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Short communication: dietary oregano essential oil supplementation promotes tibial bone mass and size but not density or strength in growing lambs

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Bone development is essential for the growth, welfare, and long-term productivity of growing lambs. This study aimed to assess the effects of dietary oregano essential oil (OEO) supplementation on tibial morphology, mineralization, and mechanical properties in lambs. Twelve healthy, 3-month-old male lambs (Small-tailed Han × Black-boned cross, average liveweight 30.8 ± 0.4 kg) were randomly assigned to either a control group or a group receiving OEO at 0.5 g/kg of feed for 76 days. Tibiae were collected post-slaughter for morphometric and mechanical analysis. Tibiae were assessed for weight, length, bone mineral content (BMC), bone mineral density (BMD), bone area, bone volume, and breaking strength. Data were analyzed using independent sample *t*-tests, with significance set at $P < 0.05$. OEO supplementation significantly increased tibia weight (14.4%), BMC (19.7%), bone area (12.2%), and bone volume (19.6%) ($P < 0.05$). Tibia length, BMD, and breaking strength showed numerical increases but were not statistically significant. These findings suggest that bone mass and size may be more responsive to short-term dietary intervention than density or mechanical strength. This study provides original evidence that short-term OEO supplementation in lambs primarily promotes bone mass accretion and structural expansion rather than immediate improvements in density or mechanical strength. These findings expand the understanding of plant-derived feed additives in small ruminant bone health and suggest a potential strategy to enhance skeletal growth, warranting further research on long-term functional outcomes.

KEYWORDS

ovine, oregano, essential oil, bone, tibia, phytogetic feed additive, lamb

1 Introduction

Efficient skeletal development in growing lambs is essential for animal welfare, structural soundness, and productivity, particularly near marketing weight (Popkin et al., 2012). Healthy bone structure reduces the risk of locomotor disorders and improves carcass yield (Prache et al., 2022). Modern nutritional strategies increasingly emphasize not only adequate minerals and protein supply but also the inclusion of bioactive natural compounds that may influence bone metabolism while supporting overall health (Miranda et al., 2019).

Among these compounds, oregano essential oil (OEO), derived from *Origanum vulgare* L., has gained attention in animal nutrition because of its phenolic monoterpenes, particularly carvacrol and thymol, which account for most of its biological activity (Zhou et al., 2019). These compounds exhibit antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties, making them attractive natural alternatives to synthetic feed additives (Caroprese et al., 2023).

Both carvacrol and thymol have been linked to bone metabolism. *In vitro* studies have indicated that carvacrol inhibits osteoclastogenesis by reducing tartrate-resistant acid phosphatase activity and RANKL-induced osteoclast formation via NF- κ B, MAPK, and Akt pathways (Janani et al., 2021). Thymol similarly attenuates inflammatory bone loss in murine models by suppressing osteoclast differentiation and pro-inflammatory cytokines (Elbahnasawy et al., 2019).

Beyond direct bone effects, OEO modulates the rumen and gut microbiota, enhances nutrient absorption, and reduces systemic inflammation (Zhang et al., 2021). These factors might indirectly support skeletal development. Dietary supplementation in ruminants increases beneficial cellulolytic bacteria while reducing protozoa, improving fermentative efficiency (Zhou et al., 2019). Meta-analyses also report improved intestinal villus height, barrier function, and reduced pro-inflammatory cytokine expression, which collectively enhance nutrient utilization and immune resilience (Jia et al., 2022).

In calves and lambs, OEO supplementation has been associated with improved growth performance, increased antibody titers, and enhanced feed conversion efficiency, which was attributed to its immune-supportive and digestive-modulating effects (Luo et al., 2024). While these studies robustly support OEO's systemic benefits, there remains a gap concerning direct bone-related outcomes in young ruminants, particularly regarding tibia mineralization, structural morphology, and mechanical strength.

We hypothesized that dietary OEO supplementation could enhance tibia mass and structural expansion in growing lambs, which may precede measurable improvements in bone density and mechanical strength. Thus, the objectives of this study were 1) to determine whether OEO supplementation results in significant increases in tibia mass, mineral content, and structural parameters in lambs, and 2) to evaluate whether such increases coincide with improvements in tibia mechanical strength and mineral density, which reflect functional bone quality. This pilot study provides data on its effects on tibia growth and quality,

forming a basis for future investigations on dose-response and long-term impacts.

