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Research Article

Impact of antibiotic-induced gut dysbiosis model on bone mineralization and biomechanical properties in post-weaning rats

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ABSTRACT

The gut microbiota plays a crucial role in regulating host metabolism and homeostasis through immune, endocrine, and metabolic pathways. This study investigated the impact of antibiotic-induced microbial disruption during the critical post-weaning period on bone biomechanical properties and mineral composition and evaluated whether fecal microbiota transplantation (FMT) could reverse these alterations in rats. Following weaning, Sprague Dawley rats were allocated to control (CON), dysbiosis (DYS), and FMT groups. To induce experimental intestinal dysbiosis, rats in the DYS and FMT groups were administered a broad-spectrum antibiotic mixture for 14 days following an established protocol. Subsequently, the FMT group received daily fecal suspensions from healthy donor rats for 10 days. At 8 weeks of age, tibial biomechanical integrity was assessed via three-point bending tests, and bone mineral composition was determined through crude ash, calcium (Ca), and phosphorus (P) analyses. The results demonstrated that antibiotic-induced DYS significantly reduced tibial ultimate force and stiffness while increasing displacement, indicating impaired bone mechanical integrity. In addition, DYS significantly decreased crude bone ash content as well as Ca and P levels. While histopathological examination did not reveal marked structural alterations among the groups, the FMT intervention partially restored bone mechanical strength and mineral composition toward control levels. These findings suggest that antibiotic-induced perturbations during early development negatively affect bone mineralization and biomechanics, while the partial recovery following FMT supports the concept of a functional gut–bone axis.

Keywords: Bone biomechanics, Bone mineralization, Dysbiosis, Gut–bone axis, Rat

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Introduction

Bone is a dynamic tissue that undergoes continuous remodeling through the coordinated actions of osteoblasts, osteoclasts, and osteocytes. This tightly regulated process ensures the maintenance of skeletal integrity and mineral homeostasis throughout life. In recent years, increasing attention has been directed toward systemic factors that influence bone metabolism beyond traditional endocrine regulators such as parathyroid hormone, vitamin D, and sex steroids. Among these emerging regulators, the intestinal microbiota has been recognized as an important modulator of bone physiology through what is now referred to as the gut–bone axis (4, 11).

The gut microbiota consists of trillions of microorganisms that contribute to host metabolism, immune regulation, and nutrient absorption. A balanced microbial community is essential for maintaining physiological homeostasis, whereas disturbances in microbial composition—referred to as dysbiosis (DYS)—have been implicated in numerous metabolic and inflammatory disorders (9, 20). Increasing evidence indicates that the gut microbiota also plays a significant

role in skeletal health by influencing mineral absorption, modulating systemic inflammation, and regulating immune pathways that control osteoclastogenesis (14, 17). Experimental studies have demonstrated that germ-free mice exhibit altered bone mass and immune cell populations, highlighting the importance of microbial colonization in normal skeletal development (21, 22, 24).

Several mechanisms have been proposed to explain how intestinal microbiota influence bone metabolism. Microbial fermentation products such as short-chain fatty acids (SCFAs) can regulate osteoclast differentiation and bone resorption by modulating immune cell activity (11). Additionally, gut bacteria affect calcium (Ca) and phosphorus (P) bioavailability, which are critical minerals for bone mineralization as also demonstrated in animal nutrition models (14). DYS has also been associated with increased intestinal permeability and systemic low-grade inflammation, both of which can stimulate osteoclast activity and ultimately compromise bone strength (13). Consequently, alterations in gut microbial composition may lead not only to changes in bone mineral density but also to deterioration of bone mechanical properties.

Early life represents a particularly critical window for the development of both the gut microbiota and the skeletal system. The post-weaning period is characterized by rapid microbial colonization and significant skeletal growth, making this stage highly sensitive to environmental perturbations such as antibiotic exposure (19). Antibiotic-induced DYS during early development has been shown to disrupt microbial diversity and metabolic signaling pathways, potentially leading to long-term physiological consequences (15, 28). Despite growing interest in the gut–bone axis, relatively few studies have investigated how DYS during the post-weaning period affects bone biomechanical properties and mineral composition.

Fecal microbiota transplantation (FMT) has emerged as a promising strategy for restoring microbial balance following DYS. By transferring fecal microbial communities from healthy donors to recipients, FMT can reestablish a functional microbial ecosystem and improve host metabolic and immune functions (9). Although FMT has been extensively studied in gastrointestinal and metabolic disorders, its potential effects on skeletal health remain incompletely understood. Experimental evidence suggests that restoration of a healthy microbiota may reverse some of the metabolic and inflammatory consequences of DYS, thereby potentially improving bone quality (1).

