ORIGINAL RESEARCH ARTICLE

Effects of probiotic modulation on bone health in estrogen-deficient female Sprague–Dawley rats

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Abstract

Postmenopausal osteoporosis is a common metabolic disorder caused mainly by estrogen deficiency, which accelerates bone resorption, suppresses bone formation, and increases fracture risk in aging women. Emerging evidence suggests that the gut microbiota influences skeletal homeostasis through the gut–bone axis, indicating that probiotic supplementation may provide a novel nutritional strategy for bone protection. This study evaluated the osteoprotective effects of two probiotic strains, *Bifidobacterium breve* i1088 and *Bifidobacterium longum* subsp. longum i772, in an ovariectomized Sprague–Dawley rat model of estrogen-deficiency–induced osteoporosis. Female rats were randomly allocated to sham-operated control, ovariectomized model, probiotic treatment, and positive control groups. After intervention, bone mineral density, femoral calcium content, and trabecular microarchitecture were assessed. Compared with untreated ovariectomized rats, probiotic supplementation significantly increased bone mineral density and bone calcium content, improved trabecular thickness, and reduced trabecular separation ($P < 0.01$). These findings demonstrated that probiotic administration attenuated bone loss and preserved skeletal microstructure under estrogen-deficient conditions. The results provide preclinical evidence that *Bifidobacterium*-based probiotic formulations may serve as a promising adjunctive nutritional approach for maintaining bone health in postmenopausal women. Further clinical studies are required to confirm their preventive and therapeutic potential in postmenopausal osteoporosis. *(Afr J Reprod Health 2026; 30 [11]: 127-139).*

Keywords: Postmenopausal women; Probiotics; *Bifidobacterium* breve; *Bifidobacterium* longum; Estrogen deficiency; Osteoporosis

Résumé

L'ostéoporose postménopausique est un trouble métabolique fréquent principalement causé par une carence en œstrogènes, qui accélère la résorption osseuse, inhibe la formation osseuse et augmente le risque de fractures chez les femmes âgées. Des données récentes suggèrent que le microbiote intestinal influence l'homéostasie osseuse par l'intermédiaire de l'axe intestin–os, indiquant qu'une supplémentation en probiotiques pourrait constituer une nouvelle stratégie nutritionnelle de protection osseuse. Cette étude a évalué les effets ostéoprotecteurs de deux souches probiotiques, Bifidobacterium breve i1088 et Bifidobacterium longum subsp. longum i772, dans un modèle de rates Sprague–Dawley rat ovariectomisées reproduisant l'ostéoporose induite par la carence œstrogénique. Les animaux ont été répartis aléatoirement en groupes témoin simulé, modèle ovariectomisé, traitement probiotique et contrôle positif. Après l'intervention, la densité minérale osseuse, la teneur en calcium fémoral et la microarchitecture trabéculaire ont été évaluées. Comparativement aux rates ovariectomisées non traitées, la supplémentation probiotique a significativement augmenté la densité minérale osseuse et la teneur en calcium osseux, amélioré l'épaisseur trabéculaire et réduit la séparation trabéculaire ($P < 0,01$). Ces résultats montrent que l'administration de probiotiques atténue la perte osseuse et préserve la microarchitecture squelettique en condition de carence œstrogénique. Cette étude fournit des preuves précliniques suggérant que des formulations probiotiques à base de Bifidobacterium pourraient représenter une approche nutritionnelle adjuvante prometteuse pour maintenir la santé osseuse chez les femmes ménopausées. Des études cliniques supplémentaires sont nécessaires pour confirmer leur potentiel préventif et thérapeutique dans l'ostéoporose postménopausique. *(Afr J Reprod Health 2026; 30 [11]: 127-139).*

Mots-clés Femmes postménopausées ; Probiotiques ; Bifidobacterium breve ; Bifidobacterium longum ; Carence en œstrogènes ; Ostéoporose

Introduction

Osteoporosis (OP) is a systemic skeletal disorder characterized by decreased bone mass, deterioration of bone microarchitecture, increased bone fragility, and an elevated risk of fractures.[1] Clinically, it is commonly observed in postmenopausal women and older adults.[2] Global epidemiological surveys indicate that more than 200 million individuals are affected by OP, among whom postmenopausal osteoporosis (PMOP) accounts for a substantial proportion. The pathogenesis of PMOP primarily involves estrogen deficiency, which enhances osteoclast activity, increases bone resorption, and reduces bone formation, ultimately resulting in bone loss and diminished bone strength.[3,4] Further data show that the global prevalence of OP is approximately 23.1% in women, markedly higher than 11.7% in men. With accelerating population aging, the number of patients with OP in the European Union is projected to increase from 27.5 million in 2010 to 33.9 million by 2025.

By 2050, more than 50% of osteoporotic fractures worldwide are expected to occur in Asia, highlighting the rapid growth of this disease burden in developing countries. Moreover, the total number of incident OP cases worldwide is estimated to reach 263.2 million during 2030–2034, with women constituting the majority (approximately 154.4 million). This trend not only increases healthcare costs but also compromises patients' quality of life and intensifies socioeconomic pressure.[5] Conventional treatments mainly include hormone replacement therapy and bisphosphonates. For example, alendronate sodium can inhibit osteoclast activity and increase bone mineral density; however, long-term administration is often associated with adverse effects such as gastrointestinal discomfort, increased tumor risk, and osteonecrosis of the jaw, which limits its broader clinical use.[6] Therefore, identifying safe and effective intervention strategies has become a key research focus.