2 Materials and methods

2.1 Animals, experimental design, and diets

The study was conducted at Dongming Shangbang Livestock Farming Cooperative, Heze City, Shandong Province, China, as part of a larger project. All experimental procedures were reviewed and approved by the Animal Ethics and Welfare Committee of Jilin Agricultural Science and Technology College (Approval No. 2023001).

Twelve crossbred male lambs (Small-tailed Han $\times$ Black-boned sheep), aged 3 months and averaging 30.8 ± 0.4 kg, were randomly assigned to two dietary groups ($n = 6$ per group): Control, basal total mixed ration (TMR); OEO group, basal TMR supplemented with 0.5 g/kg OEO additive. The additive, containing 3.6% carvacrol and 0.13% thymol, was sourced from Guangzhou Meritech Bioengineering Technology Corporation (Guangzhou, Guangdong Province, China). Diets were formulated according to the Feeding Standards of Meat-producing Sheep and Goats (Ministry of Agriculture of China, 2005) and processed into pellets. The diets were composed of (as fed) 27% corn, 15% soybean meal, 30% peanut vine, 14.4% wheat middlings, 10% *Leymus chinensis* hay, 2% wheat bran, 0.44% calcium hydrogen phosphate, 0.4% limestone, 0.4% sodium chloride, and 0.36% premix; and contained (as % of dry matter) 12.3% ash, 15.1% crude protein, 3.0% ether extract, 37.3% neutral detergent fiber, and 18.3% acid detergent fiber.

All lambs were housed indoors in pens with slatted floors, with free access to water. Ambient temperature and ventilation were maintained in accordance with welfare standards. The animals had ad libitum access to feed, which was provided twice daily at 06:30 and 18:00.

The trial lasted for 92 days, including a 16-day adaptation and a 76-day feeding trial period, and was conducted in spring. At the end of the trial, all lambs were slaughtered.

2.2 Sample collection and measurements

Lambs were fasted for 16 h, deprived of water for 2 h, and humanely slaughtered by exsanguination via the jugular vein. The right tibiae were excised immediately after slaughter, and all adhering muscles, fat, and connective tissues were carefully removed. Each bone was rinsed and wrapped with gauze soaked in normal saline to remove residual blood and then briefly dried with absorbent paper. The cleaned, fresh tibiae were weighed using an analytical balance, and the wet weight was recorded. The length of each tibia was measured by placing the bone on a flat surface and determining the distance from the proximal end of the tibial plateau to the distal end of the medial malleolus using a flexible ruler with millimeter accuracy.

Bone mineral density (BMD) and bone mineral content (BMC) were determined using a Dual-energy X-ray Absorptiometry (DXA)

bone densitometer (InAlyzer, Seoul, South Korea). Prior to scanning, the cleaned tibiae were wrapped in saline-soaked gauze to maintain moisture. Each bone was positioned on the DXA scanner bed with the medial side facing upward, and scanning was performed using the small animal mode. The DXA software automatically calculated the BMD (g/cm^2) and BMC (g) for each tibia.

Bone area and bone volume were evaluated using micro-computed tomography (micro-CT; SkyScan 1176, Bruker, Kontich, Belgium). The cleaned tibiae were wrapped in parafilm to prevent moisture loss and placed in the scanner tube. Scanning was performed at a voxel resolution of 10–20 μm , and three-dimensional reconstruction and analysis were conducted using CTAn software (version 1.17.7.2, Bruker, Kontich, Belgium) to calculate bone cross-sectional area (cm^2) and total bone volume (cm^3).

Tibia strength was measured using an electronic universal testing machine (Jinan Testing Group Co., Ltd., Jinan, China) following a three-point bending protocol. Fresh tibiae were positioned horizontally on two supports with a span equivalent to 40%–50% of the tibia length. A downward force was applied to the midpoint of the bone shaft at a constant loading rate of 2 mm/min until fracture occurred. The maximum load (kN) recorded at the point of failure was defined as the breaking strength of the tibia.

2.3 Statistical analysis

Data were analyzed using Student's *t*-test (SPSS 26.0; IBM Corp., Armonk, NY, USA). Normality and homogeneity of variance were confirmed by Shapiro–Wilk and Levene's tests. Results are expressed as mean $\pm$ SEM, and differences were considered significant at $P < 0.05$.

