Probiotics as a New Regulator for Bone Health: A Systematic Review and Meta-Analysis

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1.Introduction

Bone health is critically important to the overall health and quality of life and depends on the balance of bone resorption and bone formation [1]. Low bone mineral density (BMD) value is an indicator of osteoporosis or fracture and one of the major public health problems [2]. Significant disability, increased dependency, reduced quality of life, and increased economic burden to the health care system are the most consequences of reduced BMD [3–5]. BMD is affected by
2. Materials and Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement with the aim of assessing the relation between probiotic consumption and bone health parameters and has been recorded in PROSPERO.

2.1. Search Strategy. Previous publications on the effect of probiotic consumption and bone health status were selected through searching in PubMed/Medline, ISI Web of Science, SCOPUS, Cochrane, and EMBASE up to December 2020. The following key words were used in this search: probiotics OR symbiotic OR Lactobacillus OR Bifidobacterium for the intake of probiotics, and osteoporosis OR fracture OR “bone mineral density” OR BMD OR “bone mineral content” OR “alkaline phosphatase” OR osteocalcin OR “procollagen type I N-terminal propeptide” OR hydroxyproline OR “NF-κB ligand” for bone health status (Supplemental Table 1). In PubMed, keywords were searched through [tiab] and [MeSH] tags. No limitation was applied during the search. The reference lists of retrieved papers were also examined to avoid missing any published data. Finally, articles in the English language were included.

2.2. Inclusion and Exclusion Criteria. Two investigators independently selected the articles through the mentioned search strategy. Publications that fulfilled the following criteria were eligible for inclusion: (1) all studies with clinical trial design or experimental design (animal studies); (2) studies that examined the relationship between probiotic consumption and bone health status parameters; and (3) those that reported quantity findings for probiotic consumption and bone health parameters. We excluded letters, comments, reviews, meta-analyses, ecological, and in vitro studies as well as duplicate studies. Inclusion criteria based on PICOS include the following: Population, adults (for human studies) and other animals for experimental studies; Intervention/Exposure, probiotic consumption; Comparison, consumption and nonconsumption of probiotics; Outcome: bone health parameters; Study design: clinical trial design or experimental design (animal studies).

2.3. Data Extraction. For each eligible study, the following information was extracted: first author, year of publication, study design, country, age range, gender, sample size (number of participants in each group), type of intervention, duration of intervention, the dose of probiotic intake in the intervention group, characteristics of the control group, outcome variables, the mean and standard deviation of bone health parameters in the intervention and control groups, and quality score.

2.4. Quality Assessment of Studies. The risk of bias for the included studies was evaluated using the Cochrane quality assessment tool for RCTs. Two independent investigators assessed the quality of studies using the following seven criteria: (i) random sequence generation, (ii) allocation concealment, (iii) blinding of participants and personnel, (iv) blinding of outcome assessment, (v) incomplete outcome data, (vi) selective reporting, and (vii) other probable sources of biases. To evaluate the quality of studies, each study was allocated a label (yes, no, or unclear) indicating that it was judged as low risk, high risk, or unknown risk of bias, respectively [27].
All steps of the methods were performed by two investigators independently including searching, article screening, and data extracting, and checking the quality of articles. Disagreements between the two investigators were resolved by discussion and consensus.

2.5. Statistical Analysis. Mean differences ± SDs of measures such as chemical bone health parameters and BMD, comparing probiotic consumption to control, were used to calculate the overall effect sizes. When mean differences ± SDs were not reported, we calculated them by considering changes in each parameter throughout the study. In addition, these parameters were reported in different units across the studies. We converted them to the same units. We over all effects size was calculated by using different units across the studies. We converted them to the study. In addition, these parameters were reported in different units across the studies. We converted them to the same units. The overall effect size was calculated by using a random effects model, which takes between-study variation into account. Cochran’s Q test and I² statistic were used to assess between-study heterogeneity. Sensitivity analysis was used to explore the extent to which inferences might depend on a particular study or group of studies. Publication bias was examined by visual inspection of funnel plots and the application of Egger’s and Begg’s tests. We used kappa statistics to assess the consonant between investigators. All statistical analyses were conducted by using STATA version 14.2 (StataCorp). P values < 0.05 were considered significant.

3. Results

In total, 1123 articles were found in our initial search. After exclusion of duplicate studies and screening nonrelated articles based on title and abstract, 75 articles were remained. We further excluded 31 papers because of the following reasons: (1) those that examined the effect of probiotic consumption on gut microbiota without considering the effects of probiotics on bone health status or assessed the relationship between gut microbiota and bone health parameters without intervention (n = 16); (2) publications in which no effect sizes were reported (n = 3); and (3) those that had observational design (cohort, case-control, or cross-sectional design) (n = 12). After these exclusions, 44 papers remained for the current systematic review (Figure 1). Two investigators independently selected the articles through the mentioned search strategy and they had high agreement (0.90). The disagreement between the two investigators was resolved by the opinion of the third one.

3.1. Animal Studies. Characteristics of 37 animal studies on the effects of probiotics on bone parameters are presented in Table 1. These investigations are published between 2004 and 2020. Most of them were performed on rats except for four studies performed on chocks and hens [28–31]. Target species of rats were Sprague-Dawley in 10 studies [32–41], C57BL/6 mice in 9 studies [42–50], Wistar rat in 5 studies [51–55], and BALB/c mice in 5 studies [56–59]. The other studies used senescence-accelerated mouse (SAMP) [60, 61], virgin fisher rat [62], and N4 Swiss Webster retired breeder mice [63]. Out of 37 studies, 17 publications were performed on male [29, 33, 35, 38, 41, 45, 46, 48, 49, 52–55, 60, 61, 63, 64], 17 on female [30, 32, 34, 36, 37, 39, 40, 42, 44, 50, 51, 56, 57, 59, 62, 65], and 3 on both gender [28, 31, 43]. In fourteen investigations, female rats had ovariectomy surgery that induced osteoporosis [36, 37, 39, 40, 42, 44, 47, 51, 56, 57, 59, 62, 66], and in two studies, diabetic rats were included [38, 45]. Animals were fed by L. reuteri [30, 31, 40, 43, 45, 46, 56, 57, 64], L. casei [34, 38–40, 51, 63], L. paracasei [42, 44, 54, 65], L. plantarum [42, 47, 63, 65], L. acidophilus [40, 55, 59, 67], B. bifidum [40, 53, 55, 63], B. longum [37, 47, 52, 53], B. subtilis [28, 29, 55], L. helveticus [32, 37, 41], L. bulgaricus [33, 35, 63], Enterococcus faecium [30, 31, 55], and L. rhamnosus [48, 49, 64]. Other studies used B. breve [53, 63], B. animalis [30, 31], Streptococcus thermophilus [33, 35], Pediococcus acidilactici [30, 31], Escherichia coli [49, 64], Lactococcus lactis [60, 61], Bacillus licheniformis [28], Clostridium butyrium [29], Bacillus coagulans [40], and Pasteurized Akkermansia muciniphila [50]. The dosage and complete name and species of probiotics were reported in Table 2. The sample size varied from 1 [61] to 120 [30] in each group. The duration of intervention was between 9 days [33, 35] and 11 months [61]. Although probiotic feeding had increased calcium [39], phosphorus [42, 48], 25-OH-D [40, 50], PTH [33], osteocalcin (OC) [33, 36, 44, 50, 51], and alkaline phosphatase (ALP) [40] levels in some investigations, reduced levels of ALP [39], acid phosphatase (ACP) [65], urinary calcium [41], and phosphorus [48] were observed in others. These different findings might be due to different age, sex, estrogen status, duration of intervention, and sample sizes. In terms of BMD, increased BMD in different sites were reported in previous publications, total [34, 35, 44, 51, 55], tibia [12, 30–32, 35, 54, 59], femur [30, 31, 49, 59], and calcaneus [35]. An increase in BMC was also reported in five investigations [30, 31, 40, 43, 57]. Trabecular thickness [34, 39, 43–45, 50, 51, 56, 59], bone volume [32, 34, 36, 38, 39, 42–44, 49, 50, 57, 59, 65], tibia length [29], femur weight [38, 65], and bone phosphorus [28, 52] and bone calcium [52, 53] were also affected by probiotic feeding in animals. In eight publications, probiotic feeding showed no effects on bone parameters [37, 46, 47, 60–64].

3.2. Clinical Trials. Characteristics of seven clinical trials regarding the effects of probiotic consumption on bone health status presented in Table 2. These studies are published between 2004 and 2020. Three of the publications were performed in European countries [20, 21, 68], two in Asian countries [19, 23], and two in the USA and Canada [22, 69]. All studies had randomized study design except for two of them [22, 68]. Out of seven included clinical trials, three studies were conducted on healthy postmenopausal women [19, 21, 68], two on postmenopausal women with osteopenia [20, 23], one in hypercholesterolemic adults [69], and one in overweight and obese adults [22]. Sample sizes were varied from 10 to 66 in the intervention group and 10 to 61 in the control group. The dosage and complete name and species of probiotics are reported in Table 2. Supplements contained 1.5 * 10⁸ to 5 *
10^10 CFU of probiotics per dose. Several types of probiotics were consumed: *L. helveticus* [21], *L. reuteri* [68, 69], *B. subtilis* [19], and combination of various species [20, 22, 23]. The duration of intervention was between 1 day [21] and 12 months [20, 68]. Increased calcium [21] and 25-OH-D [69] level, and decreased parathyroid hormone [21, 23, 70], collagen type 1 cross-linked C-telopeptide (CTX) [20, 23], and bone-specific alkaline phosphatase (BALP) [23] level were demonstrated in these publications. Also, increased total hip BMD [19], and reduced BMD loss in L2-L4 [20], femoral neck [20], trochanter [20], and tibia [68] were indicated in these investigations. Almost all of the publications had high-quality score according to the Cochrane quality assessment tool for RCTs, except for one of them [22]. This study assessed the effect of symbiotic and probiotic consumption and BMC. Due to lack of effect sizes, we could not perform meta-analyses in BMC. Therefore, all high-quality score studies were included in different meta-analyses.