Therefore, the present study aimed to investigate the effects of antibiotic-induced DYS during the post-weaning period on bone strength, mineral composition, and histological characteristics of rats, and to determine whether FMT can mitigate DYS-induced skeletal alterations. By integrating biomechanical testing, mineral analysis, and histological evaluation, this study aims to provide further insight into the role of the gut microbiota in regulating bone integrity during early development.

Materials and Methods

Animals and Experimental Protocol: Twenty-eight newly weaned 4-week-old (70–76 g), male Sprague Dawley rats, obtained from the Bursa Uludağ University Experimental Animal Production Center, were used in this study. The sample size was determined based on a power analysis conducted using G*Power software (v3.1.9.7). Based on previous studies evaluating bone biomechanical properties in rat models, an effect size (f) of 0.65 was anticipated. With an α level of 0.05 and a desired statistical power (1-β) of 0.80 for one-way ANOVA, the minimum required total sample size was calculated as 24–27 animals. To account for potential experimental attrition and to ensure robust histopathological and mineral analyses, a total of 28 rats (n = 8–10 per group) were included in the study.

Rats were housed under pathogen-free conditions at 20–24°C with a 12-h light/12-h dark cycle throughout the experiment. Animals were allocated to three groups according to body weight: control (CON, n = 8), DYS (n = 10), and FMT (n = 10). To induce intestinal DYS, rats in the DYS and FMT groups received a broad-spectrum antibiotic mixture consisting of vancomycin (8 mg/kg) and amoxicillin (60 mg/kg) administered via drinking water for 14 consecutive days starting at 4 weeks of age to 6 weeks of age, as previously described (19). Antibiotics (Bostonchem Co., MA, USA) were freshly prepared daily based on the average water intake (10–12 mL/100 g body weight/day) to ensure accurate dosing, resulting in final concentrations of vancomycin and amoxicillin of 0.08 mg/mL and 0.6 mg/mL, respectively. After DYS was induced in the FMT group, FMT was performed using freshly collected feces from healthy CON male donors. Donor fecal pellets were pooled and suspended in sterile saline (100 mg/mL). Twenty-four hours after the final antibiotic exposure, rats in the FMT group received 1 mL of the fecal suspension intragastrically once daily for 10 consecutive days. (2). Rats in the CON group received antibiotic-free water throughout the study. All animals were allowed ad libitum access to standard pelleted chow throughout the study.

At 8 weeks of age, rats were anesthetized with sevoflurane (2–4% in 100% O_2) and euthanized by cervical dislocation. Both tibiae were then excised, carefully dissected free of surrounding soft tissues, wrapped in gauze moistened with phosphate-buffered saline (PBS), and stored at −20°C until further analyses. Prior to mechanical testing and other measurements, all

specimens were thawed at room temperature. The tibia was selected as a representative weight-bearing long bone and a well-established model for assessing bone morphology and mechanical strength in rats.

Biomechanical Testing of Tibial Bones: The structural properties and mechanical integrity of the harvested tibiae were evaluated in specimens from the CON (n=8), DYS (n=10), and FMT (n=10) groups using a standardized three-point bending test. All mechanical assessments were conducted using a universal testing machine (MNR-010, UVE Measurement Equipment, Bursa, Türkiye) specifically calibrated for low-strength biological materials. The system was equipped with an integrated 1 kN load cell to ensure high precision during data acquisition. Prior to testing, specimens were thawed at room temperature and kept moistened with phosphate-buffered saline (PBS) to maintain their viscoelastic properties. Each tibia was positioned horizontally on two polished metal supports with a defined support span length of 20 mm. The bone was oriented so that the load was applied to the mid-diaphysis (the midpoint of the tibial shaft) to simulate physiological loading conditions in a weight-bearing long bone. Mechanical loading was performed at a constant crosshead speed of 10 mm/min until catastrophic failure (complete fracture) occurred. The system's software continuously recorded the relationship between the applied force and the resulting deformation. From the generated load-displacement curves, the following primary biomechanical parameters were derived. Ultimate Force (N) was measured as the maximum load-bearing capacity of the bone before fracture. Stiffness (N/mm) was calculated from the slope of the linear (elastic) portion of the load-displacement curve, representing the bone's resistance to elastic deformation. Displacement (mm) is the total deformation or travel distance of the crosshead at the point of fracture. All tests were performed by a single investigator to ensure procedural consistency across the groups.