In recent years, the "gut–bone axis" concept has revealed the pivotal role of the gut microbiota in the regulation of bone metabolism.[7] Gut dysbiosis may indirectly modulate bone homeostasis by influencing immune responses, nutrient absorption, and the production of microbial metabolites. Studies have shown that patients with OP exhibit reduced gut microbial diversity, accompanied by decreases in beneficial genera such as *Lactobacillus* and *Bifidobacterium*, and increases in potentially harmful genera such as *Serratia*.[8] This imbalance may exacerbate bone loss by promoting pro-inflammatory cytokine release, disrupting calcium and vitamin D metabolism, or altering endocrine signaling. Probiotics, as live microorganisms capable of modulating the gut microecology, have demonstrated bone-protective potential in OP. Among them, members of the genus *Bifidobacterium* are major beneficial commensals in the intestine that contribute to host health and participate in the regulation of multiple physiological and pathological processes.[9] For instance, *Bifidobacterium animalis* LPL-RH significantly increased bone mineral density and improved bone biomechanical properties in ovariectomized rat models by modulating the OPG/RANK/RANKL pathway, upregulating osteoprotegerin (OPG) expression and downregulating receptor activator of nuclear factor-κB ligand (RANKL).[10] Following intervention with calcium-fortified fresh milk in ovariectomized rats, bone metabolic disturbances were markedly alleviated, as evidenced by increased bone mineral density and bone mineral content, reduced osteoclast-related markers, and elevated osteoblast-related markers, thereby delaying bone loss. These effects may be attributable to improved calcium and vitamin D nutritional status and restoration of bone remodeling balance, potentially involving regulation of bone metabolism–related signaling pathways such as RANKL/RANK/OPG.[11] In addition, *Bifidobacterium bifidum* TMC3115 ameliorated glucocorticoid-induced OP by inhibiting the oxidative stress–JAK2/STAT3 pathway and reducing the protein expression of p22, gp91, p-Syk, p-Src, p-JAK2, and p-STAT3.[12] Ferroptosis, a recently recognized form of regulated cell death, can exert bone-protective effects by modulating the expression of ferroptosis-related molecules, including FTH1, SLC7A11, and GPX4.[13]

Clinical evidence further suggests that probiotic combinations can reduce bone resorption markers in postmenopausal women.[14,15] Although

fecal microbiota transplantation (FMT) can reconstruct the gut microbial structure, its impact on host immune homeostasis may be bidirectional. Studies indicate that gut microbiota remodeling can enhance T helper 17 (Th17) cell responses, and Th17-associated inflammation has been shown to mediate bone loss by promoting osteoclast activation. Therefore, under certain pathological conditions, FMT may carry a potential risk of inducing Th17-related bone loss.[16] The mechanisms by which lactic acid bacteria facilitate calcium absorption involve lowering intestinal pH, activating calcium ion channels, and promoting short-chain fatty acid production.[17] The dual roles of the gut microbiota in OP suggest that targeted modulation of beneficial bacteria may represent a novel therapeutic strategy. Probiotic supplementation has been reported to significantly increase bone mineral density in women with PMOP, with a more pronounced effect during the osteopenic stage. Daily supplementation for 24 weeks to 12 months can reduce the bone turnover marker C-terminal telopeptide of type I collagen (CTX), increase serum 25-hydroxyvitamin D levels, promote calcium absorption, and reduce bone loss.[18] Collectively, these findings indicate that probiotic interventions targeting the gut–bone axis are characterized by low risk and high adherence and may serve as adjunctive therapies for PMOP.[12]

In our previous work, two novel probiotic strains were isolated and identified from healthy infant feces: *Bifidobacterium breve* i1088 and *Bifidobacterium longum* subsp. *longum* i772. Based on morphological characterization, API 50 assays, and identification using 16S rRNA and *tuf* genes, i1088 was found to be sensitive to 13 antibiotics, exhibited a survival rate >92% under pH 3.0 and 0.3% bile salt conditions, promoted skeletal development and alleviated osteoporosis in a zebrafish model, and improved viscosity and palatability in fermented milk.

The i772 strain was sensitive to 25 antibiotics, showed a survival rate of 3.22% in simulated gastrointestinal fluids, promoted pro-inflammatory cytokine expression in THP-1 cells, and increased cohesiveness in fermented milk. These characteristics suggest that i1088 and i772 possess tolerance to gastrointestinal conditions, immunomodulatory activity, and potential bone health–promoting effects, which can be further enhanced when combined with milk calcium and colostrum basic protein.[19,20] Based on an ovariectomized rat model, the present study evaluated the effects of *B. breve* i1088, *B. longum* subsp. *longum* i772, and their composite formulations (milk calcium + colostrum basic protein) on OP. Bone-related indices including bone mineral density (BMD), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and bone calcium content were assessed, aiming to provide evidence for the application of probiotics in the prevention and management of PMOP.