3.3. Meta-Analysis. Some chemical parameters such as level of serum calcium, serum phosphorus, PTH, and urinary calcium had enough effect sizes (at least 3 effect sizes) to perform a meta-analysis to calculate combined results of probiotic consumption on bone health parameters. Most of clinical trials in this regard assessed the relation between probiotic consumption and bone health parameters in women 50 years and older. Combining four effect sizes of three studies indicated that probiotic consumption had significantly increased serum calcium levels (weighted mean difference (WMD): 3.82 mmol/l; 95% CI: 1.05, 6.59 mmol/l; I²-square = 98.0%, P < 0.0001) (Figure 2(a)) [21, 23, 69]. Although significant heterogeneity was reported, low number of included studies did not let us to perform subgroup analysis and find source of heterogeneity. Combining four effect sizes of three studies, we did not find any significant effect of probiotic consumption on serum phosphorus levels (WMD: 1.14 mmol/l; 95% CI: −0.44, 2.73 mmol/l) (Figure 2(b)) [21, 23, 69]. In terms of PTH levels, probiotic consumption significantly decreases PTH levels (WMD: −5.53 ng/l; 95% CI: −9.83, −0.86 ng/l, I²-square = 98.2%, P < 0.0001) (Figure 3(a)) [21, 23]. Combining three effect sizes of two studies, we found that probiotic consumption significantly influences urine calcium levels (WMD: 4.85 mmol/l; 95% CI: 1.16,
Table 1: Characteristics of studies that reported the relationship between probiotic consumption and bone health in animals.

| Author (year)          | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results ± SD (Int vs. ctrl) |
|------------------------|----------------|-----------------|--------------------|----------------|---------------|----------|---------|-----------------------------|
| Narva et al. (2004)    | 6-week-old, male, Sprague-Dawley rats | 5 groups (n=10-11 per group) | 1. L. helveticus (LBK16H)-fermented milk + solid content of the same milk 2. Saccharomyces-fermented milk (Saccharomyces cerevisiae and L. helveticus bacteria) | 1: 150kJ/100g 2: 200kJ/100g | 1-sour milk fermented with a Lactococcus sp.-mixed culture 2:skim milk 3:water | 14 weeks | BMC (g) | Int1: 0.451 ± 0.010  Int2: 0.409 ± 0.009 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 0.469 ± 0.008  Ctrl2: 0.460 ± 0.008 |
|                        |                |                 |                    |                |               |          | RMD (g/cm³) | Int1: 0.228 ± 0.003  Int2: 0.248 ± 0.002 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 0.240 ± 0.005  Ctrl2: 0.240 ± 0.005 |
|                        |                |                 |                    |                |               |          | U-Ca (mg/d) | Int1: 2.3 ± 0.5  Int2: 1.7 ± 0.3 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 0.8 ± 0.1  Ctrl2: 1.6 ± 0.2 |
|                        |                |                 |                    |                |               |          | Bone weight (g) | Int1: 0.9 ± 0.2  Int2: 0.9 ± 0.2 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 0.9 ± 0.1  Ctrl2: 1.0 ± 0.1 |
|                        |                |                 |                    |                |               |          | Bone volume (cm³) | Int1: 3.0 ± 0.2  Int2: 3.0 ± 0.2 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 2.9 ± 0.1  Ctrl2: 3.0 ± 0.1 |
|                        |                |                 |                    |                |               |          | Bone length (cm) | Int1: 3.5 ± 0.3  Int2: 3.5 ± 0.3 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 3.6 ± 0.3  Ctrl2: 3.6 ± 0.3 |
|                        |                |                 |                    |                |               |          | Ca (mg/g) | Int1: 162.0 ± 1.3  Int2: 164.7 ± 1.3 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 169.9 ± 1.9  Ctrl2: 168.6 ± 1.9 |
|                        |                |                 |                    |                |               |          | P (mg/g) | Int1: 74.6 ± 0.7  Int2: 75.8 ± 1.1 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 75.0 ± 0.8  Ctrl2: 74.6 ± 0.8 |
| Muñoz et al. (2006)   | 1-day-old broiler chicks (both genders) | 50 (n=25 per each group) | Diet (corn, soybean meal, and wheat) + Bacillus licheniformis and Bacillus subtilis (each containing 2.3 × 10⁸ CFU/g of spore) | 1: 150kJ/100g 2: 200kJ/100g | 1: sour milk fermented with a Lactococcus sp.-mixed culture 2:skim milk 3:water | 6 weeks | Modulus of elasticity (kg/cm²) | Int1: 5192  Int2: 4487 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 5192  Ctrl2: 4487 |
|                        |                |                 |                    |                |               |          | Yield stress (kg/cm²) | Int1: 83.48  Int2: 81.56 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 11.26  Ctrl2: 10.06 |
|                        |                |                 |                    |                |               |          | Bone-P (%) | Int1: 23.63  Int2: 22.52 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 23.63  Ctrl2: 22.52 |
|                        |                |                 |                    |                |               |          | Bone-Ca (%) | Int1: 23.63  Int2: 22.52 |
|                        |                |                 |                    |                |               |          |                     | Ctrl1: 23.63  Ctrl2: 22.52 |
| Author (year)       | Target species          | Sample size (n) | Intervention group                                                                 | Probiotic dose                                                                 | Control group                  | Duration | Outcome | Results (mean ±SD (Int vs. ctrl)) |
|---------------------|-------------------------|-----------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------|----------|---------|----------------------------------|
| Mathy et al. (2007) | 90-day-old female Wistar rats | 70              | Soy protein free powdered semipurified diet (control group)                          | Soy protein free powdered semipurified diet + genistein                        | Soybean-protein-free powdered semipurified diet | 90 days  |         |                                  |
|                     |                         |                 | 2. Soy protein free powdered semipurified diet + genistein                          | Soy protein free powdered semipurified diet + daidzein                         | Soybean-protein-free powdered semipurified diet |         |         |                                  |
|                     |                         |                 | 3. Soy protein free powdered semipurified diet + daidzein                          | Soy protein free powdered semipurified diet + equol                          | Soybean-protein-free powdered semipurified diet |         |         |                                  |
|                     |                         |                 | 4. Soy protein free powdered semipurified diet + equol                          | Soy protein free powdered semipurified diet + daidzein + short chain ROS       | Soybean-protein-free powdered semipurified diet |         |         |                                  |
|                     |                         |                 | 5. Soy protein free powdered semipurified diet + daidzein + short chain ROS       | Soy protein free powdered semipurified diet + Lactobacillus case               | Soybean-protein-free powdered semipurified diet |         |         |                                  |

**Outcome Measures**

- **Total BMD (g/cm²)**
  - OVX: 0.220
  - G: 0.234
  - D: 0.239
  - E: 0.239
  - D + FOS: 0.245
  - D + L: 0.241
  - OVX: 0.209
  - G: 0.221
  - D: 0.225
  - E: 0.228
- **Diaphyseal BMD (g/cm²)**
  - OVX: 0.209
  - G: 0.221
  - D: 0.225
  - E: 0.228
  - D + FOS: 0.230
  - D + L: 0.232
  - OVX: 0.222
  - G: 0.246
  - D: 0.248
  - E: 0.251
- **Metaphyseal BMD (g/cm²)**
  - OVX: 0.222
  - G: 0.246
  - D: 0.248
  - E: 0.251
  - D + FOS: 0.260
  - D + L: 0.262
  - OVX: 0.287
  - G: 0.302
  - D: 0.304
  - E: 0.306
- **Femoral failure load**
  - OVX: 110.6
  - G: 119.8
  - D: 119.8
  - E: 119.8
  - D + FOS: 119.8
  - D + L: 119.8
  - OVX: 110.6
  - G: 119.8
  - D: 119.8
  - E: 119.8
  - D + FOS: 119.8
  - D + L: 119.8
- **OC (ng/ml)**
  - OVX: 2.91
  - G: 5.93
  - D: 5.93
  - E: 5.93
  - D + FOS: 5.93
  - D + L: 5.93
  - OVX: 2.93
  - G: 5.95
  - D: 5.95
  - E: 5.95
  - D + FOS: 5.95
  - D + L: 5.95
- **Trabecular thickness (um)**
  - OVX: 3.91
  - G: 4.89
  - D: 4.89
  - E: 4.89
  - D + FOS: 4.89
  - D + L: 4.89
  - OVX: 3.91
  - G: 4.89
  - D: 4.89
  - E: 4.89
  - D + FOS: 4.89
  - D + L: 4.89
  - OVX: 4.89
  - G: 6.95
  - D: 6.95
  - E: 6.95
  - D + FOS: 6.95
  - D + L: 6.95

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### Table 1: Continued.

| Author (year)          | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome                  | Results (mean ± SD or n) |
|------------------------|----------------|-----------------|--------------------|----------------|---------------|----------|--------------------------|--------------------------|
| Narva et al. (2007)    | 3-month-old female Sprague-Dawley rats | 50 (10 in each group) | 1. Tap water | 60ml | Sham-operated group receiving tap water (sham) | 12 weeks | Cortical BMD of tibia (mg/cm²) | Sham: 105.25 vs. O VX: 112.15 |
|                        |                |                  | 2. 30 mg/L synthesized VPP peptide in water |                |               |          | Trabecular BMD of proximal tibia (mg/cm³) | Sham: 245.75 vs. O VX: 105.30 |
|                        |                |                  | 3. 60 mg/L synthesized VPP peptide in water |                |               |          | Cortical BMD of proximal tibia (mg/cm³) | Sham: 126.61 vs. O VX: 125.6 |
|                        |                |                  | 4. Lactobacillus helveticus-fermented milk containing VPP peptide 21 mg/L |                |               |          | Bone volume per tissue volume (%) | Sham: 3.73 vs. O VX: 3.51 |
|                        |                |                  |                    |                |               |          | Tibial thickness (µm) | Sham: 44.00 vs. O VX: 46.65 |
|                        |                |                  |                    |                |               |          | Tibial cortical thickness (µm) | Sham: 38.51 vs. O VX: 40.65 |

| Kimoto-Nira et al. (2007) | SAMP male, 6 months | 2 (± 1 in each group) | Cornmeal+wheat+fishmeal+soy+oil+calcium carbonate+vitamin/mineral (control) + strain H61 Lactobacillus lactis subsp. cremoris H61 (strain H61) | 8 months | Bone density (mg/cm²) | 44.42 vs. 38.49 |

| Kimoto-Nira et al. (2009) | Male, 1-month-old, SAMP 6 mice | Eighteen per experimental group | Cornmeal+wheat+fishmeal+soy+oil+calcium carbonate+vitamin/mineral (control) + strain H61 Lactobacillus lactis G50 | 11 months | Bone density (mg/cm²) | 37.7 ± 0.88 vs. 36.5 ± 0.68 |

| Houshmand et al. (2010) | 1-day male broiler chicks | 150 (6 groups, n = 25) | Low-calcium diet + probiotic | 1 × 10⁸ CFU/kg Bacillus subtilis and 1 × 10⁸ CFU/kg Lactobacillus butyricum | Basal diet containing recommended level of calcium (0.9%) | 21 days | Tibial length (cm) | Not detected in all groups |
|                        |                            |                          | 2. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × Pre: 0.09 ± 0.09 |
|                        |                            |                          | 3. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × Sym: 0.05 ± 0.09 |
|                        |                            |                          | 4. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 5. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 6. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 7. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 8. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 9. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |

|                        |                            |                          | 1 × 10⁸ CFU/kg Bacillus subtilis and 1 × 10⁸ CFU/kg Lactobacillus butyricum | Basal diet containing recommended level of calcium (0.9%) | 21 days | Tibial length (cm) | Not detected in all groups |

|                        |                            |                          | 2. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × Pre: 0.09 ± 0.09 |
|                        |                            |                          | 3. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × Sym: 0.05 ± 0.09 |
|                        |                            |                          | 4. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 5. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 6. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 7. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 8. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
|                        |                            |                          | 9. Low-calcium diet + probiotic |                |               |          | Tibial length (cm) | LC × OA: 0.05 ± 0.09 |
### Table 1: Continued.