Tibia Length, Weight, and Cortical Area Measurements: Following thawing at room temperature, tibial specimens from the CON (n=8), DYS (n=10), and FMT (n=10) groups were gently blotted dry to remove excess surface moisture. Tibia length was measured as the distance from the proximal tibial plateau to the distal medial malleolus using a digital caliper with 0.01 mm precision. Each measurement was performed in triplicate, and the mean value was recorded for analysis. Tibia weight was determined using an analytical balance with 0.001 g sensitivity after removal of all remaining soft tissues and drying of the bone surface. For cortical area determination, standardized transverse sections were obtained at the mid-diaphysis (50% of tibial length). Cross-sections were analyzed using image analysis software (ImageJ, NIH, USA) to calculate the cortical area (Ct.Ar), which was expressed in mm^2. All analyses were conducted by the same investigator to minimize inter-observer variability.

Crude Ash Content Determination in Bone Samples: Crude ash content in bone samples was determined using a gravimetric dry ashing method adapted from a previously described protocol (28) in specimens from the CON (n = 8), DYS (n = 10), and FMT (n = 10) groups. Briefly, porcelain crucibles were pre-ashed in a muffle furnace at 550 ± 50 °C for 1 h, cooled in a desiccator, and weighed to a constant mass (W_0). The entire cleaned and finely ground tibia from each animal was placed into a pre-weighed crucible, dried at 105°C for 7 days, and then weighed (W_1). To minimize sample loss due to spattering, the samples were first gently pre-charred at low temperature and then transferred to a muffle furnace, where they were ashed at 550 ± 50 °C for 4–6 h until a light gray/white ash residue was obtained. After ashing, crucibles were removed from the furnace, cooled to room temperature in a desiccator, and weighed (W_2). When necessary, the ashing and weighing steps were repeated until a constant weight was achieved. Crude ash percentage was calculated using the following equation: % Crude Ash = $[(W_2 - W_0) / (W_1 - W_0)] \times 100$, where W_0 is the weight of the empty crucible, W_1 is the weight of the crucible containing the sample, and W_2 is the weight of the crucible containing the ash.

Bone Ca concentration was determined spectrophotometrically using a well-established colorimetric method based on the formation of a calcium–o-cresolphthalein complex, with absorbance measured at 575 nm. Bone P concentration was determined spectrophotometrically using a standard phosphomolybdate colorimetric method based on the formation of a phosphomolybdenum blue complex, with absorbance measured at 720 nm. Standard calibration curves were prepared using known concentrations of Ca and P standards. Results were expressed as total content mg per dry bone, and the Ca/P ratio was calculated from the measured calcium and phosphorus values.

Histopathological Examination of Tibia Bone: For histopathological evaluation, tibial specimens from the CON (n=8), DYS (n=10), and FMT (n=10) groups were fixed in 10% neutral-buffered formalin for 48 h. Samples were then decalcified in 10% (w/v) EDTA (pH 7.2–7.4) at room temperature for approximately 15 days, with the decalcifying solution renewed every 5 days. Following decalcification, tissues were dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin. Serial sections were cut at 4 µm thickness using

a rotary microtome, mounted on glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) according to standard protocols (12). Histological assessment was performed under a light microscope, and representative images were captured for documentation.

Statistical Analyses: All data are presented as mean ± standard error of the mean (SEM). Prior to statistical comparisons, the normality of data distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variance was evaluated using Levene's test. All data met the assumptions for parametric analysis. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $P<0.05$.

Results

Effects of DYS and FMT on Tibial Biomechanical Parameters: Mechanical testing revealed that DYS adversely affected tibial strength. In terms of ultimate force, the CON group exhibited a mean value of approximately 37.5 N, whereas this parameter was significantly reduced in the DYS group to approximately 34.3 N ($P<0.05$). Notably, the FMT group showed an ultimate force of approximately 36.5 N, which was close to CON levels (Figure 1).

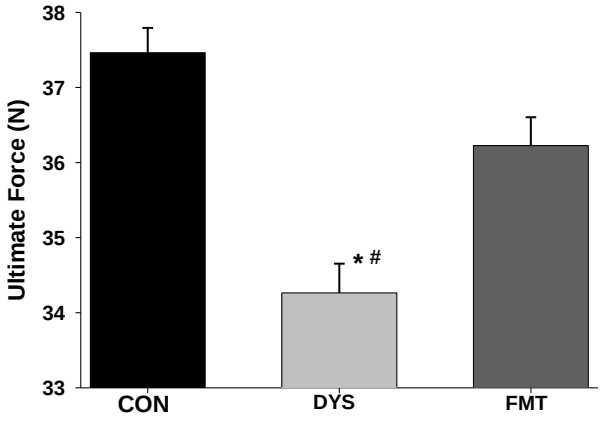


Figure 1. Effects of DYS and FMT on tibial ultimate force.

Ultimate force of tibiae was measured by a three-point bending test in CON (n=8), DYS (n=10), and FMT (n=10) groups. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Symbols indicate statistical significance ($P<0.05$): (*) vs. CON and (#) vs. FMT. CON: Control; FMT: Fecal microbiota transplantation; DYS: Dysbiosis.