Methods

Extraction, identification, and culture of probiotics

Based on previously reported methods, Bifidobacterium breve i1088 and Bifidobacterium longum subsp. longum i772 were isolated from feces of healthy infants and identified accordingly.[19,20] The i1088 composite formulation consisted of milk calcium (26.4 mg/kg) + colostrum basic protein (5 mg/kg) + viable i1088 (1 mg/kg). The i772 composite formulation consisted of milk calcium (26.4 mg/kg) + colostrum basic protein (5 mg/kg) + viable i772 (5 mg/kg). After compounding, the gavage volume was 2 mL per rat, which corresponds to a human equivalent daily intake of milk calcium 792 mg + colostrum basic protein 60 mg + 1 × 10^9 CFU.[21]

Establishment of the animal model

Female Sprague–Dawley (SD) rats (10–12 weeks old; approximately 200–250 g) were purchased from SPF (Suzhou) Biotechnology Co., Ltd. (Certificate No.: SCXK (Su) 2022-0006). Animals were housed in a specific pathogen-free (SPF) facility under controlled conditions (22–25°C, 50%–60% humidity, 12 h light/dark cycle) with ad libitum access to standard chow and water. All rats were acclimated for 1 week before experiments.A total of 72 rats were randomly assigned into six groups (n = 12 per group): (1) normal control group (Sham): sham operation + saline gavage; (2) model

control group (OVX): ovariectomy (OVX) + saline gavage; (3) i1088 composite group (OVX + i1088): OVX + i1088 composite gavage; (4) i772 composite group (OVX + i772): OVX + i772 composite gavage; (5) i1088 + i772 composite group (OVX + combination): OVX + combined probiotic composite gavage; and (6) positive control group (OVX + positive drug): OVX + positive drug (e.g., alendronate sodium) gavage. Randomization was performed using a random number table to ensure balanced allocation among groups.

OVX-induced osteoporosis model

Anesthesia was induced and maintained with isoflurane (induction 4%–5%, maintenance 1.5%–2.5%) in oxygen at a flow rate of 1–2 L/min. After inhalational anesthesia, a midline abdominal incision of approximately 2 cm was made through the skin and muscle layers to expose the ovaries. Following ligation of the ovarian artery, bilateral ovaries were excised. The muscle and skin were sutured with 6-0 absorbable sutures. In the sham group, ovaries were exposed but not removed. Penicillin was administered postoperatively to prevent infection.

Experimental intervention

Interventions were initiated 12 weeks after OVX surgery (stable model period). Rats in probiotic intervention groups received daily gavage of the corresponding composite formulations (doses as described in Section 2.1) at a volume of 2 mL per rat, using a three-section rat gavage needle (Zhongke Life, China). The normal control group and OVX model control group received an equal volume of saline (0.9% sodium chloride injection; Sichuan Kelun Pharmaceutical Co., Ltd.) The positive control group received alendronate sodium by gavage (dose adjusted according to the literature to 1 mg/kg, once weekly). The intervention lasted for 28 consecutive days (once daily). Body weight and general condition were monitored during the intervention. After the final administration, rats were euthanized by overdose anesthesia, and femoral samples were collected.

Measurement of outcomes

Determination of bone calcium content (BMC)

After the final administration, rats were euthanized and the right femur was harvested. Surrounding muscles and connective tissues were removed. After recording the wet weight, femora were dried in an oven at 110°C for 2 h and reweighed to obtain the dry weight, and the dry-to-wet weight ratio was calculated. Dried femora were then carbonized and ashed in a muffle furnace at 800°C for 2 h.

After cooling, ash weight was recorded. A 1000 mg/L calcium standard stock solution (Sigma-Aldrich) was used to prepare calcium standard solutions of 10.0, 15.0, and 30.0 mg/L. Absorbance was measured using an atomic absorption spectrophotometer (PinAAcle 900T, PerkinElmer, USA) to generate a standard curve. For each rat, 0.1 g of ash was weighed and digested in 10 mL of 5% nitric acid at room temperature for 48 h. The digest was then measured for absorbance, and bone calcium content was calculated from the standard curve and expressed as mg/g ash weight.

Assessment of BMD, BV/TV, Tb.Th, and Tb.Sp

The left femur was fixed in paraformaldehyde fixative (G1101; Servicebio, Wuhan, China) for 24 h. Sample preparation included washing with PBS three times, blotting dry, and sealing with parafilm. Femora were scanned using a micro-computed tomography system (Skyscan 1276, Bruker, USA) under the following parameters: resolution 18 μm, voltage 50 kV, current 200 μA, and rotation angle 180°.Three-dimensional reconstruction was performed using CTvox software, and femoral morphology images were exported.

CTAn software was used to analyze parameters in the femoral condyle region, including bone mineral density (BMD, g/cm^3), bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), and trabecular separation (Tb.Sp, μm). The region of interest (ROI) was defined as the distal femur 1–3 mm.

Statistical analysis

Data were analyzed using SPSS software (version 26.0). Normality and homogeneity of variance were assessed prior to group comparisons. When data met assumptions of normality and homoscedasticity, independent-samples t tests were used for two-group comparisons, and one-way ANOVA was applied for multiple-group comparisons, followed by Tukey's HSD test or Dunnett's t test as appropriate. When data were not normally distributed or did not meet homogeneity of variance, the Mann–Whitney U test was used for two-group comparisons, and the Kruskal–Wallis one-way ANOVA test was used for multiple-group comparisons. If variance information was unavailable, homogeneity of variance was assumed by default. Quantitative data are presented as mean ± standard deviation ($\bar{x} \pm s$). A P value < 0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Animal Ethics Committee of Animal Center of Junlebao Dairy Group Co., Ltd. (Approval no. 24-015; Approval Date: 16th June, 2024).