| Author (year)     | Target species | Sample size (n) | Intervention group                                                                                                                                  | Probiotic dose                                                  | Control group                          | Duration | Outcome | Results mean ± SD (Int vs. ctrl) |
|-------------------|----------------|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------|----------|---------|----------------------------------|
| Chiang and Pan (2011) | Female 2-month-old C57BL/6J mice | 48 (40 mice were OVX, and 8 SH mice) | 1. Phosphate-buffered saline (OVX)  
2. Fosormin, 0.2 mg of alendronic acid, 8 units of calcitriol per week (FOS)  
3. 0.1 g of freeze-dried powder of soy skim milk fermented by *L. plantarum* NTU 101 (NTU 101F)  
4. 0.1 g of freeze-dried powder of soy skim milk fermented by *L. plantarum* NTU 102 (NTU 102F)  
5. 0.1 g of freeze-dried powder of nonfermented soy skim milk (NFSM) | Phosphate-buffered saline (n=8, sham) | 8 weeks | ACP (U/L) | Phosphate-buffered saline | 8 weeks | ACP (U/L) | Phosphate-buffered saline | 8 weeks | ACP (U/L) |
|                   |                |                 |                                                                                                                                                    |                                                                                                              |                                            |          |         |                                  |          |         |
| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results (mean ± SD; Int vs. ctrl) |
|--------------|----------------|----------------|--------------------|----------------|---------------|----------|---------|---------------------------------|
| Chiang et al. (2011) | Female BALB/c mice, 3 months old | 20 each group (n = 5) | 1. 0.05 g freeze-dried powder of soy skim milk fermented by *L. plantarum* NTU 101 (NTU 101F) 2. 0.05 g freeze-dried powder of soy skim milk fermented by *L. plantarum* NTU 102 (NTU 102F) 3. 0.05 g freeze-dried powder of nonfermented soy skim milk (NFSM) | *Lactobacillus* paracasei subsp. paracasei strain NTU 101F (3.0 × 10^{11} CFU/g) & NTU 102F (3.9 × 10^{11} CFU/g) | Phosphate-buffered saline (PBS; control, ctrl) | 10 months | Right femur weight (%) | NUT101F: 0.7 ± 0.0 NUT102F: 0.6 ± 0.1 NFSM: 0.7 ± 0.0 Ctrl: 1.5 ± 0.1 NUT101F: 1.6 ± 0.0 NUT102F: 1.6 ± 0.1 NFSM: 1.6 ± 0.1 Ctrl: 3.9 ± 0.5 NUT101F: 9.8 ± 0.3 NUT102F: 9.5 ± 0.3 NFSM: 10.2 ± 0.5 Ctrl: 7.0 ± 0.7 | Right femur length (cm) 1.6 ± 0.0 1.6 ± 0.1 1.6 ± 0.1 3.9 ± 0.5 9.8 ± 0.3 9.5 ± 0.3 10.2 ± 0.5 7.0 ± 0.7 | Ca (mg/dL) | Ctrl: 9.8 ± 0.5 NUT101F: 9.0 ± 0.3 NUT102F: 9.1 ± 0.3 NFSM: 10.2 ± 0.5 Ctrl: 7.0 ± 0.7 | Cup: 0.0 ± 0.0 | P (mg/dL) | Ctrl: 7.0 ± 0.7 NUT101F: 7.3 ± 0.7 NUT102F: 7.8 ± 0.5 NFSM: 8.8 ± 0.5 Ctrl: 78.7 ± 8.2 | ALP (U/L) | Ctrl: 76.2 ± 6.4 NUT101F: 69.2 ± 9.3 NUT102F: 70.0 ± 6.4 Ctrl: 52.2 ± 0.4 NUT101F: 3.9 ± 1.1 NUT102F: 3.6 ± 0.7 NFSM: 3.6 ± 0.1 Ctrl: 0.751 | ACP (U/L) | Ctrl: 0.723 NUT101F: 0.799 NUT102F: 0.799 NFSM: 0.736 Ctrl: 1.08 | BMD (g/cm²) | NUT101F: 0.946 NUT102F: 1.088 NFSM: 1.095 Ctrl: 0.969 | Trabecular bone volume (%) | Ctrl: 0.0 ± 0.0 | NUT101F: 0.0746 NUT102F: 2.38 NFSM: 1.95 | Trabecular thickness (mm) | Ctrl: 0.0 ± 0.0 | NUT101F: 0.0776 NUT102F: 0.735 |
Table 1: Continued.

| Author (year)          | Target species                    | Sample size (n) | Intervention group | Probiotic dose | Control group            | Duration | Outcome | Results’ mean ± SD (Int vs. Ctrl) |
|------------------------|-----------------------------------|-----------------|--------------------|----------------|--------------------------|----------|---------|-----------------------------------|
| Takasugi et al. (2011) | Malerats, aged 3 weeks, Sprague–Dawley rats | 32 (8 in each group) | 1. DFL             | Dairy product fermented by Lactobacillus helveticus and Streptococcus thermophilus (DFL) | 1. Casein-based diet | 9 days |                                      |
|                        |                                   |                 | 2. DFL + PPI       |                |                          |          |         | CTD: 599 ± 12                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 599 ± 12                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 402 ± 9                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 506 ± 8                       |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 555 ± 9                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 296 ± 7                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 303 ± 6                       |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 303 ± 6                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 200 ± 5                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 251 ± 8                       |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 257 ± 16                |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 357 ± 9                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 265 ± 6                       |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 265 ± 6                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 169 ± 6                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 236 ± 6                       |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 252 ± 8                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 13 ± 0.5                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 197 ± 0.6                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 197 ± 0.6                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 151 ± 3                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 208 ± 0.7                     |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 242 ± 5                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 13 ± 0.5                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 175 ± 0.4                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 175 ± 0.4                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 146 ± 3                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 183 ± 0.4                     |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 163 ± 4                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 104 ± 2                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 105 ± 2                       |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 105 ± 2                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 142 ± 3                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 147 ± 0.4                     |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 131 ± 3                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 16 ± 0.2                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CTD: 90 ± 2                        |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 90 ± 2                        |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 115 ± 3                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 117 ± 0.4                     |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 107 ± 2                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 143 ± 9                      |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | Total P (mg/ml)                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 150 ± 6                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 196 ± 8                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 196 ± 8                       |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 194 ± 8                 |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 796 ± 47                     |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | OC (ng/ml)                         |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 965 ± 60                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 956 ± 60               |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 705 ± 26                      |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 784 ± 55                |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 78 ± 4.10                    |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | CIX (ng/ml)                        |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 199 ± 6.6                    |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 215 ± 3                |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 57 ± 7                        |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 60 ± 5                  |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 41 ± 2.1                     |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | PTH (pg/ml)                        |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 713 ± 16                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 165 ± 40               |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 165 ± 40                      |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 36 ± 1.8                     |
|                        |                                   |                 |                    |                |                          |          |         |                                      |
|                        |                                   |                 |                    |                |                          |          |         | 25-OH-D (nmol/l)                   |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 53 ± 2.2                     |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl + PPI: 53 ± 2.2               |
|                        |                                   |                 |                    |                |                          |          |         | DFL: 58 ± 1.7                      |
|                        |                                   |                 |                    |                |                          |          |         | DFL + PPI: 58 ± 0.5                |
|                        |                                   |                 |                    |                |                          |          |         | Ctrl: 32 ± 0.9                     |