Consistent with these findings, stiffness values also indicated that dysbiotic rats had compromised bone mechanical integrity The mean stiffness was 195 N/mm in the CON group, and significantly lower in the DYS group (160 N/mm, $P<0.05$). FMT partially ameliorated this reduction, with the stiffness in the FMT group reaching approximately 180 N/mm, showing no significant difference compared with CON (Figure 2).

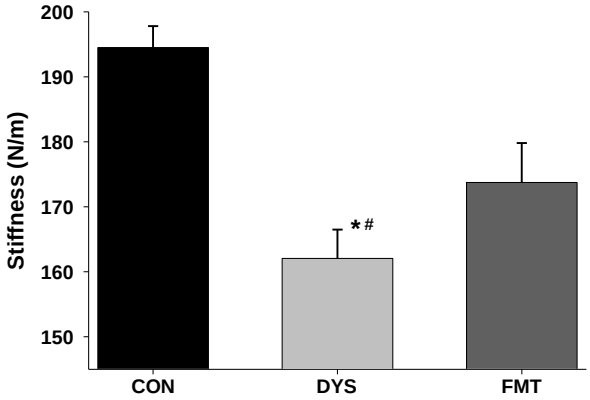


Figure 2. Effects of DYS and FMT on tibial stiffness.

The stiffness was measured by a three-point bending test in CON (n=8), DYS (n=10), and FMT (n=10) groups. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Symbols indicate statistical significance ($P<0.05$): (*) vs. CON and (#) vs. FMT. CON: Control; FMT: Fecal microbiota transplantation; DYS: Dysbiosis.

Regarding displacement, the DYS group exhibited a significantly higher displacement than the CON group (2.12 mm vs. 1.95 mm, $P<0.05$), suggesting increased deformation and reduced mechanical resistance. In the FMT group, displacement was measured at approximately 2.05 mm, which was comparable to the CON group (Figure 3).

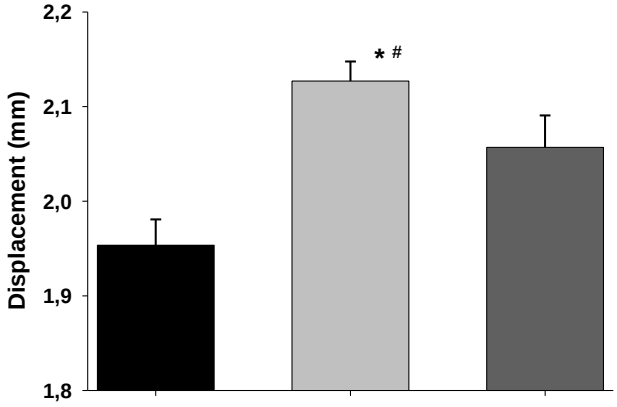


Figure 3. Effects of DYS and FMT on tibial displacement.

Maximum displacement (mm) of tibial bones was measured by a three-point bending test in CON (n=8), DYS (n=10), and FMT (n=10) groups. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Symbols indicate statistical significance ($P<0.05$): (*) vs. CON and (#) vs. FMT. CON: Control; FMT: Fecal microbiota transplantation; DYS: Dysbiosis

Effects of DYS and FMT on Tibial Length, Weight, Cortical Area, and Bone Crude Ash Content: Tibial morphometric and compositional parameters are presented in Table 1. No significant differences were observed in cortical area among the CON, DYS, and FMT

Table 1. Effects of DYS and FMT on tibial morphometric parameters and crude ash content.

	CON	DYS	FMT	P (DYS vs CON)	P (DYS vs FMT)
Cortical Area (mm^2)	3.05 ± 0.12	3.23 ± 0.19	2.94 ± 0.25	0.462	0.318
Length (mm)	35.3 ± 0.3	34.9 ± 0.4*	35.1 ± 0.6	0.044	0.048
Weight (g)	0.64 ± 0.02	0.60 ± 0.03*	0.63 ± 0.04	0.036	0.049
Crude Ash (%)	43.3 ± 1.7	41.1 ± 0.5*	42.9 ± 1.3	0.014	0.022
Bone Ca (mg/bone)	44.3 ± 1.2	39.4 ± 0.4*	43.2 ± 1.5	0.013	0.017
Bone P (mg/bone)	20.8 ± 0.7	18.5 ± 0.2*	20.3 ± 0.9	0.011	0.015
Ca/P ratio	2.13 ± 0.04	2.12 ± 0.03	2.13 ± 0.05	0.874	0.912

Values are presented as mean ± SEM. Differences among groups were analyzed by one-way ANOVA followed by Tukey's post hoc test. *, P<0.05 vs. CON and FMT; CON: Control; FMT: Fecal microbiota transplantation; DYS: Dysbiosis.

groups (Table 1). In contrast, tibial length was slightly but significantly reduced in the DYS group compared with CON (P<0.05), whereas the FMT group showed values comparable to the CON group (Table 1). Similarly, tibial weight was significantly lower in the DYS group than in the CON group (P<0.05), while the FMT group exhibited values close to those of CON (Table 1).