Results

Validation of model establishment

To confirm successful establishment of the ovariectomy-induced osteoporosis model, bone calcium content (BMC), bone mineral density (BMD), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were compared between the model control group (ovariectomized rats without intervention) and the normal control group (intact rats). The results showed that, compared with the normal control group, the model control group exhibited a significant reduction in femoral BMC ($p < 0.001$), BMD ($p < 0.001$), BV/TV ($p < 0.001$), and Tb.Th ($p < 0.001$), accompanied by a significant increase in Tb.Sp ($p < 0.001$). These findings indicate pronounced osteoporotic changes after ovariectomy, confirming successful model establishment.

Effects of interventions

Bone calcium content

No significant differences were observed in the femoral dry-to-wet weight ratio among groups ($p > 0.05$). Compared with the model control group, the B. breve i1088 composite group (milk calcium + colostrum basic protein + viable i1088) and the B. longum subsp. longum i772 composite group (milk calcium + colostrum basic protein + viable i772) showed significantly higher bone calcium content ($p < 0.001$). Compared with the normal control group, bone calcium content was significantly decreased in the model control group ($p < 0.001$). Detailed results are presented in Table 1, as well as in Figure 1 and Figure 2, which are bar charts illustrating the changes in femoral dry-to-wet weight ratio and bone calcium content across the experimental groups. (bar charts showing changes in femoral dry/wet weight ratio and bone calcium content across groups).

Bone mineral density

Compared with the model control group, BMD was significantly increased in both the B. breve i1088 composite group and the B. longum subsp. longum i772 composite group ($p < 0.001$). Compared with the normal control group, BMD was significantly reduced in the model control group ($p < 0.001$). No significant differences in BMD were observed among experimental groups A, B, C, and D (corresponding to different composite formulations) ($p > 0.05$). These results are visualized in Figure 3, a bar chart depicting BMD levels across groups, and Figure 4, which presents micro-CT images of the trabecular microarchitecture in the femoral condyle region.

Bone volume fraction

Compared with the model control group, BV/TV was significantly increased in the B. breve i1088 composite group and the B. longum subsp. longum i772 composite group ($p < 0.001$). Compared with the normal control group, BV/TV was significantly decreased in the model control group ($p < 0.001$). No significant differences in BV/TV were observed

Table 1: Measurements of femoral dry-to-wet weight ratio and bone calcium content in rats from each group

Group	Femoral dry-to-wet weight ratio (Mean ± SD)	Bone calcium content （mg/g, Mean ± SD）
Normal control group	0.714 ± 0.020	251 ± 24.4
Model control group	0.686 ± 0.017	117 ± 12.6***
Positive drug group	0.685 ± 0.014	221 ± 26.3###
Experimental group A	0.687 ± 0.052	178 ± 24.5###
Experimental group B	0.688 ± 0.021	225 ± 21.3###
Experimental group C	0.679 ± 0.018	183± 19.6###
Experimental group D	0.674 ± 0.030	213 ± 40.0###

Data are presented as mean ± standard deviation (SD) (n = number of rats per group, determined according to the experimental design). Groups included the blank control group, model control group, composite group with viable *Bifidobacterium breve* i1088, composite group with viable *Bifidobacterium longum* subsp. *longum* i772, etc. No significant differences were observed in femoral dry-to-wet weight ratio among groups ($p > 0.05$); bone calcium content was significantly higher in the composite groups than in the model control group ($p < 0.001$). Statistical annotations: *** $p < 0.001$ vs. blank control group; ### $p < 0.001$ vs. model control group.