**Evidence-Based Complementary and Alternative Medicine**
| Author(s) (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results (mean ± SD) |
|-----------------|----------------|---------------|--------------------|----------------|---------------|----------|---------|-------------------|
| Rodrigues et al. (2012) | Male Wistar rats, aged 6 months | 32 (n = 8 in each group) | 1. Yacon flour (Y) 2. Diet + L. longum (B) 3. Yacon flour + L. longum (YB) | Bifidobacterium longum; 10^9 CFU/mL | 1. Ctrl: AIN-93G diet 28 days |  |  |  |
| Shim et al. (2012) | Sprague-Dawley female rats, 10 weeks | 38 (sham, n = 8; OVX, n = 40) | 1. Bilaterally OVX (OVX) 2. Bilaterally OVX + 0.3 g/kg of HRT (HRT-0.3) 3. Bilaterally OVX + 1.0 g/kg of HRT (HRT-1.0) 4. Bilaterally OVX followed by 0.3 g/kg of HRT (OVX-HRT-0.3) 5. Bilaterally OVX + 10 g/kg of HRT (HRT-10) | Lactobacillus casei KFRI-127; 1.5 x 10^9 CFU/mL | Sham operated (sham); normal diet without supplement 3 months |  |  |  |
| McCabe et al. (2013) | C57Bl/6 mice, 16 weeks (both gender) | 20 (n = 10 in each group) | Normal diet with supplementation formula | Lactobacillus rhamnosus ATCC 8475; 50(μl) of 1 x 10^9 CFU/mL | Normal diet without supplement 4 weeks |  |  |  |
Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results | Notes |
|---------------|----------------|----------------|--------------------|----------------|---------------|----------|---------|---------|-------|
| Takasugi et al. (2013) | 3-week-old male Sprague–Dawley rats | 56 (n=8 in each group) | 1. Normal (ctrl diet+vehicle) 2. Low GOL (HO) + PPI 3. High GOL (HO) + PPI 4. DFL + PPI 5. DFL + HO + PPI | Dairy product fermented by Lactobacillus bulgaricus and Streptococcus thermophiles (DFL) | 1. Ctrl: modified AIN-93G diet + PPI | 9 days | Cortical BMD (mg/cm³) | Normal: 518 ± 36 Ctrl: 475 ± 21 HO: 483 ± 21 DFL: 540 ± 21 DFL + HO: 535 ± 13 DFL + HO+ PPI: 542 ± 30 |  |
| Britton et al. (2014) | BALB/c mice 12 weeks of age, non-OVX and OVX, female | 24 (n=8 in each group) | 1. OVX with normal diet 2. OVX + L. reuteri with normal diet | 300μl (1 x 10^8 CFU/ml) L. reuteri ATCC PTA 6675 or MRS gavaging 15 μl 100 CFU/ml L. reuteri added water | Non-OVX (ctrl) with normal diet | Four weeks | Femur trabecular BMD (mg/cc) | Ctrl: 246 ± 1.2 OVX: 179 ± 7 OVX + Lr: 222 ± 11 |  |
Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results (mean ± SD) |
|---------------|----------------|----------------|-------------------|----------------|---------------|----------|---------|---------------------|
| Ohlsson et al. (2014) | Six-week-old C57BL/6N female mice | 60 (n = 10 in each group) | 1. OVX vehicle 2. OVX L. para 3. OVX L. mix | Lactobacillus strain, L. paracasei DSM13454 (L. para) or a mixture of three strains, L. paracasei DSM13454, L. plantarum DSM 15312 and DSM 15313 supplementation | 1. Vehicle sham 2. L. para sham 3. L. mix sham | 6 weeks | Trabecular bone volume (%) | OVX vehicle 13.2 ± 0.7 L. para sham 13.2 ± 0.7 L. mix sham 13.2 ± 0.7 |
| | | | | | | | | | Vehicle sham 32.2 ± 0.9 L. para sham 32.2 ± 0.9 L. mix sham 32.2 ± 0.9 |
| | | | | | | | | BMD (mg/cm²) | Vehicle sham 40 ± 0.7 L. para sham 40 ± 0.7 L. mix sham 40 ± 0.7 |
| | | | | | | | | Trabecular thickness (mm) | Vehicle sham 40 ± 0.7 L. para sham 40 ± 0.7 L. mix sham 40 ± 0.7 |
| | | | | | | | | OC (ng/ml) | Vehicle sham 105 ± 1.0 L. para sham 105 ± 1.0 L. mix sham 105 ± 1.0 |
| | | | | | | | | Ca (mg/dl) | Vehicle sham 105 ± 1.0 L. para sham 105 ± 1.0 L. mix sham 105 ± 1.0 |
| | | | | | | | | 25(OH)D3 (ng/ml) | Vehicle sham 105 ± 1.0 L. para sham 105 ± 1.0 L. mix sham 105 ± 1.0 |
Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results (mean ± SD) |
|---------------|----------------|----------------|-------------------|----------------|---------------|----------|---------|---------------------|
| Parvaneh et al. (2015) | 10-week-old female mature Sprague-Dawley rats | 24 (n = 8) | G2: OVX, G3: OVX + B. longum (OVX + B. longum). | 1 mL of B. longum (10^8-10^9 CFU/mL) | G1: sham-ovariectomized (sham) | 16 weeks | Osteocalcin (ng/ml) | Sham: 184.61 ± 6.93, OVX: 76.81 ± 4.44, OVX + B.: 101.31 ± 9.21, Sham: 2.27 ± 0.12 |
| | | | | | | | Ca (mmol/L) | Sham: 2.27 ± 0.06, OVX: 2.27 ± 0.06, Sham: 2.06 ± 0.14, OVX: 2.40 ± 0.36, OVX + B.: 2.40 ± 0.36, Sham: 7.96 ± 0.45, OVX: 8.01 ± 0.51, Sham: 7.93 ± 0.65, OVX: 4.53 ± 0.27 |
| | | | | | | | Trabecular thickness (mm) | Sham: 106 ± 0.02, OVX: 99.9 ± 0.07, Sham: 0.99 ± 0.06 |
| | | | | | | | Bone volume (%) | Sham: 22.4 ± 2.1, NS: 20.9 ± 2.8, DSI: 18.4 ± 2.3, NS: 4.8 ± 2.2 |
| | | | | | | | Femur trabecular thickness (μm) | DSI: 45 ± 2, NS: 47 ± 3, DSI: 50 ± 2, NS: 49 ± 17, DSI: 95 ± 32, NS: 64 ± 10, DSI: 82 ± 32, NS: 14 ± 5.5, DSI: 16 ± 9.5, NS: 14 ± 7.3, DSI: 16 ± 2.98 |
| | | | | | | | BMC (mg) | Sham: 2.27, NS: 2.27, DSI: 2.27, NS: 2.27, DSI: 2.27 |
| | | | | | | | Bone volume (%) | Sham: 31.2 ± 0.5, NS: 32.0 ± 0.5, DSI: 34.8 ± 0.5, NS: 36.3 ± 0.5, DSI: 38.9 ± 0.5 |
| | | | | | | | Trabecular thickness (um) | Sham: 42.5 ± 0.7, NS: 42.5 ± 0.7, DSI: 42.5 ± 0.7, NS: 42.5 ± 0.7, DSI: 42.5 ± 0.7 |
| Zhang et al. (2015) | Adult (14 weeks old) C57BL/6 male mice | 40 (n = 10 in each group) | 1. Normal diet + gavage L. reuteri (control mice) 2. Normal diet + gavage L. reuteri (diabetic mice) 3. Normal diet (diabetic mice) | Lactobacillus reuteri ATCC PTA 6475 (10^9 colony-forming units/mL) Normal diet without gavage (control mice) | 4. weeks | BMD (mg/cc) | Control: 223.05, Diabetic: 177.23, Control + L. reuteri: 238.62, Diabetic + L. reuteri: 237.75 |
| | | | | | | | BMC (mg) | Control: 0.581, Diabetic: 0.502, Control + L. reuteri: 0.634, Diabetic + L. reuteri: 0.657 |
| | | | | | | | Trabecular thickness (mm) | Control: 42.5 ± 0.7, Diabetic: 42.5 ± 0.7, Control + L. reuteri: 42.5 ± 0.7, Diabetic + L. reuteri: 42.5 ± 0.7 |
| | | | | | | | Bone volume (%) | Control: 31.2 ± 0.5, Diabetic: 21.8 ± 1.7, Control + L. reuteri: 35.7 ± 1.7, Diabetic + L. reuteri: 35.7 ± 1.7 |
| Collins et al. (2016) | Female BALB/c mice 11 weeks of age | 4 group (n = 9-18 per group) | 1. Non-surgery (NS) + L. reuteri 2. Dorsal surgical incision (DSI) + L. reuteri | Lactobacillus reuteri ATCC PTA 6475 (10^9 colony-forming units/mL) | 4 or 8 weeks | BMD (mg/cc) | NS: 22 ± 2.1, NS + L. reuteri: 20.9 ± 2.8, DSI: 18.4 ± 2.3, DSI + L. reuteri: 18.4 ± 2.3, NS: 4.8 ± 2.2 |
| | | | | | | | BMC (mg) | NS: 2.2 ± 0.5, NS + L. reuteri: 2.2 ± 0.5, DSI: 2.2 ± 0.5, DSI + L. reuteri: 2.2 ± 0.5 |
| | | | | | | | Bone volume (%) | NS: 31.2 ± 0.5, NS + L. reuteri: 32.0 ± 0.5, DSI: 34.8 ± 0.5, DSI + L. reuteri: 36.3 ± 0.5 |
| | | | | | | | Trabecular thickness (μm) | NS: 42.5 ± 0.7, NS + L. reuteri: 42.5 ± 0.7, DSI: 42.5 ± 0.7, DSI + L. reuteri: 42.5 ± 0.7 |

Evidence-Based Complementary and Alternative Medicine
Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results mean ±SD (Int vs. ctrl) |
|---------------|----------------|----------------|--------------------|----------------|----------------|----------|---------|---------------------------------|
| Scholz-Ahren et al. (2016) | Virgin female Fisher-344 rats, 21-week | 80 (n=15-16 in each group) | 1. PRO: L. acidophilus NCC90 | f1–5×10⁸ CFU per 100g | Sham OVX | 16 weeks | Trabecular thickness (mm) | OVX: 0.020 ± 0.002  PRO: 0.020 ± 0.004  PRE: 0.020 ± 0.003  SYN: 0.023 ± 0.003  ONX: 0.015  PRO: 0.016  PRE: 0.015  SYN: 0.014  ONX: 0.012  PRO: 0.012  PRE: 0.012  SYN: 0.011  |
|              |                |                |                    |                |                |          | U-Ca (mg/7d) | OVX: 9.07 (4.07 vs. 4.40)  PRO: 9.83 (4.75 vs. 4.40)  PRE: 10.42 (5.25 vs. 4.60)  SYN: 10.77 (5.37 vs. 4.60)  |
|              |                |                |                    |                |                |          | Ca-femur (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | Ca-lumber vertebra (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | U-Ca (mg/7d) | OVX: 9.07 (4.07 vs. 4.40)  PRO: 9.83 (4.75 vs. 4.40)  PRE: 10.42 (5.25 vs. 4.60)  SYN: 10.77 (5.37 vs. 4.60)  |
|              |                |                |                    |                |                |          | Ca-femur (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | Ca-lumber vertebra (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | U-Ca (mg/7d) | OVX: 9.07 (4.07 vs. 4.40)  PRO: 9.83 (4.75 vs. 4.40)  PRE: 10.42 (5.25 vs. 4.60)  SYN: 10.77 (5.37 vs. 4.60)  |
|              |                |                |                    |                |                |          | Ca-femur (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | Ca-lumber vertebra (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | U-Ca (mg/7d) | OVX: 9.07 (4.07 vs. 4.40)  PRO: 9.83 (4.75 vs. 4.40)  PRE: 10.42 (5.25 vs. 4.60)  SYN: 10.77 (5.37 vs. 4.60)  |
|              |                |                |                    |                |                |          | Ca-femur (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | Ca-lumber vertebra (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | U-Ca (mg/7d) | OVX: 9.07 (4.07 vs. 4.40)  PRO: 9.83 (4.75 vs. 4.40)  PRE: 10.42 (5.25 vs. 4.60)  SYN: 10.77 (5.37 vs. 4.60)  |
|              |                |                |                    |                |                |          | Ca-femur (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
|              |                |                |                    |                |                |          | Ca-lumber vertebra (mg) | OVX: 8.41 (4.10 vs. 4.05)  PRO: 8.64 (4.30 vs. 4.25)  PRE: 10.04 (5.03 vs. 4.95)  SYN: 10.40 (5.20 vs. 5.05)  |
| Bayat et al. (2018) | Male Sprague-Dawley rats (aged 3-4 months) | 65 (n=13 in each group) | 1. Diabetic group (STZ): they were fed 1 ml/day of distilled water | | | | Tibia weight (mg) | Ctrl: 405 ± 14  D: 229 ± 22  SM: 310 ± 75  PSM: 312 ± 70  OP: 316 ± 74  |
|              |                |                |                    |                |                |          | Tibia volume (mm³) | Ctrl: 243 ± 18  D: 124 ± 20  SM: 178 ± 46  PSM: 179 ± 49  OP: 181 ± 50  |
|              |                |                |                    |                |                |          | Tibia trabeculae volume (mm³) | Ctrl: 119 ± 23  D: 57 ± 8  SM: 72 ± 25  PSM: 74 ± 28  OP: 86 ± 30  |
|              |                |                |                    |                |                |          | Vertebra weight (mg) | Ctrl: 318 ± 34  D: 239 ± 50  SM: 275 ± 31  PSM: 293 ± 29  OP: 315 ± 41  |
|              |                |                |                    |                |                |          | Vertebra volume (mm³) | Ctrl: 207 ± 42  D: 116 ± 22  SM: 154 ± 49  PSM: 155 ± 57  OP: 161 ± 57  |
|              |                |                |                    |                |                |          | Vertebra trabeculae volume (mm³) | Ctrl: 86 ± 23  D: 45 ± 10  SM: 50 ± 25  PSM: 51 ± 14  OP: 60 ± 17  |
| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results |
|--------------|----------------|----------------|-------------------|--------------|---------------|----------|---------|---------|
| Blanton et al. (2018) | Male 8-month-old NIH Swiss Webster retired breeder mice | 18 | 1–10% grape powder + 1% probiotic | Bifidobacterium breve, B. breve, Lactobacillus casei, L. plantarum, and L. bulgaricus at a concentration of 1.0×10^11 CFU/g | 5% probiotic | 6 months | Tibia bone volume (Tb.BV/TV (%)) | Ctrl: 5.9 ± 3.7
20%G: 6.4 ± 3.3
10%G + 1%P: 6.1 ± 3.2
20%G + 1%P: 5.4 ± 1.8
15%G: 6.4 ± 2.7
10%G: 0.0002 ± 0.007
20%G: 0.042 ± 0.007
15%G + 1%P: 0.003 ± 0.003

Tibia thickness (Tb.Th (mm)) | Ctrl: 6.1 ± 0.06
20%G: 6.4 ± 0.06
10%G + 1%P: 6.3 ± 0.17
15%G: 7.4 ± 0.5
10%G: 0.000 ± 0.000
20%G: 0.000 ± 0.000
15%G + 1%P: 0.000 ± 0.000

Femur bone volume (F.BV/TV (%)) | Ctrl: 5.6 ± 1.7
20%G: 6.4 ± 2.6
10%G + 1%P: 6.2 ± 3.7
15%G: 7.4 ± 1.5
10%G: 0.000 ± 0.000
20%G: 0.000 ± 0.000
15%G + 1%P: 0.000 ± 0.000

