edited, and verified all content to ensure accuracy and integrity. The authors take full responsibility for the content of the publication.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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