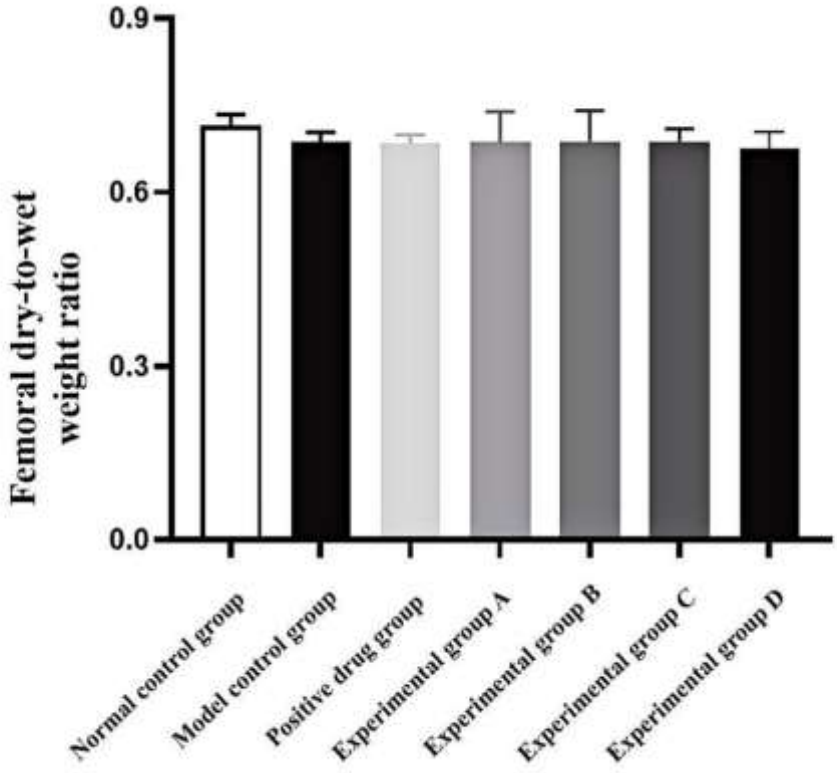

Figure 1: Effects of the two probiotics and their composite formulations on femoral dry and wet weights in osteoporotic rats *** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group (no significant annotations are shown in this figure).

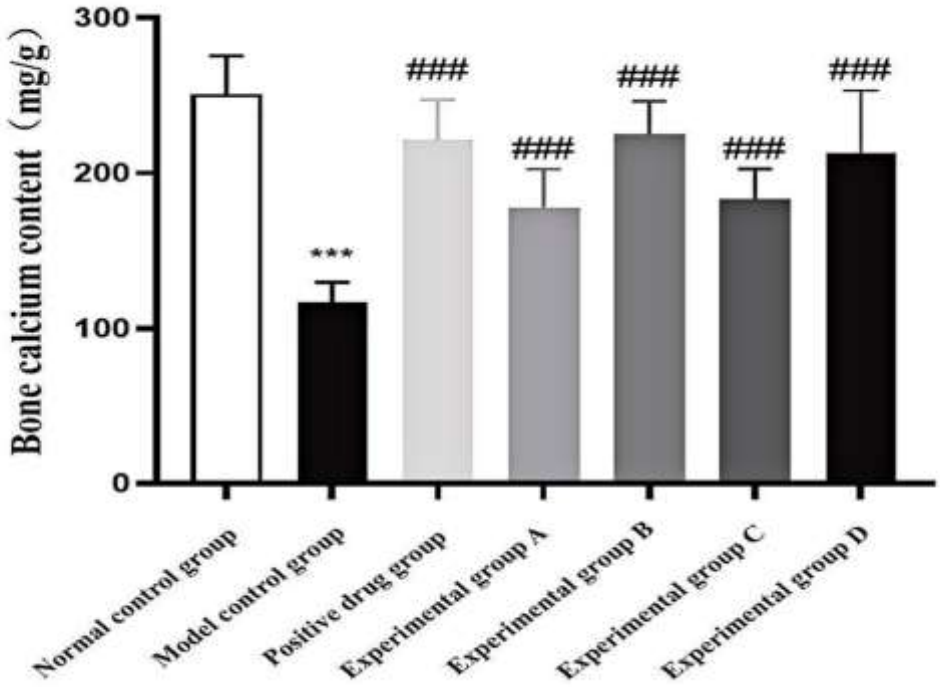

Figure 2: Effects of the two probiotics and their composite formulations on bone calcium content in osteoporotic rats
*** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group.

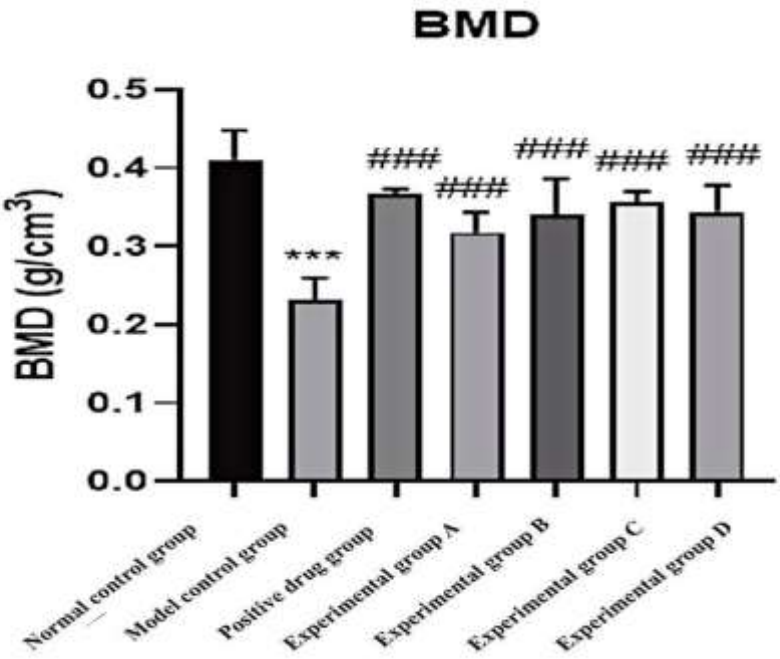

Figure 3: Effects of the two probiotics and their composite formulations on bone mineral density in osteoporotic rats
*** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group.