Femur thickness (F.Th (mm)) | Ctrl: 6.4 ± 2.7
20%G: 6.4 ± 2.6
10%G + 1%P: 6.4 ± 2.0
15%G: 7.4 ± 3.7
10%G: 0.000 ± 0.000
20%G: 0.000 ± 0.000
15%G + 1%P: 0.000 ± 0.000

| Dar et al. (2018) | Female mice (BALB/c) of 8–10 weeks | 30 (n = 10 in each group) | Group B: OVX | 200 μl of 10^7 CFU/mL Lactobacillus acidophilus (LA) ATCC 6066 | Group A: no probiotic + sham-operated | 6 weeks | BMD of LV (mgHA/cm^3) | Sham: 2.14 ± 0.07
OVX: 1.74 ± 0.20

BMD of femur trabecular (mgHA/cm^3) | Sham: 3.25 ± 0.06
OVX: 1.77 ± 0.00

BMD of tibia trabecular (mgHA/cm^3) | Sham: 3.35 ± 0.14
OVX: 1.54 ± 0.13

BMD of femur cortical (mgHA/cm^3) | Sham: 3.32 ± 0.08
OVX: 1.77 ± 0.09

BMD of tibia cortical (mgHA/cm^3) | Sham: 3.35 ± 0.14
OVX: 1.54 ± 0.13

Bone volume of LV5 (Bone volume of LV5 (%)) | Sham: 23.3 ± 0.04
OVX: 1.83 ± 0.04

Bone volume of femur trabecular (Bone volume of femur trabecular (%)) | Sham: 35.5 ± 0.04
OVX: 1.83 ± 0.04

Bone volume of tibia trabecular (Bone volume of tibia trabecular (%)) | Sham: 35.5 ± 0.04
OVX: 1.83 ± 0.04

Thickness of LV5 (mm) | Sham: 1.65 ± 0.00
OVX: 1.64 ± 0.16

Thickness of femur trabecular (mm) | Sham: 1.73 ± 0.04
OVX: 1.74 ± 0.04

Thickness of tibia trabecular (mm) | Sham: 1.70 ± 0.04
OVX: 1.74 ± 0.04

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| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results’ mean (SD) (Int vs. ctrl) |
|---------------|----------------|----------------|-------------------|----------------|---------------|----------|---------|---------------------------------|
| Eaimworawuthikul et al. (2018) | Six-week-old male Wistar rats | 48 (n=6 in each group) | 1. High fat diet + vehicle (HFV) 2. High fat diet + probiotic (HFPO) 3. High fat diet + prebiotic (HFPE) 4. High fat diet + symbiotic (HFS) | Lactobacillus paracasei HII01 1 x 10^8 CFU/ml/day | 1. Normal diet + vehicle (NDV) 2. Normal diet + probiotic (NDPO) 3. Normal diet + prebiotic (NDPE) 4. Normal diet + symbiotic (NDS) | 24 weeks | Trabecular volumetric BMD (g/cm³) | NDV: 0.978 NDPO: 0.881 NDPE: 0.878 NDS: 0.872 HFV: 0.881 HFPO: 0.877 HFPE: 0.883 HFS: 0.85 NDV: 24.11 NDPO: 32.39 NDPE: 35.37 NDS: 34.56 HFV: 20.72 HFPO: 19.98 HFPE: 21.71 HFS: 22.14 NDV: 0.325 NDPO: 0.327 NDPE: 0.340 NDS: 0.399 HFV: 0.306 HFPO: 0.245 HFPE: 0.267 HFS: 0.427 | Bone volume (%) | NDV: 24.11 NDPO: 32.39 NDPE: 35.37 NDS: 34.56 HFV: 20.72 HFPO: 19.98 HFPE: 21.71 HFS: 22.14 NDV: 0.325 NDPO: 0.327 NDPE: 0.340 NDS: 0.399 HFV: 0.306 HFPO: 0.245 HFPE: 0.267 HFS: 0.427 | Trabecular thickness (mm) | NDV: 0.325 NDPO: 0.327 NDPE: 0.340 NDS: 0.399 HFV: 0.306 HFPO: 0.245 HFPE: 0.267 HFS: 0.427 |
| Author/year | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results (mean ± SD) (Int vs. ctrl) |
|-------------|----------------|----------------|-------------------|---------------|---------------|----------|---------|-----------------------------------|
| Montazeri et al. (2018) | Adult female Sprague-Dawley rats (2-14 weeks old) | 49 (n = 7 in each group) | 1. OVX (normal diet) 2. OVX + Lactobacillus acidophilus 3. OVX + L. rhamnosus 4. OVX + Bifidobacterium 5. OVX + Lactobacillus reuteri | Lactobacillus, Streptococcus, Bifidobacterium 1.5×10^8 CFU/mL | Ctrl: normal diet 4 weeks | | | Ctrl: 19.96  
OVX: 14.49  
LBA: 16.14  
LBR: 16.47  
BB: 15.40  
LBA: 16.36  
Ctrl: 6.48  
OVX: 5.36  
LBA: 413  
LBC: 6.57  
BCO: 4.49  
BB: 5.11  
LBR: 5.35  
Ctrl: 10.06  
OVX: 9.45  
LBA: 9.77  
LBC: 304.13  
BCO: 4.72  
LBR: 70.87  |
| Parvani et al. (2018) | Mature Sprague-Dawley rats, aged 10 weeks, female | 24 (n = 8 in each group) | G2: OVX  
G3: OVX + L. helveticus | Lactobacillus helveticus (ATCC 27053) 1 mL of 10^9 CFU/mL of L. helveticus in phosphate buffer saline | G1: sham | 16 weeks | Bone volume (%)  
Bone mineral density (g/cm^3)  
Trabecular thickness (mm) | Sham: 78±9.50  
OVX: 49±5.7  
OVX + L: 75±6.6  
Sham: 78±5.5  
OVX: 5.3±0.4  
OVX + L: 5.8±0.5  
Sham: 107±0.22  
OVX: 97±6.08  
OVX + L: 94±0.03  |
| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results' mean (SD) | Int vs. ctrl |
|--------------|----------------|----------------|--------------------|----------------|---------------|----------|---------|-------------------|-------------|
| Yan et al. (2018) | 1-day-old Ross broiler chicks, both gender | 360 (n = 120 in each group) | 1. 0.5X regular diet + 0.5g/kg symbiotic | Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis, and Lactobacillus reuteri | Ctrl: regular diet | 40 days | Tibia BMD (g/cm²) | Ctrl: 0.209 | 0.5X: 0.216 |
| | | | | | | | Tíbula BMC (g) | Ctrl: 2.82 | 0.5X: 3.02 |
| | | | | | | Femur BMD (g/cm²) | Ctrl: 0.199 | 0.5X: 0.211 |
| | | | | | | Femur BMC (g) | Ctrl: 1.60 | 0.5X: 1.62 |
| | | | | | | Femur BMC (g) | Ctrl: 0.215 | 0.5X: 0.214 |
| | | | | | | Femur BMC (g) | Ctrl: 1X | 0.5X: 1.92 |
| | | | | | | Femur BMC (g) | Ctrl: 2X | 0.5X: 2.21 |
| | | | | | | Femur BMC (g) | Ctrl: 3X | 0.5X: 2.53 |
| Yan et al. (2018) | 56-week-old White Leghorn laying hens of the Hy-Line W-36 strain | 96 (n = 24 in each group) | 1-0.5X: 0.5g/kg (0.5 × 10⁶ CFU/g) | Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis, and Lactobacillus reuteri | Ctrl: regular diet | 7 weeks | Tibia BMC (g) | Ctrl: 2.25 | 0.5X: 2.35 |
| | | | | | | Femur BMC (g) | Ctrl: 1.04 | 0.5X: 1.07 |
| | | | | | | Femur BMC (g) | Ctrl: 4X | 0.5X: 1.07 |
| | | | | | | Femur BMC (g) | Ctrl: 2X | 1X: 1.10 |
| | | | | | | Keel BMC (g) | Ctrl: 2X | 1X: 0.73 |
| | | | | | | Keel BMC (g) | Ctrl: 3X | 0.5X: 0.71 |
| | | | | | | Keel BMC (g) | Ctrl: 4X | 0.5X: 0.71 |
| | | | | | | Keel BMC (g) | Ctrl: 8X | 0.5X: 0.71 |
| | | | | | | Keel BMC (g) | Ctrl: 1X | 0.5X: 0.97 |
| | | | | | | Keel BMC (g) | Ctrl: 2X | 1X: 1.05 |
| | | | | | | Keel BMC (g) | Ctrl: 3X | 2X: 0.99 |
| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results’ mean (SD) (Int vs. ctrl) |
|--------------|----------------|----------------|-------------------|---------------|---------------|----------|---------|----------------------------------|
| Achiet al. (2019) | Male Wistar rats, aged = NM | 48 (n = 8 in each group) | | 1- B. breve NCIM 5671, 2- B. longum NCIM 5672, 3- B. bifidum NCIM 5697 | Ctrl: regular diet | 15 days | | Ca-bone (mg/g) |
| | | | | | | | | B. breve: 182.94 ± 21.28 | B. longum: 160.86 ± 18.71 | B. bifidum: 163.20 ± 7.76 |
| | | | | | | | | Arthritic: 139.15 ± 18.49 | Piroxicam: 204.85 ± 14.02 |
| | | | | | | | | Ctrl: 204.85 ± 4.15 |
| | | | | | | | | K-bone (mg/g) |
| | | | | | | | | B. breve: 3.013 ± 0.60 | B. longum: 2.575 ± 0.68 | B. bifidum: 2.839 ± 0.83 |
| | | | | | | | | Arthritic: 2.007 ± 0.44 | Piroxicam: 3.143 ± 0.56 |
| | | | | | | | | Ctrl: 3.143 ± 0.56 |
| Coffins et al. (2019) | Male mice (12 weeks of age) wild-type (C57BL/6) and Rag1 knockout (Rag1tm1Mm, C57BL/6 background) | 42 (n = 11-11 in each group) | | 1-WT + LR, 2-RK | 3.3 × 10^8 CFU/ml of Lactobacillus reuteri ATCC PT 6475 | 4 weeks | | Femur trabecular bone volume (%) |
| | | | | | | | | WT + LR: 2.28 ± 3.33 | RK: 4.65 ± 2.23 |
| | | | | | | | | WT + LR: 265 ± 6.27 | RK: 260 ± 8.44 |
| | | | | | | | | WT + LR: 0.52 ± 0.011 | RK: 0.33 ± 0.011 |
| | | | | | | | | WT + LR: 0.06 ± 0.004 | RK: 0.05 ± 0.002 |
| | | | | | | | | WT + LR: 0.06 ± 0.004 | RK: 0.07 ± 0.006 |
| | | | | | | | | WT + LR: 7.97 ± 7.17 | RK: 7.96 ± 1.14 |
| | | | | | | | | WT + LR: 0.01 ± 0.002 | RK: 0.02 ± 0.003 |
| Lee et al. (2019) | Eight-week-old female Sprague-Dawley (SD) rats | 40 (n = 10 per group) | | 1-Sham-operated (sham), 2-O VX, 17- beta-estradiol (E2), 3: 500 mg/kg LAB-extract treated O VX (LAB) | OVX-control (OVX) | 8 weeks | | Alp (U/L) |
| | | | | | | | | Sham: 112.53 | OVX: 160.00 |
| | | | | | | | | E2: 62.95 | LAB: 75.21 |
| | | | | | | | | Sham: 44.06 | OVX: 17.28 |
| | | | | | | | | E2: 9.98 | LAB: 22.78 |
| | | | | | | | | Sham: 71.14 | OVX: 426.2 |
| | | | | | | | | E2: 54.62 | LAB: 52.13 |
| | | | | | | | | Sham: 16.83 | OVX: 4.20 |
| | | | | | | | | E2: 8.50 | LAB: 7.45 |
| | | | | | | | | Sham: 0.105 | OVX: 0.096 |
| | | | | | | | | E2: 0.111 | LAB: 0.018 |
Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome |
|---------------|----------------|-----------------|--------------------|----------------|---------------|---------|---------|
| Levi et al. (2019) | Male Wistar rats (Rattus norvegicus albinus), aged ≥ NM | 40 (n = 10 per group) | Group PROB (control + probiotic); group CSI (cigarette smoke inhalation); group CSI + PROB (CSI + probiotic) | Lactobacillus acidophilus (1 x 10^9 CFU/kg); Enterococcus faecium (2 x 10^9 CFU/kg); Bacillus subtilis (2.9 x 10^9 CFU/kg); Bifidobacterium bifidum (2 x 10^9 CFU/kg) | Group ctrl (control, without CSI and probiotic) | 6 months | Bone mineral density (BMD) | Ctrl: 0.780 ± 0.010; PROB: 0.901 ± 0.012; CSI: 0.478 ± 0.014; CSI + PROB: 0.713 ± 0.010 |
|               |                |                 |                    |                |               |         | Bone mineral volume (%) | Ctrl: 79.74 ± 2.010; PROB: 80.10 ± 2.010; CSI: 72.10 ± 2.010; CSI + PROB: 74.56 ± 2.010 |
| Liu et al. (2019) | Six-week-old male C57BL/6J mice | 5 group (n = 10–12) | 1. Sham group: normal saline; 2. LGG group: Tryptose phosphate broth (TDB) (43mg/kg) and LGG | Lactobacillus rhamnosus GG (5 x 10^8 CFU, ATCC) | 1. TDF group (negative control); 2. ZOL (zoledronate) group (positive control) | 8 weeks | Calcium (Ca mmol/L) | Sham: 2.02 ± 0.01; LGG: 1.98 ± 0.01; TDF: 1.97 ± 0.01; ZOL: 1.97 ± 0.01 |
|               |                |                 |                    |                |               |         | Phosphorous (P mmol/L) | Sham: 3.86 ± 0.01; LGG: 3.86 ± 0.01; TDF: 3.08 ± 0.01; ZOL: 3.18 ± 0.01 |
|               |                |                 |                    |                |               |         | Mandibular BMD (mg/cm²) | Sham: 1.29 ± 0.16; LGG: 1.177 ± 0.14; TDF: 1.011 ± 0.14; ZOL: 1.286 ± 0.14 |
|               |                |                 |                    |                |               |         | Mandibular BMC (mg) | Sham: 1.23 ± 0.16; LGG: 1.177 ± 0.14; TDF: 1.011 ± 0.14; ZOL: 1.286 ± 0.14 |
|               |                |                 |                    |                |               |         | Bone volume (%) | Sham: 58.65 ± 0.14; LGG: 57.56 ± 0.14; TDF: 64.92 ± 0.14; ZOL: 64.18 ± 0.14 |
|               |                |                 |                    |                |               |         | Trabecular thickness (mm) | Sham: 0.06 ± 0.01; LGG: 0.06 ± 0.01; TDF: 0.06 ± 0.01; ZOL: 0.07 ± 0.01 |
| Liu et al. (2019) | Mice aged 6 weeks and male (C57BL/6 ) | 5 group (n = 10–12) | (a) Sham group: normal saline (NS); (b) LGG + TDF group: 0.066mg TDF (84mg/kg body weight) + 5 x 10^8 CFU LGG; (c) E. coli + TDF group: TDF (43mg/kg body weight) + 5 x 10^8 CFU E. coli; (d) LGG (accession number KM815037); Escherichia coli (accession number KJ299222); (e) ZOL + TDF group: TDF (43mg/kg body weight) + ZOL (100μg/kg as a positive control) | LGG (accession number KM815037) | 1. TDF group (negative control); 2. ZOL (zoledronate) group (positive control) | 8 weeks | Femur BMD (mg/cm³) | Sham: 541.57 ± 2.01; LGG + TDF: 566.59 ± 2.01; E. coli + TDF: 415.57 ± 2.01; TDF: 421.57 ± 2.01; ZOL + TDF: 254.07 ± 2.01 |
|               |                |                 |                    |                |               |         | Femur BMC (mg) | Sham: 8.06 ± 0.01; LGG + TDF: 8.30 ± 0.01; E. coli + TDF: 3.86 ± 0.01; TDF: 3.86 ± 0.01; ZOL + TDF: 2.38 ± 0.01 |
|               |                |                 |                    |                |               |         | Bone volume (%) | Sham: 25.47 ± 2.01; LGG + TDF: 29.87 ± 2.01; E. coli + TDF: 16.7 ± 2.01; TDF: 16.7 ± 2.01; ZOL + TDF: 9.02 ± 2.01 |
|               |                |                 |                    |                |               |         | Trabecular thickness (mm) | Sham: 0.045 ± 0.01; LGG + TDF: 0.044 ± 0.01; E. coli + TDF: 0.039 ± 0.01; TDF: 0.038 ± 0.01; ZOL + TDF: 0.194 ± 0.01 |