3 Results

Dietary supplementation with OEO was associated with improvements in several tibial characteristics in growing lambs compared with the control group (Table 1). Tibia weight increased significantly from 118.97 g to 136.13 g ($P = 0.034$), and BMC rose from 11.28 g to 13.50 g ($P = 0.010$). Similarly, bone area and bone volume were significantly enhanced by OEO supplementation, increasing by 12.2% ($P = 0.023$) and 19.6% ($P = 0.010$), respectively. In contrast, tibial length, bone mineral density (BMD), and tibial strength increased numerically with OEO supplementation but did not reach statistical significance ($P > 0.05$).

4 Discussion

The present study examined the impact of dietary OEO on tibia development and mechanical properties in growing lambs. The findings indicate that short-term supplementation of OEO for 76 days primarily stimulated bone mass accretion and structural

TABLE 1 Effects of 76 days of dietary oregano essential oil supplementation on tibial characteristics in growing lambs ($n = 6$ per treatment).

Item	CON ¹	OEO	SEM	Difference	<i>P</i> -value
Tibia weight (g)	118.97	136.13	4.216	14.4%	0.034
Tibia length (cm)	21.85	22.46	0.283	2.8%	0.301
Bone mineral density (BMD, g/cm^2)	0.21	0.23	0.006	9.5%	0.276
Bone mineral content (BMC, g)	11.28	13.50	0.472	19.7%	0.010
Bone area (cm^2)	52.90	59.33	1.499	12.2%	0.023
Bone volume (cm^3)	6.82	8.16	0.286	19.6%	0.010
Tibia strength (kN)	2.99	3.19	0.092	6.7%	0.293

¹ CON, no supplement; OEO, oregano essential oil additive supplemented at 0.5 g/kg of feed.

expansion, whereas changes in density and mechanical strength were not significant. This pattern suggests that OEO may initially influence bone modelling and mineral deposition, although longer-term studies are needed to determine whether functional properties are subsequently affected.

The observed increases in tibia weight, bone mineral content, bone area, and bone volume indicate that OEO promotes bone growth and mineral accretion. OEO is rich in phenolic monoterpenes, particularly carvacrol and thymol, which exhibit antimicrobial, anti-inflammatory, and antioxidant properties (Botsoglou et al., 2003). These compounds have been shown to influence bone metabolism directly. For instance, carvacrol was found to inhibit osteoclastogenesis by suppressing the NF- κ B pathway and inducing apoptosis in mature osteoclasts, thereby reducing bone resorption (Deepak et al., 2016). Similarly, thymol attenuates inflammatory bone loss by suppressing osteoclast differentiation and pro-inflammatory cytokines in murine models (Elbahnasawy et al., 2019). These mechanisms may have contributed to the increased bone mass observed in our study, as reduced bone resorption allows for greater retention of bone tissue. In addition to direct effects on bone cells, OEO may enhance bone health indirectly through its impact on gut health and nutrient absorption. OEO supplementation modulates the rumen and gut microbiota, increasing beneficial cellulolytic bacteria and improving fermentative efficiency, which enhances nutrient utilization in ruminants (Zou et al., 2016). A meta-analysis showed that OEO, particularly in microencapsulated forms, improves intestinal villus height, barrier integrity, and reduces pro-inflammatory cytokine expression in sheep (Andri et al., 2020). Enhanced absorption of key minerals such as calcium and phosphorus could support bone mineral accretion, contributing to the significant increases in bone mineral content and bone volume observed in our study.

Despite the significant improvements in bone mass parameters, bone mineral density and breaking strength did not significantly change. Bone mineral density, defined as the amount of mineral per unit area (g/cm^2), reflects the concentration of mineral within the

bone. The lack of significant change in bone mineral density suggests that the increase in bone size (area and volume) was proportional to the increase in mineral content, resulting in a similar density. This is consistent with the findings of Zhang et al. (2022), who reported that dietary supplementation with carvacrol and thymol in laying hens improved tibia mechanical properties without significantly altering bone ash content, indicating that structural modelling, rather than mineral density, was primarily affected. Breaking strength, a measure of the bone's mechanical integrity, depends on both the quantity and quality of bone tissue, including its mineral content, collagen matrix, and microarchitecture. The lack of a significant increase in breaking strength in our study may be attributed to the short duration of supplementation (76 days), which may not have been sufficient to induce measurable changes in bone quality. This is supported by studies suggesting that improvements in bone strength often require longer-term interventions to allow for remodeling processes (Turner, 2002). Additionally, the specific dose of OEO (0.5 g/kg) used in our study may not have been optimal for enhancing mechanical properties, as higher doses in other studies have shown varied effects. For example, Canbolat et al. (2018) reported that higher concentrations of OEO (up to 1200 mg/kg) in lambs negatively affected growth performance, possibly due to a depressed rumen fermentation, highlighting the importance of dose optimization.