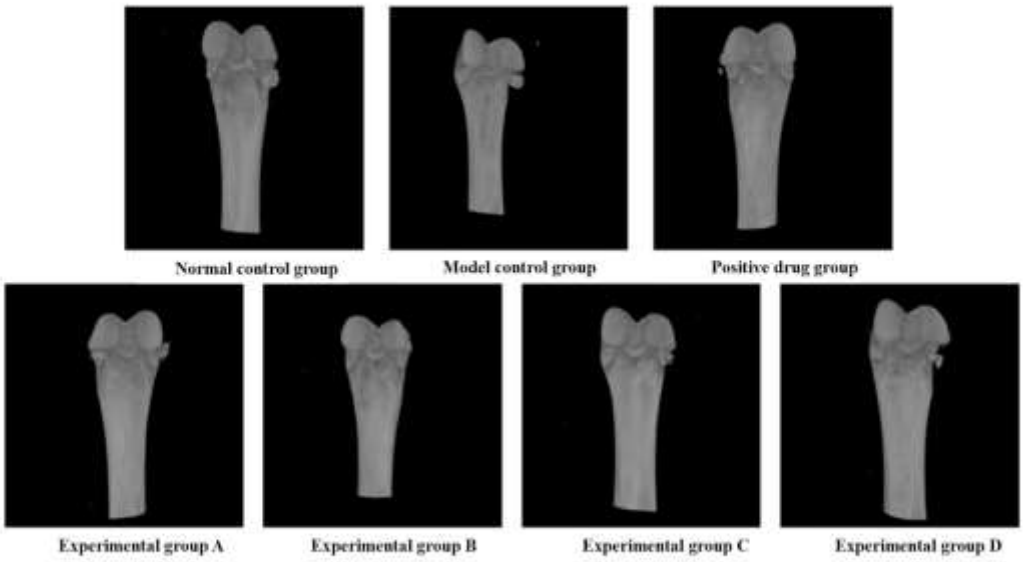

Figure 4: Three-dimensional reconstructed micro-CT images of the femur in each group
The 3D reconstructed images illustrate changes in femoral bone microarchitecture across groups. Compared with the normal control group, the model group exhibited marked damage to the femoral head morphology and prominent osteoporotic features. In experimental groups A, B, C, and D, the femoral 3D reconstructions showed varying degrees of structural recovery, suggesting that the experimental interventions provided protective effects on bone

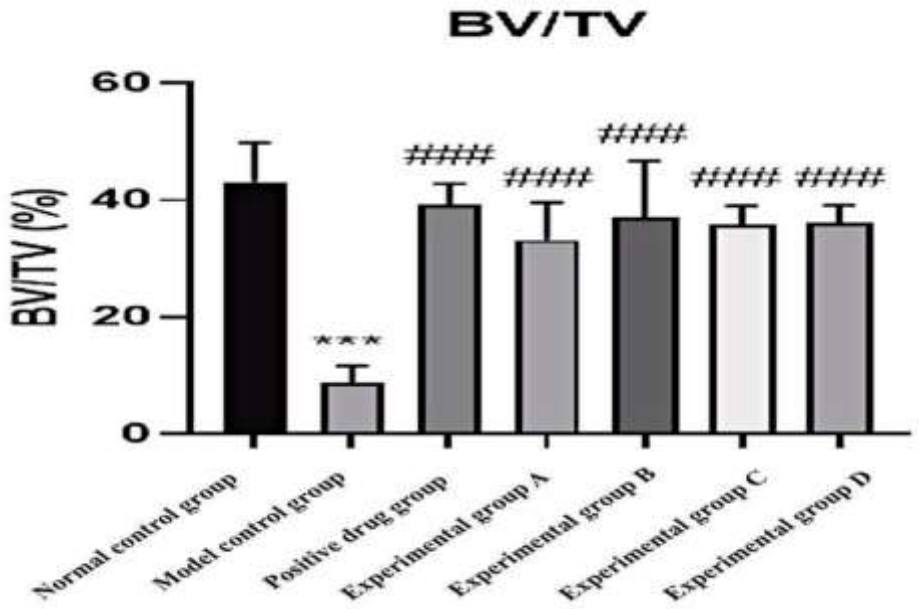

Figure 5: Effects of the two probiotics and their composite formulations on bone volume fraction (BV/TV) in osteoporotic rats
*** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group.

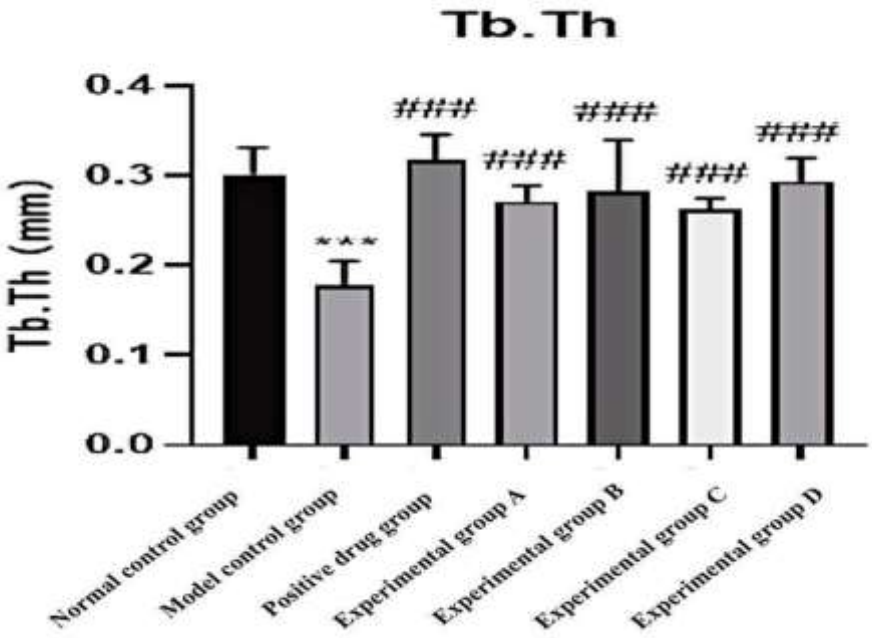

Figure 6: Effects of the two probiotics and their composite formulations on trabecular thickness (Tb.Th) in osteoporotic rats *** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group.