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Table 1: Continued.

| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Results’ mean ± SD (Int vs. ctrl) |
|---------------|----------------|----------------|-------------------|----------------|---------------|----------|---------|----------------------------------|
| Schepp et al. (2019) | Eleven-week-old male BALB/c mice | 88 (n = 10–18 per group) | 1. ABX-antibiotic-treated (ampicillin 1.0 g/L and neomycin 0.5 g/L) 2. ABX + LR 3. ABX + LGG 4. ABX + EC 5. ABX + MDY (high-molecular-weight polymer) | 300 mL of L. reuteri 6475 (LR), Lactobacillus rhamnosus (LGG), nonpathogenic Escherichia coli (EC; ATCC O6; B1) 10^9 CFU/mL | Ctrl: normal diet | 4 week | BMC (mg) |
|               |                |                |                   |                |               |          |         | Ctrl 0.022 ± 0.001 ARX 0.022 ± 0.0005 ARX + 1.8 0.022 ± 0.0001 ARX + LGG 0.022 ± 0.001 ARX + EC 0.022 ± 0.0007 ARX + MDY 0.022 ± 0.001 | 0.029 ± 0.001 ARX 0.029 ± 0.0005 ARX + 1.8 0.29 ± 0.003 ARX + LGG 0.29 ± 0.005 ARX + EC 0.29 ± 0.005 ARX + MDY 0.29 ± 0.005 |
| Author (year)                  | Target species         | Sample size (n) | Intervention group       | Probiotic dose                  | Control group | Duration | Outcome | Results |
|-------------------------------|------------------------|-----------------|--------------------------|--------------------------------|---------------|----------|---------|---------|
| Lawenius et al. (2019)        | Twelve-week-old female C57BL/6 mice | 8–12/group      | Sham + pAkk              | Pasteurized Akkermansia muciniphila (pAkk) (2 x 10^8 CFU/150 µl) | Sham + Veh    | 4 week   |         | Femur length (mm) |
|                               |                        |                 |                          |                                |               |          |         | Sham: 25.9 ± 1.3 |
|                               |                        |                 |                          |                                | Sham: 25.7 ± 1.2 |
|                               |                        |                 |                          |                                | Sham: 25.8 ± 1.1 |
|                               |                        |                 |                          |                                | Sham: 25.6 ± 1.0 |
|                               |                        |                 |                          |                                | Sham: 25.4 ± 0.9 |
|                               |                        |                 |                          |                                | Sham: 25.2 ± 0.8 |
|                               |                        |                 |                          |                                | Sham: 25.1 ± 0.7 |
|                               |                        |                 |                          |                                | Sham: 25.0 ± 0.6 |
|                               |                        |                 |                          |                                | Sham: 24.9 ± 0.5 |
|                               |                        |                 |                          |                                | Sham: 24.8 ± 0.4 |
|                               |                        |                 |                          |                                | Sham: 24.7 ± 0.3 |
|                               |                        |                 |                          |                                | Sham: 24.6 ± 0.2 |
|                               |                        |                 |                          |                                | Sham: 24.5 ± 0.1 |

### Femur Cortical Thickness (mm)

- Sham + Veh: 0.197 ± 0.002
- Sham + pAkk: 0.192 ± 0.002
- OVX + Veh: 0.184 ± 0.001
- OVX + pAkk: 0.184 ± 0.002

### Vertebral Length (mm)

- Sham + Veh: 15.37 ± 0.15
- Sham + pAkk: 15.19 ± 0.07
- OVX + Veh: 15.61 ± 0.08
- OVX + pAkk: 15.27 ± 0.15

### Femur Cortical Thickness (mm)

- Sham + Veh: 4.02 ± 0.02
- Sham + pAkk: 4.00 ± 0.02
- OVX + Veh: 3.96 ± 0.001
- OVX + pAkk: 3.96 ± 0.001

### Vertebral Cortical Thickness (mm)

- Sham + Veh: 0.197 ± 0.002
- Sham + pAkk: 0.192 ± 0.002
- OVX + Veh: 0.184 ± 0.001
- OVX + pAkk: 0.184 ± 0.002

### 25(OH)D3 (ng/ml)

- Sham + Veh: 44.5 ± 1.3
- Sham + pAkk: 42.9 ± 1.0

### OC (ng/ml)

- Sham + Veh: 96.8 ± 3.8
- Sham + pAkk: 101.4 ± 3.6

### BMD (mg/cm²)

- Sham + Veh: 70.24
- Sham + pAkk: 70.54

### Bone Volume (%)

- Sham + Veh: 14.34
- Sham + pAkk: 14.03

### trabecular thickness (mm)

- Sham + Veh: 0.0478
- Sham + pAkk: 0.0442

### PTH (pg/ml)

- Sham + Veh: 235
- Sham + pAkk: 230

### Ca (mg/dl)

- Sham + Veh: 9.44
- Sham + pAkk: 9.44

### U-Ca

- Sham + Veh: 0.149
- Sham + pAkk: 0.120
| Author (year) | Target species | Sample size (n) | Intervention group | Probiotic dose | Control group | Duration | Outcome | Result mean (SD) (int vs. ctrl) |
|--------------|----------------|----------------|-------------------|----------------|---------------|----------|---------|--------------------------------|
| Kim et al. (2019) | Female C57BL/6 mice (6 weeks old for GV-induced BV and 11 weeks old for ovariectomy-induced osteoporosis) | 56 (n = 8 in each group) | 1. SH: vehicle (1% maltose) 2. OVX: vehicle (1% maltose) 3. OLP: 1 x 10⁹ CFU LP 4. OBL: 1 x 10⁹ CFU BL 5. OMX: 1 x 10⁹ CFU LP + BL 6. OCP: 1 mg/kg beta-estradiol | 1 x 10⁹ CFU Lactobacillus plantarum NK3 and Bifidobacterium longum NK49 | Ctrl: vehicle (1% maltose) | 2 weeks (6 days per week) | Femur weight (g) | Ctrl: 0.178, SH: 0.173, OVX: 0.180 |
| | | | | | | | | | Femur weight (g) | |
| | | | | | | | | | OLP: 0.178, OBL: 0.176, OMX: 0.175, OCP: 0.179, Ctrl: 0.280, SH: 0.286, OVX: 0.176 |
| | | | | | | | | | Ca (mmol/L) | OLP: 0.269, OBL: 0.304 |
| | | | | | | | | | OMX: 0.288, Ctrl: 0.265 |
| | | | | | | | | | P (mmol/L) | Ctrl: 0.971, SH: 0.920, OVX: 0.688 |
| | | | | | | | | | P (mmol/L) | OLP: 0.961, OBL: 0.962, OMX: 0.945, Ctrl: 0.862, SH: 0.498, OVX: 0.309 |
| | | | | | | | | | OC (pg/ml) | OLP: 0.699, OBL: 0.641, OMX: 0.570, Ctrl: 0.643 |