In terms of mineral composition, bone crude ash content was significantly decreased in the DYS group compared with CON (P<0.05), whereas the FMT group demonstrated crude ash values similar to the CON group (Table 1). Consistent with these findings, both bone Ca and P levels were significantly reduced in the DYS group (P<0.05), while values in the FMT group were comparable to those in the CON group (Table 1). The Ca/P ratio remained similar among all groups (Table 1).

Effects of DYS and FMT on Tibia Histopathology: For quantitative assessment, transverse sections were imaged across four independent fields per slide using an Olympus CX43 light microscope. Histological examination of the experimental groups revealed no structural abnormalities within the bone tissue. Both the growth plate and the compact bone architecture maintained a normal morphological appearance (Figure 4).

Although minor variations in bone thickness were noted across different regions, these fluctuations did not result in observable differences between the groups. Comparisons between the CON, DYS, and FMT groups confirmed that bone thickness and tissue integrity remained consistent across all experimental cohorts. This uniformity in bone structure is further demonstrated in the representative microscopic images provided (Figure 4).

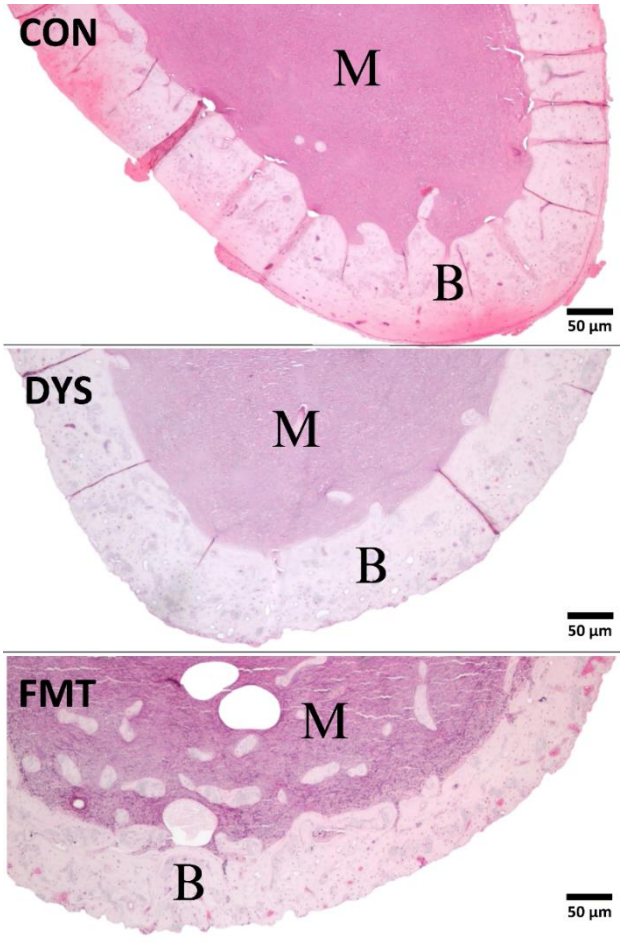


Figure 4. Representative histological sections of the tibia across experimental groups.

Hematoxylin and eosin (H&E)-stained transverse tibial sections from the CON (n=8), DYS (n=10), and FMT (n=10) groups showed preserved histological architecture, with apparent qualitative structural deviations. Cortical bone thickness and overall tissue integrity appeared comparable among the groups, consistent with the absence of statistically significant differences in quantitative measurements. M, medullary area; B, bony structures. Images were acquired using an Olympus CX43 light microscope with a 10× objective lens. Scale bar = 50 µm. CON: Control; FMT: Fecal microbiota transplantation; DYS: Dysbiosis

Discussion and Conclusion

The present study investigated the effects of antibiotic-induced DYS during the post-weaning period on bone biomechanical properties and mineral composition and evaluated whether FMT could reverse these alterations. The findings demonstrated that DYS significantly impaired bone mechanical strength and reduced mineral content, whereas FMT partially restored these parameters toward CON levels. These results provide further evidence supporting the existence of a functional gut–bone axis and highlight the importance of gut microbiota during early developmental periods for normal skeletal physiology.