Our findings are consistent with previous research demonstrating the positive effects of OEO and its components on bone health in various animal models. In growing rabbits, dietary supplementation with 0.2% oregano extract increased bone weight, particularly in the femur, compared to vitamin E supplementation (Cardinali et al., 2015). Similarly, Zhang et al. (2022) found that a combination of carvacrol or thymol with cinnamaldehyde improved tibia mechanical properties in post-peak laying hens, associated with reduced inflammation-mediated bone resorption. In rats with low calcium intake, thyme supplementation (containing thymol) ameliorated osteoporosis by modulating bone mineral content and histomorphometric parameters (Elbahnasawy et al., 2019). Collectively, these studies suggest that OEO and its bioactive components have broad potential to enhance skeletal development across species, likely by reducing bone resorption and improving nutrient absorption. The anti-inflammatory properties of OEO may further contribute to its bone-protective effects. Chronic inflammation is known to stimulate osteoclast activity and inhibit osteoblast function, leading to bone loss (Hotamisligil, 2006). By suppressing lipopolysaccharide-induced activation of NF- κ B and reducing pro-inflammatory cytokines in macrophages, OEO may create a more favorable environment for bone formation (Cheng et al., 2018). Although inflammatory markers were not measured in our study, the established anti-inflammatory effects of OEO suggest that this mechanism could play a role in the observed outcomes.

The increases in bone mass and structural parameters observed in this pilot study indicate the potential of OEO as a natural feed additive in small ruminant production systems. Enhanced skeletal development can improve animal welfare by reducing the risk of locomotor disorders and increasing mechanical soundness, which is

critical for lambs approaching marketing weight (Suttle, 2010). Moreover, the use of OEO aligns with the growing demand for antibiotic-free and sustainable nutritional strategies in livestock production. Unlike synthetic additives, OEO offers a residue-free alternative with fewer side effects, making it an attractive option for improving productivity while maintaining consumer safety (Hao et al., 2014).

Several limitations should be considered when interpreting our results. The small sample size ($n = 6$ per group) may have limited the statistical power to detect significant differences in bone mineral density and breaking strength. In addition, the study was conducted on a crossbreed (Small-tailed Han $\times$ Black-boned sheep), and the findings may not be generalizable to other breeds or production systems. The 76-day feeding period, while sufficient to observe changes in bone mass, may not have been long enough to detect improvements in bone quality, which involves slower remodeling processes. Future research should focus on larger sample sizes and extended supplementation periods to determine whether initial gains in bone mass translate into improvements in bone density and mechanical strength. Dose-response studies are also warranted to identify the optimal OEO concentration for maximizing bone health benefits without negatively affecting growth performance, as observed in some studies with higher doses (Canbolat et al., 2018). Furthermore, investigating the molecular mechanisms underlying OEO's effects on bone metabolism, such as changes in bone turnover markers or gene expression in osteoblasts and osteoclasts, could provide deeper insights into its mode of action. Exploring the synergistic effects of OEO with other phytochemical additives, as demonstrated in hens (Zhang et al., 2022), could also enhance its efficacy in ruminants.

In conclusion, our study provides preliminary evidence that dietary OEO supplementation at 0.5 g/kg was associated with greater tibia weight, bone mineral content, bone area, and bone volume in growing lambs, likely through direct effects on bone cells and indirect effects via improved gut health and reduced inflammation. These findings support the potential of OEO as a natural, sustainable feed additive that may improve skeletal health in small ruminants, contributing to enhanced animal welfare and productivity. Further research is needed to optimize dosing regimens and elucidate the long-term effects and mechanisms of OEO on bone development in ruminant production systems.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Animal Ethics and Welfare Committee of Jilin Agricultural Science and Technology College.

The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XW: Writing – review & editing, Software, Investigation, Validation, Data curation, Formal Analysis. GZ: Methodology, Resources, Writing – review & editing, Conceptualization, Project administration, Supervision. AJ: Visualization, Writing – review & editing. XS: Project administration, Supervision, Formal Analysis, Visualization, Funding acquisition, Conceptualization, Writing – review & editing, Writing – original draft, Resources.

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