Tibiotarsal index = (diaphysis diameter – medullary canal diameter)/diaphysis diameter × 100 (Barnet and Nordin, 1980). Robusticity index = bone length/cube root of bone weight (Reisenfeld, 1972). Calcium (Ca), phosphorus (P), urinary calcium (U-Ca), parathyroid hormone (PTH), osteocalcin (OC), isoleucyl-prolyl-proline (IPP), valyl-prolyl-proline (VPP), collagen type 1 cross-linked C-telopeptide (CTX), 25-hydroxyvitamin D (25-OH-D), alkaline phosphatase (ACP), bone mineral density (BMD), bone mineral content (BMC), Hwangyoun- haedok-tang (HRT), fermented HRT (HHR T), sham-operated (SH), ovariectomized (OVX), tibia dyschondroplasia (TD), senescence-accelerated mouse (SAMP), and dorsal surgical incision (DSI). *Final results in each group were reported in the Results column. **Results with red color are significant (P value <0.05).
| Author (year) | Country | Study design | Population sex and age (y) | Sample size (n) | Intervention group (strain of probiotics) | Probiotic dose | Control group | Duration | Outcome | Results mean ± SD (Int vs. ctrl) | Quality score |
|--------------|---------|--------------|-----------------------------|----------------|------------------------------------------|----------------|--------------|----------|---------|---------------------------------|--------------|
| Narva et al. (2004) | Finland | Randomized double-blind crossover study | Postmenopausal women 50 to 78 y (65 y) | 20 | *Lactobacillus helveticus* LBK-16H bacteria/milk fermented 14.5 g/100 g IPP & VPP | Portion size 220 ml | Normal sour milk fermented with a *Lactococcus* sp. mixed culture (420 ml) | 1 day Int 6 day washout 1 day Int | | Ca (mmol/l) 0.09 ± 0.01 vs. 0.05 ± 0.02 (↑) | H |
| | | | | | | | | | | P (mmol/l) −0.09 ± 0.02 vs. −0.09 ± 0.02 | |
| | | | | | | | | | iCa (mmol/l) 0.03 ± 0.005 vs. 0.03 ± 0.004 | |
| | | | | | | | | | PTH (ng/l) −20.8 ± 5.3 vs. −15.4 ± 6.4 (↑) | |
| | | | | | | | | | ICTP (ug/l) −1.09 ± 0.28 vs. −1.15 ± 0.20 | |
| | | | | | | | | | U-Ca (mmol/l) 0.17 ± 0.5 vs. 0.10 ± 0.02 vs. 0.07 ± 0.01 (↓) | |
| | | | | | | | | | iCa (mmol/l) 0.005 ± 0.04 vs. 0.026 ± 0.005 | |
| | | | | | | | | | PTH (ng/l) −13.0 ± 4.7 vs. −23.7 ± 5.9 (↑) | |
| | | | | | | | | | ICTP (ug/l) −1.10 ± 0.10 vs. −1.08 ± 0.18 | |
| | | | | | | | | | U-Ca (mmol/l) 0.21 ± 0.07 (↓) | |
| Jones et al. (2013) | Canada | Double-blind, placebo-controlled, randomized, parallel-arm study | Hypercholesterolemic adults 34–64 y (50 y)/both gender 127 (61 Pbo) (66 Int) | 2 capsules (130 mg *L. reuteri* (2.9 × 10^8) + 170 mg MD) | Capsule supplement/ *Lactobacillus reuteri* NCIMB 30242 | 300 mg MD | 13 week | | | Ca (umol/l) −0.01 ± 0.001 vs. 0.01 ± 0.02 vs. −0.03 ± 0.001 | H |
| | | | | | | | | | P (umol/l) −0.01 ± 0.04 vs. 14.73 ± 13.38 | |
| | | | | | | | | | 25 (OH) D (nmol/L) −3.19 ± 5.63 (↑) | |
| Author (year) | Country | Study design                  | Population sex and age (y) | Sample size (n) | Intervention group (strain of probiotics) | Probiotic dose | Control group | Duration | Outcome | Quality score |
|---------------|---------|-------------------------------|-----------------------------|-----------------|------------------------------------------|----------------|---------------|----------|---------|---------------|
| Jafarnejad et al. (2017) | Iran | Randomized, double-blind, placebo-controlled study | Women with osteopenia 50–72 y (58 y) | 4L (20 Int) (21 Pbo) | Multispecies probiotic capsules (GeriLact) (7 bacteria species: *Lactobacillus casei* 1.3 × 10^10, *Bifidobacterium longum* 5 × 10^10, *Lactobacillus acidophilus* 1.5 × 10^10, *Lactobacillus rhamnosus* 3.5 × 10^8, *Lactobacillus bulgaricus* 2.5 × 10^8, *Bifidobacterium breve* 1 × 10^10, and *Streptococcus thermophilus* 1.5 × 10^6) | 1 capsule 500 mg | Placebo capsule 500 mg of corn starch | 6 months | | H |

Results mean ± SD (Int vs. ctrl) |
- Spinal BMD (g/cm^2) 0.001 ± 0.01 vs. 0.002 ± 0.01
- Total hip BMD (g/cm^2) −0.015 ± 0.01 vs. −0.016 ± 0.02
- BALP (U/L) −3.12 ± 0.76 vs. 0.82 ± 0.06
- OC (ng/ml) −0.16 ± 0.07 vs. −1.62 ± 0.2
- CTX (ng/ml) −0.03 ± 0.001 vs. −2.87 ± 0.14
- PTH (pg/ml) 7.4 ± 0.7 vs. 7.5 ± 0.4
- ALP (U/L) 0.75 ± 0.12 vs. 0.52 ± 0.04
- Ca (mg/dl) 0.29 ± 0.04 vs. 0.05 ± 0.001
- 25 (OH) D (ng/ml) 28.20 ± 0.97 vs. 29.08 ± 1
- U-Ca (mg/dl) 14.5 ± 0.5 vs. 3.8 ± 0.3
| Author et al. (year) | Country | Study design | Population sex and age (y) | Sample size (n) | Intervention group (strain of probiotics) | Probiotic dose | Control group | Duration | Outcome | Results mean ± SD (Int vs. ctrl) | Quality score |
|----------------------|---------|--------------|----------------------------|----------------|-----------------------------------------|---------------|---------------|----------|---------|---------------------------------|--------------|
| Lambert et al. (2017) | Denmark | Double-blind, parallel design, placebo-controlled, randomized controlled trial | Postmenopausal osteopenic women 59–64 y (61 y) | 78 (38 Int) (40 Pbo) | 60 mg isoflavone aglycones and acid lactic probiotics, cold fermentation | 2 Sachet 95 ml (RCE extract + probiotic) | 2 Sachet 95 ml (water + food color) | 12 month | | CTX (ng/ml) | −0.05 ± 0.13 vs. 0.03 ± 0.16 (†) | H |
| | | | | | | | | | | P1NP (ng/ml) | 1.56 ± 0.53 vs. 0.7 ± 0.56 | |
| | | | | | | | | | | OC (ng/ml) | −0.03 ± 0.29 vs. 0.69 ± 0.22 | |
| | | | | | | | | | | BMD changes L2–L4 (g/cm²) | −0.0085 (−0.017, 0.00006) vs. −0.022 (−20.032, 20.012) (†) | |
| | | | | | | | | | | BMD changes FN (g/cm²) | −0.004 (−0.01, 0.004) vs. −0.02 (−0.03, −0.15) (†) | |
| | | | | | | | | | | BMD changes troch (g/cm²) | −0.004 (−0.01, 0.004) vs. −0.017 (−0.025, −0.008) (†) | |
| Takimoto et al. (2018) | Japan | Randomized, placebo-controlled, double-blind clinical trial | Healthy women 50-69 y (57 y) | 61 (31 Int, 30 Pbo) | Tables contain soybean oil residue (0.34 mg) + *Bacillus subtilis* C-3102 | 0.19 g (3.4 × 10⁶) | 0.19 g | 26 weeks | | BMD changes L2–L4 (g/cm²) | 0.18 ± 0.50 vs. −0.68 ± 0.63 | H |
| | | | | | | | | | | BMD changes total hip (g/cm²) | 2.53 ± 0.52 vs. 0.83 ± 0.35 (†) | |

*Table 2: Continued.*
| Author (year)       | Country      | Study design                        | Population sex and age (y) | Sample size (n) | Intervention group (strain of probiotics) | Probiotic dose | Control group | Duration | Outcome |
|---------------------|--------------|-------------------------------------|----------------------------|----------------|-------------------------------------------|----------------|---------------|----------|---------|
| Nilsson et al. (2018) | Sweden       | Double-blind, placebo-controlled study | Women 75–80 y (76 y)        | 90 (45 Int, 45 Pbo) | Capsules contain *Lactobacillus reuteri* ATCCPTA 6475 + MD | 2 capsules $5 \times 10^9$ MD | 12 months       | Tibia total vBMD changes | 
|                     |              |                                     |                            |                |                                           |                |               |          | −0.83 (−1.47, −0.19) vs. −1.85 (−2.64, −1.07) (1) | H |
|                     |              |                                     |                            |                |                                           |                |               | BMD changes L2–L4 (g/cm²) | 0.78 (−0.54, 2.10) vs. 0.08 (−1.02, 1.19) |
|                     |              |                                     |                            |                |                                           |                |               | total hip vBMD changes (g/cm²) | −0.13 (−1.33, 1.07) vs. −0.90 (−2.07, 0.27) |
|                     |              |                                     |                            |                |                                           |                |               | BAP (U/L) | −4.83 (−13.8, 13.1) vs. 5.43 (−12.8, 22.0) |
| Sergeev et al. (2020) | The USA      | Placebo-controlled clinical trial    | Overweight and obese adults 31–62 y (47 y)/ both gender | 20 (10 Int, 10 Pbo) | Capsule (*Lactobacillus acidophilus* DDS-1, *Bifidobacterium lactis* UABla-12, *Bifidobacterium longum* UABl-14, and *Bifidobacterium bifidum* UABB-10) + a trans-galactooligosaccharide (GOS) | 69 mg ($1.5 \times 10^9$) 5.5g | Placebo capsule 3 month | BMC changes (kg) | 0.75 ± 0.05 vs. 0.16 ± 0.01 | L |
|                     |              |                                     |                            |                |                                           |                |               |          |                     |