In the present study, the antibiotic-induced model of DYS significantly reduced tibial ultimate force and stiffness while increasing displacement, indicating a deterioration in bone mechanical integrity. While gut microbiota composition was not directly assessed, these findings are consistent with accumulating evidence suggesting that alterations in intestinal homeostasis can influence bone metabolism and skeletal health (5, 31). The observed skeletal impairment may reflect the systemic consequences of the experimental DYS model, which has been reported to disrupt the gut–bone axis in post-weaning rats. The gut microbiota contributes to bone homeostasis through several mechanisms, including regulation of immune responses, production of microbial metabolites, and modulation of nutrient absorption (3, 13). Disruption of microbial communities has been reported to promote systemic inflammation and osteoclast activation, which can result in increased bone resorption and reduced bone strength (17). Experimental studies have also demonstrated that animals with altered or depleted gut microbiota display significant changes in bone mass and microarchitecture, further supporting the importance of microbial colonization for normal skeletal development (16, 22).

A potentially important mechanism underlying microbiota-mediated regulation of bone metabolism involves microbial fermentation products including SCFAs such as acetate, propionate, and butyrate. These metabolites are thought to play essential roles in bone homeostasis by regulating immune responses and suppressing osteoclast differentiation (11). In various experimental models, SCFAs have been shown to inhibit osteoclastogenesis through metabolic reprogramming of osteoclast precursors and suppression of key transcription factors involved in osteoclast differentiation (11, 33). In addition, previous studies suggest that SCFAs may modulate immune cell differentiation, including regulatory T cells, and influence signaling pathways such as Wnt signaling, thereby promoting osteoblast activity and bone formation (23, 30). Although SCFAs were not measured in this study, antibiotic-induced DYS may reduce their production, which could contribute to the impaired bone remodeling and decreased bone strength observed in the DYS group.

In addition to metabolite-mediated mechanisms, DYS may impair intestinal barrier integrity and increase systemic inflammatory responses. Increased intestinal permeability allows microbial products and inflammatory mediators to enter the circulation, which can stimulate osteoclast activity and disrupt the balance between bone formation and resorption (13). Recent research has also suggested that gut microbiota-associated inflammatory pathways may interact with oxidative stress-related cellular mechanisms, including ferroptosis in osteoblasts, which has been implicated in bone metabolic disorders such as osteoporosis (8, 25, 32). These emerging mechanisms may further contribute to the complex interactions between gut microbiota and skeletal metabolism.

Consistent with the biomechanical findings, the present study also demonstrated that DYS significantly decreased crude bone ash content and Ca and P levels. Since bone ash primarily reflects the mineralized component of bone tissue, reductions in mineral content indicate impaired bone mineralization. Previous studies have shown that gut microbiota plays a critical role in regulating mineral absorption and metabolism, particularly Ca and P, which are essential for skeletal development (4, 6). Microbiota-derived metabolites can enhance intestinal mineral absorption and influence endocrine pathways that regulate bone formation and mineral deposition.

Interestingly, despite the observed changes in bone mechanical properties and mineral composition, histopathological examination did not reveal pronounced structural abnormalities in bone architecture. This observation suggests that functional deterioration in bone strength may occur before detectable morphological changes. Similar findings have been reported in experimental studies where early metabolic disturbances in bone were reflected in biomechanical parameters prior to visible histological alterations (17, 26), indicating that mechanical testing may represent a sensitive indicator of early skeletal impairment.

Another important finding of the present study is that FMT partially reversed DYS-induced alterations in bone parameters. Animals receiving FMT exhibited improvements in bone strength, stiffness, mineral composition, and morphometric parameters compared with DYS animals. FMT is increasingly recognized as an effective strategy for restoring microbial diversity and functional metabolic activity within the intestinal ecosystem. Restoration of beneficial microbial populations may enhance SCFA production, reduce intestinal inflammation, and improve nutrient absorption,

thereby positively influencing host metabolic pathways involved in bone metabolism (9, 23, 27).

The post-weaning period represents a critical developmental window during which both microbiota maturation and skeletal growth occur simultaneously. Antibiotic exposure during this stage may disrupt microbial colonization and metabolic signaling pathways, potentially leading to long-term physiological consequences (7, 18). Recent evidence also suggests that early-life disturbances in gut microbiota can significantly influence host metabolic and immune development, thereby affecting bone growth and skeletal integrity later in life (10, 31). The partial recovery observed following FMT in the present study further supports the concept that microbiota restoration strategies may mitigate some of the adverse skeletal effects of early-life DYS.

The present study has several limitations. First, gut microbiota composition was not directly characterized; therefore, the presence of antibiotic-induced DYS was inferred from the established experimental model and previous studies using the same protocol. Second, microbial metabolites, including SCFAs, were not measured, and the proposed mechanisms underlying the observed skeletal alterations are based on previous experimental evidence. Finally, the relatively short duration of the study precludes conclusions regarding the long-term effects of antibiotic-induced DYS and FMT on skeletal development.