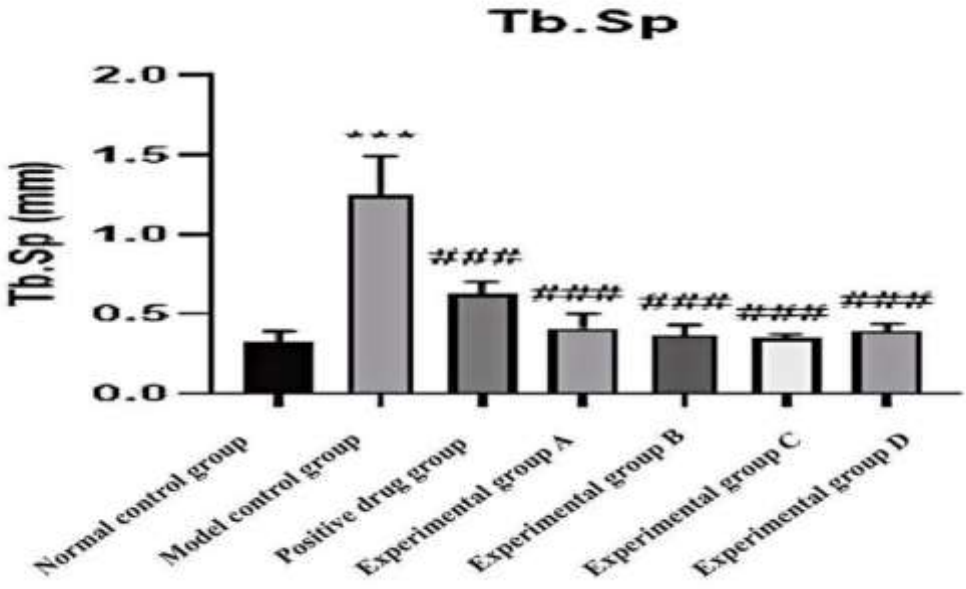

Figure 7: Effects of the two probiotics and their composite formulations on trabecular separation (Tb.Sp) in osteoporotic rats *** $p < 0.001$ vs. Normal control group; ### $p < 0.001$ vs. model control group

among experimental groups A, B, C, and D ($p > 0.05$). These findings are illustrated in Figure 5, a bar chart showing BV/TV levels across groups, alongside the micro-CT images of trabecular architecture presented in Figure 4.

Trabecular thickness

Compared with the model control group, Tb.Th was significantly increased in the B. breve i1088 composite group and the B. longum subsp. longum i772 composite group ($p < 0.001$). Compared with the normal control group, Tb.Th was significantly reduced in the model control group ($p < 0.001$). No significant differences in Tb.Th were observed among experimental groups A, B, C, and D ($p > 0.05$). Corresponding results are shown in Figure 6, a bar chart of Tb.Th across groups, and the micro-CT visualization in Figure 4.

Trabecular separation

Compared with the model control group, Tb.Sp was significantly decreased in the B. breve i1088 composite group and the B. longum subsp. longum i772 composite group ($p < 0.01$). Compared with the normal control group, Tb.Sp was significantly increased in the model control group ($p < 0.001$). No significant differences in Tb.Sp were observed among experimental groups A, B, C, and D ($p > 0.05$). These changes are depicted in Figure 7, a bar chart of Tb.Sp across groups, and the micro-CT images in Figure 4

Discussion

The beneficial effects of probiotics on osteoporosis may involve multiple biological processes. Their acid and bile salt tolerance provides a basis for intestinal colonization and the generation of bioactive metabolites. Such metabolites, including short-chain fatty acids (SCFAs), may enhance calcium absorption by lowering intestinal pH, or indirectly influence bone metabolism by modulating gut barrier function.[9] In addition, studies on calcium-fortified fresh milk suggest that calcium supplementation can inhibit osteoclast activity via the RANKL/RANK/OPG pathway.[11] In the present study, the combination of milk calcium with probiotics may have further potentiated this effect. Moreover, the involvement of the oxidative stress–JAK2/STAT3 pathway in osteoporosis has been demonstrated in studies of *Bifidobacterium bifidum* TMC3115, which improved trabecular microarchitecture by reducing the expression of p-JAK2 and p-STAT3. The bone-protective efficacy observed for our composite formulation may also be attributable, at least in part, to similar antioxidant mechanisms; future work should verify this hypothesis by assessing oxidative stress–related markers (e.g., reactive oxygen species [ROS] levels or GPX4 expression).[22] Colostrum basic protein is rich in bioactive peptides and may exert effects by promoting osteoblast differentiation or suppressing osteoclast activity.[23] Its synergistic interaction with probiotics may amplify bone protection by enhancing intestinal nutrient absorption efficiency. Furthermore, the fermentation characteristics of i1088 and i772 provide a practical basis for developing functional dairy products, aligning with nutritional optimization strategies such as calcium fortification in fresh milk, and offering new directions for industrial translation.