Intervention (Int), placebo (Pbo), calcium (Ca), phosphate (P), serum ionised calcium (iCa), parathyroid hormone (PTH), carboxyterminal telopeptide of type I collagen (ICTP), urinary calcium (U-Ca), isoleucyl-prolyl-proline (IPP), valyl-prolyl-proline (VPP), maltodextrin (MD), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), control (ctrl), collagen type I cross-linked C-telopeptide (CTX), bone mineral density (BMD), bone mineral content (BMC), alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BAP), femoral neck (FN), lumbar spine (L), trochanter (Troch), procollagen type I N-terminal propeptide (PINP), 25-hydroxy vitamin D (25-OH-D), volumetric bone mineral density (vBMD), red clover extract (RCE), year (y), and gram (g). *Results are presented with mean differences and standard deviation, and red color results are significant ($P_{value} < 0.05$).
8.53 mmol/l; I-square = 97.6%, P < 0.0001) (Figure 3(b)) [21, 23]. Bone mineral density was calculated in different locations such as spinal, total hip, femoral neck, and troch. BMD at spinal and total hip had enough effect sizes, and we performed meta-analysis in these parameters. Combining four effect sizes of three studies indicated that probiotic consumption did not influence spinal BMD levels (WMD: 0.65 g/cm²; 95% CI: −0.18, 1.47 g/cm²) (Figure 4(a)) [19, 20, 23, 68]. In terms of total hip BMD, combining three effect sizes of three studies had shown nonsignificant increase in BMD level of total hip (WMD: 1.45 g/cm²; 95% CI: −0.38, 3.28 g/cm²) (Figure 4(b)) [19, 23, 68].

3.4. Sensitivity Analysis. To investigate the influence of each individual study on the overall findings, we excluded studies from the analysis, stage by stage, and found no significant impact of any individual study on the overall effect sizes.

3.5. Publication Bias. The funnel plots indicated moderate asymmetry, suggesting that publication bias cannot be completely excluded as a factor of influence on the present meta-analysis (data not shown). However, Begg’s and Egger’s regression tests provided no evidence of substantial publication bias.

4. Discussion

In this systematic review, we found some effects of probiotic supplementation on bone health parameters such as serum and urinary calcium levels and PTH levels. Some strains of Bifidobacterium and Lactobacillus such as L. reuteri, L. casei, L. paracasei, L. bulgaricus, and L. acidophilus indicated beneficial effects on bone health parameters in animal experiments and clinical trials.

Probiotic consumption has been assessed in few clinical trials. In most of them, probiotic consumption had beneficial effects on bone health parameters such as BMD, serum calcium, 25 (OH) D, and PTH levels. Only one clinical trial in this regard had reported no effect of probiotic consumption on bone health parameters that has been done on overweight and obese adults [22]. There are some critical points, which should be considered in the interpretation of the results of this study. This study had no randomization or blinding, so we cannot rule out the probable risk of bias. Notably, participants were obese or overweight, and we all know that this condition could change gut microbiota [71, 72] as well as hormonal status [70]. Considering all
Clinical trials, it seems that the consumption of probiotics may have positive effects on bone health in humans. Meta-analysis indicated that probiotic consumption improved some bone health parameters such as serum calcium levels and PTH. Significant heterogeneity is reported in our findings. Due to the small number of effect sizes, we could not use subgroup analyses to find the source of heterogeneity. Although studies in this area had high quality, they had some limitation. There are some differences between the previous included publications that could be the cause of heterogeneity. It seems that the mean age of the participants, gender, bone health status, and chronic conditions can justify the heterogeneity between studies.

Compared to clinical trials, the effects of probiotics on bone health have been addressed more in the animal experiments. Although in eight articles no effects of probiotics on bone health parameters were detected, most of the previous publications had reported beneficial effects of probiotic feeding on the bone health status of animals. There are several points that could explain the lack of connection between probiotic feeding and bone health parameters in these eight articles. Species of rats included in these surveys are different; virgin fisher rat [58], SAMP rat [60, 61], and ND4 Swiss Webster retired breeder mice [63] were used, while other studies used Sprague-Dawley rat, C57BL/6J mice, Wistar rat, and BALB/c mice. Sample sizes in one of these studies are very low (one rat in each group [60]), and older rats were included in these studies [37, 46, 47]. Considering the limitations of studies, it seems that probiotic feedings have beneficial effects on bone health parameters in animal experiments.

Gut microbiota is considered as an organ involving in mucosal barrier function, immune system, endocrine system, food digestion, and energy metabolism as well as bone health and metabolism [73–77]. Gut microbiota could regulate bone metabolism through the effects on the immune system, the endocrine organs, and calcium absorption. Some species of intestinal bacteria promote the release of inflammatory mediators, such as tumor necrosis factor-α (TNF α), interleukin (IL)-1, and IL-6 which plays an important role in the formation of osteoclasts and osteoblasts [78]. Intestinal microbiota also promotes the release of endothelial nitric oxide synthase (eNOS). eNOS mRNA regulates the production of osteoblasts and osteoclasts, as well as inflammatory mediators. NO has dichotomous biological effects, and at low concentrations, NO may promote proliferation, differentiation, and survival of osteoblasts, whereas at high concentrations, NO may inhibit bone

| Author (year) | SMD (95% CI) | Weight (%) |
|---------------|--------------|------------|
| Narva (2004)  | –0.92 (–1.57, –0.27) | 37.31      |
| Narva (2004)  | 2.01 (1.24, 2.77) | 37.20      |
| Jafarnejad (2017) | –22.53 (–27.59, –17.51) | 25.49      |
| Overall (I-squared = 98.2%, p = 0.000) | –5.35 (–9.83, –0.86) | 100.00     |

Note: weights are from random effects analysis

| Author (year) | SMD (95% CI) | Weight (%) |
|---------------|--------------|------------|
| Narva (2004)  | 0.04 (–0.58, 0.66) | 39.97      |
| Narva (2004)  | –1.05 (–1.72, –0.39) | 39.91      |
| Jafarnejad (2017) | 26.10 (20.27, 31.92) | 20.12      |
| Overall (I-squared = 97.6%, p = 0.000) | 4.85 (1.16, 8.53) | 39.91      |

Note: weights are from random effects analysis

Figure 3: Forest plots for the effect of probiotic consumption on (a) PTH levels and (b) urinary calcium levels, expressed as mean differences between intervention and the control diets.
resorption and formation. Therefore, at a certain concentration range, NO can avoid osteoclast-mediated bone resorption and promote osteoblast growth [79, 80]. Probiotics could affect gut microbiota and regulate immune cells and inflammatory cytokines or hormones and growth factors by inducing the host’s production of β-defensin and IgA. Probiotics may also be able to enhance the intestinal barrier function by maintaining tight junctions and inducing mucin production. Probiotic-mediated immunomodulation may occur through the mediation of cytokine secretion signaling pathways such as NFκB and MAPKs, which plays a vital role in the formation of osteoclasts and osteoblasts [81–83]. Gut microbiota has also critical effects on the endocrine system. Levels of serum IGF-1 can promote the differentiation and growth of bone cells, including osteoblasts and chondrocytes, and enhance normal interactions among them [84]. Moreover, the IGF-1 signaling pathway is involved in the regulation of bone metabolism via both growth hormone (GH) and PTH which directly and indirectly have effects on bone growth [85]. PTH is secreted from parathyroid glands which regulate calcium levels by increasing absorption of calcium in gut, decreasing calcium absorption in kidney and increasing bone resorption. Bone remodeling is a dynamic coordination process between bone formation with osteoblasts and resorption with osteoclasts [86]. Increasing PTH level leads to more bone destruction by osteoclasts. As we indicated in meta-analysis results, greater calcium levels reduced PTH levels and osteoclast activity. Moreover, gonadal steroids, including estrogen and androgen, play key roles in the regulation of bone mass and turnover [87]. Gut microbiota regulates bone metabolism by affecting the absorption of calcium. Calcium absorption can be facilitated by vitamin D. It has been shown that a low-calcium diet alone can lead to bone resorption, high bone turnover, and impaired bone trabecular microarchitecture in multiple bones. Balanced gut microbiota leads to reduced osteoclast activity and increased osteoblast activity within the bone matrix by these strategies, which ultimately results in increased bone structure, density, and strength [88].

The present study has some strengths and limitations. It is the first study that systematically reviews the relationship between probiotic consumption and bone health parameters. In addition, a comprehensive search strategy was performed and no limitation was applied during the search. Furthermore, it is the first publication that performed meta-analysis on probiotic supplemen-
tations and bone health parameters. We considered experimental and clinical studies, and we tried to clarify the mechanism. However, some points need to be considered. Participants in the included clinical trials had different health status, for example, hyperlipidemia or obesity, which might influence the results. Moreover, different

| Author (year)  | SMD (95% CI) | Weight (%) |
|---------------|-------------|------------|
| Jafarnejad (2017) | −0.10 (−0.71, 0.51) | 24.15 |
| Lambert (2017)   | 0.00 (−0.44, 0.44)  | 25.78 |
| Takimot (2018)   | 1.94 (1.33, 2.55)  | 24.16 |
| Nilsson (2018)   | 0.78 (0.35, 1.20)  | 25.91 |
| Overall (I-squared = 90.3%, p = 0.000) | 0.65 (−0.18, 1.47) | 100.00 |

Note: weights are from random effects analysis

Figure 4: Forest plots for the effect of probiotic consumption on (a) spinal BMD levels and (b) total hip BMD levels, expressed as mean differences between intervention and the control diets.
species and dosage of probiotics were used and it might impress findings. Although different probiotic species have different effects, due to the limited publications we combined findings of all studies. In addition, we could not perform meta-analyses in all parameters because of limited number of effect sizes. High heterogeneity was reported, and subgroup analyses could not be performed. Our findings might be considered as primary findings, and further studies should be designed on different bacterial species and strains.

5. Conclusion

In conclusion, in this systematic review, we found that probiotic supplementation containing L. reuteri, L. casei, L. paracasei, L. bulgaricus, L. acidophilus, and B. subtilis might improve bone health parameters in animal and human studies. Meta-analysis of human studies indicated that probiotic consumption has significantly increased serum and urinary calcium levels and decreased PTH level. Further studies are needed to decide on the appropriate probiotic species, strain, and dosages to improve bone health status.

Data Availability

The (effect sizes) data used to support the findings of this study are included within the article. The (search strategy) data used to support the findings of this study are also included within the supplementary information file.

Ethical Approval

The study was ethically approved by the Medical Ethics Committee of the Tehran University of Medical Sciences, Tehran, Iran.

Consent

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Hanieh Malmir, Hanieh-Sadat Ejtahad, and Shirin Hasani-Ranjbar contributed in conception, design, statistical analyses, data interpretation, and manuscript drafting. Ahmad-Reza Soroush, Amir-Mohammad Mortazavian, Noushin Fahimfar, Afshin Ostovar, Ahmad Esmailzadeh, and Bagher Larijani had scientific cooperation. All the authors approved the final manuscript for submission.

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Supplementary Materials

Supplementary table 1: search strategies and the number of publications in each electronic database. (Supplementary Materials)

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