In conclusion, the present study demonstrates that antibiotic-induced DYS during the post-weaning period adversely affects bone mechanical strength and mineral composition. Importantly, FMT partially reversed these alterations, suggesting that restoration of a balanced gut microbiota may contribute to improved skeletal health. These findings further support the concept of the gut–bone axis and highlight microbiota-targeted interventions as potential strategies for preventing DYS-associated bone impairment. Further studies are warranted to clarify the underlying microbial and molecular mechanisms linking gut DYS to skeletal development.

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Ethical Statement

The experiments were performed under ethical standards approved by Bursa Uludağ University Animal Experimentation Local Ethics Committee (Approval No: 2022-12/01)

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

MY conceived the idea and planned the manuscript. BS, IA and EY contributed to sample preparation. BS, AA and EY have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant contributions by giving feedback and help shape the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative Artificial Intelligence

The authors declare that generative artificial intelligence (AI) and AI-assisted technologies were not used in the writing of this manuscript or in the preparation of figures.

Animal Welfare

The authors confirm that the study was conducted in accordance with the ARRIVE Guidelines for the protection of animals used for scientific purposes.

References

1. **Allegretti JR, Hamilton MJ** (2014): *Restoring the gut microbiome for the treatment of inflammatory bowel diseases.* World J Gastroenterol, **20**, 3468–3474. DOI: 10.3748/wjg.v20.i13.3468.
2. **Chen R, Xu Y, Wu P, et al** (2019): *Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota.* Pharmacol Res, **148**, 104403. DOI:10.1016/j.phrs.2019.104403.
3. **Chen YC, Greenbaum J, Shen H, et al** (2017): *Association between gut microbiota and bone health: Potential mechanisms and prospective.* J Clin Endocrinol Metab, **102**, 3635–3646. DOI:10.1210/jc.2017-00513.
4. **Collins FL, Rios-Arce ND, Schepper JD, et al** (2017): *The potential of probiotics as a therapy for osteoporosis.* Microbiol Spectr, **5**, 1-16. DOI:10.1128/microbiolspec.BAD-0015-2016.
5. **Han D, Wang W, Gong J, et al** (2024): *Microbiota metabolites in bone: Shaping health and confronting disease.* Heliyon, **10**, e28435. DOI:10.1016/j.heliyon.2024.e28435.
6. **Jha SS, Jeyaraman N, Jeyaraman M, et al** (2025): *Cross-talks between osteoporosis and gut microbiome.* World J Orthop, **16**, 102274. DOI: 10.5312/wjo.v16.i3.102274.
7. **Jian C, Carpén N, Helve O, et al** (2021): *Early-life gut microbiota and its connection to metabolic health in children: Perspective on ecological drivers and need for*

quantitative approach. EBioMedicine, **69**, 103475. DOI: 10.1016/j.ebiom.2021.103475.