Notably, this study has several key strengths that enhance the robustness of our findings. First, we utilized a well-validated ovariectomized rat model that closely recapitulates the pathophysiological characteristics of postmenopausal osteoporosis, providing a rigorous preclinical platform for evaluating intervention efficacy. Second, unlike many previous studies that focused on single probiotic strains, the present work investigated two novel human-derived Bifidobacterium strains, i1088 and i772, in combination with complementary nutritional components (milk calcium and colostrum basic protein), allowing us to explore the synergistic effects of a composite formulation rather than isolated interventions. Third, we comprehensively evaluated multiple key bone health parameters, including bone calcium content, BMD, BV/TV, Tb.Th, and Tb.Sp, providing a holistic assessment of the skeletal benefits of the intervention rather than focusing on a single endpoint. Finally, the strains used in this study have previously demonstrated favorable gastrointestinal tolerance and safety profiles, supporting their potential for translational application in human populations.The findings of this study carry important implications

for public health policy and clinical practice. For public health policy, the growing global burden of postmenopausal osteoporosis demands accessible, preventive nutritional strategies. Our results support the potential of probiotic-based functional foods as a population-level intervention to reduce osteoporosis risk, which could inform updated dietary guidelines for aging women. Policymakers could consider promoting the development and standardized regulation of probiotic-fortified dairy products targeting bone health, as these products are generally well-accepted by consumers and have a far more favorable safety profile compared to long-term pharmaceutical interventions. For clinical practice, these findings provide clinicians with a new adjunctive option for managing patients at risk of postmenopausal bone loss. For individuals who cannot tolerate or are unwilling to use conventional anti-osteoporotic drugs due to their associated adverse effects, probiotic composite formulations could serve as a safe, non-pharmacological alternative to slow bone loss and maintain skeletal health. Additionally, the industrial feasibility of these strains, which can be readily incorporated into fermented dairy products, means that this intervention could be easily integrated into daily dietary routines, significantly improving long-term adherence compared to complex treatment regimens.

This study has several limitations. First, metabolic differences between animal models and humans may restrict direct extrapolation of these findings; randomized controlled trials are needed to confirm efficacy in postmenopausal women. Second, mechanistic exploration remains largely at a phenomenological level, and the regulation of specific molecular pathways requires further elucidation through transcriptomic and/or proteomic approaches. Third, evidence regarding dose–response relationships and long-term safety of probiotic supplementation is still insufficient; extended intervention durations and larger sample sizes are warranted to assess clinical feasibility.

Based on the above considerations, future research may proceed in the following directions: (1) conducting clinical studies to evaluate the effects of different doses and intervention durations (e.g., 6 or 12 months) of the composite formulation on bone mineral density, together with monitoring of bone turnover markers (e.g., CTX or P1NP); (2) applying high-throughput sequencing to characterize how i1088 and i772 modulate gut microbiota composition and microbial metabolites, thereby clarifying the pathways through which they influence bone metabolism via the "gut–bone axis"; (3) investigating molecular mechanisms by measuring changes in ferroptosis-related genes (e.g., FTH1 and SLC7A11) and/or oxidative stress pathway markers to validate their potential involvement; (4) exploring combination strategies, such as co-interventions with nanomaterials or traditional Chinese medicine (e.g., Bushen Zhuangjin Decoction[8]), to assess synergistic effects on bone defect repair or fracture prevention; and (5) optimizing probiotic formulations by integrating individual gut microbiota profiles to develop personalized interventions for specific populations (e.g., older adults or postmenopausal women).

Conclusion

In this study, an ovariectomized rat model of osteoporosis was used to systematically evaluate the effects of *Bifidobacterium breve* i1088, *Bifidobacterium longum* subsp. *longum* i772, and their composite formulation with milk calcium and colostrum basic protein on skeletal health. The results demonstrated that the composite intervention significantly increased bone mineral density (BMD; $P < 0.001$), bone volume fraction (BV/TV; $P < 0.001$), trabecular thickness (Tb.Th; $P < 0.001$), and bone calcium content (BMC; $P < 0.001$), while effectively reducing trabecular separation (Tb.Sp; $P < 0.01$). The magnitude of improvement in these indices was statistically significant compared with the model control group. These findings indicate that i1088 and i772, as human-derived probiotic strains, when combined with milk calcium and colostrum basic protein, can effectively attenuate bone loss and trabecular microarchitectural deterioration, providing new experimental evidence supporting non-pharmacological interventions for osteoporosis. Consistent with micro-CT analyses and bone calcium measurements, the femoral morphology and mineralization in the intervention groups were markedly superior to those in the OVX model group.

Collectively, this work provides preliminary evidence for the application of probiotics in osteoporosis prevention and management, and the observed multi-target regulatory potential may facilitate future mechanistic studies and industrial translation.

Conflict of interests

The authors declared no conflict of interest.

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