8. **Jiang X, Stockwell BR, Conrad M** (2021): *Ferroptosis: mechanisms, biology and role in disease.* Nat Rev Mol Cell Biol, **22**, 266–282. DOI: 10.1038/s41580-020-00324-8.
9. **Kho ZY, Lal SK** (2018): *The human gut microbiome – a potential controller of wellness and disease.* Front Microbiol, **9**, 1835. DOI: 10.3389/fmicb.2018.01835.
10. **Li R, Boer CG, Oei L, et al** (2021): *The gut microbiome: A new frontier in musculoskeletal research.* Curr Osteoporos Rep, **19**, 347–357. DOI: 10.1007/s11914-021-00675-x.
11. **Lucas S, Omata Y, Hofmann J, et al** (2018): *Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss.* Nat Commun, **9**, 55. DOI: 10.1038/s41467-017-02490-4.
12. **Miyagawa K, Kozai Y, Ito Y, et al** (2011): *A novel underuse model shows that inactivity but not ovariectomy determines the deteriorated material properties and geometry of cortical bone in the tibia of adult rats.* J Bone Miner Metab, **29,** 422-436. DOI: 10.1007/s00774-010-0241-9.
13. **Ohlsson C, Sjögren K** (2015): *Effects of the gut microbiota on bone mass.* Trends Endocrinol Metab, **26**, 69–74. DOI: 10.1016/j.tem.2014.11.004.
14. **Onbaşılar İ, Yalçın S, Eser H, et al** (2025): *Combined use of essential oils with organic acids in modifying performance, intestinal health, caecal microflora, and selected blood and bone parameters in broilers.* Ankara Univ Vet Fak Derg, **72**, 377-386. DOI:10.33988/auvfd.1613810.
15. **Principi N, Esposito S** (2016): *Antibiotic administration and the development of obesity in children.* Int J Antimicrob Agents, **47**, 171–177. DOI: 10.1016/j.ijantimicag.2015.12.017.
16. **Quach D, Collins F, Parameswaran N, et al** (2018): *Microbiota reconstitution does not cause bone loss in germ-free mice.* mSphere, **3**, e00545-17. DOI:10.1128/mspheredirect.00545-17.
17. **Sjögren K, Engdahl C, Henning P, et al** (2012): *The gut microbiota regulates bone mass in mice.* J Bone Miner Res, **27**, 1357–1367. DOI:10.1002/jbmr.1588.
18. **Tulstrup MV, Christensen EG, Carvalho V, et al** (2015): *Antibiotic treatment affects intestinal permeability and gut microbial composition in Wistar rats dependent on antibiotic class.* PLoS One, **10**, e0144854. DOI: 10.1371/journal.pone.0144854.
19. **Tulstrup MV, Roager HM, Thaarup IC, et al** (2018): *Antibiotic treatment of rat dams affects bacterial colonization and causes decreased weight gain in pups.* Commun Biol, **1**, 145. DOI: 10.1038/s42003-018-0140-5.
20. **Turnbaugh PJ, Ley RE, Hamady M, et al** (2007): *The human microbiome project.* Nature, **449**, 804–810. DOI:10.1038/nature06244.
21. **Tyagi AM, Darby TM, Hsu E, et al** (2021): *The gut microbiota is a transmissible determinant of skeletal maturation.* eLife, **10**, e64237. DOI:10.7554/eLife.64237.
22. **Vahidi G, Moody M, Welhaven HD, et al** (2023): *Germ-free C57BL/6 mice have increased bone mass and altered matrix properties but not decreased bone fracture resistance.* J Bone Miner Res, **38**, 1154–1174. DOI: 10.1002/jbmr.4835.
23. **Wallimann A, Magrath W, Thompson K, et al** (2021): *Gut microbial-derived short-chain fatty acids and bone: A potential role in fracture healing.* Eur Cell Mater, **41**, 454–470. DOI:10.22203/eCM.v041a29.
24. **Wang Y, Li Y, Bo L, et al** (2023): *Progress of linking gut microbiota and musculoskeletal health: causality, mechanisms, and translational values.* Gut Microbes, **15**, 2263207. DOI: 10.1080/19490976.2023.2263207.
25. **Wang Z, Yan Q, Wang Z, et al** (2024): *Ferroptosis and its implications in bone-related diseases.* PeerJ, **12**, e18626. DOI: 10.7717/peerj.18626.
26. **Wawrzyniak A, Balawender K** (2022): *Structural and metabolic changes in bone.* Animals, **12**, 1946. DOI: 10.3390/ani12151946.
27. **Whisner CM, Castillo LF** (2018): *Prebiotics, bone and mineral metabolism.* Calcif Tissue Int, **102**, 443–479. DOI: 10.1007/s00223-017-0339-3.
28. **Williams HR, Chin TE, Tokach MD, et al** (2023): *The effect of bone and analytical methods on the assessment of bone mineralization response to dietary phosphorus, phytase, and vitamin D in nursery pigs.* J Anim Sci, **101**, skad353. DOI: 10.1093/jas/skad353.
29. **Wilkins AT, Reimer RA** (2021): *Obesity, early life gut microbiota, and antibiotics.* Microorganisms, **9**, 413. DOI: 10.3390/microorganisms9020413.
30. **Wu J, Zhang S, Wu J, et al** (2026): *Targeting the gut-bone axis through exercise: A novel approach to osteoporosis prevention and treatment.* Int J Mol Med, **57**, 91. DOI: 10.3892/ijmm.2026.5762.
31. **Wu KC, McCauley KE, Lynch SV, et al** (2024): *Alteration in the gut microbiome is associated with changes in bone metabolism after laparoscopic sleeve gastrectomy.* J Bone Miner Res, **39**, 95–105. DOI: 10.1093/jbmr/zjad017.
32. **Xu P, Lin B, Deng X, et al** (2022). *VDR activation attenuates osteoblastic ferroptosis and senescence by stimulating the Nrf2/GPX4 pathway in age-related osteoporosis*. Free Radic Biol Med, **193**, 720–735. DOI:10.1016/j.freeradbiomed.2022.11.013.
33. **Zaiss MM, Jones RM, Schett G, et al** (2019): *The gut-bone axis: how bacterial metabolites bridge the distance.* J Clin Invest, **129**, 3018–3028. DOI: 10.1172/JCI128521.

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