PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO CIÊNCIA ANIMAL

JOÃO ALBERTO ARTONI DE CARVALHO

PREBIÓTICOS MELHORAM OS INDICADORES DE OSTEOPOROSE EM MODELO PRÉ-CLÍNICO: REVISÃO SISTEMÁTICA COM META-ANÁLISE

Presidente Prudente - SP
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Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientador:
Prof. Dr. Hermann Bremer Neto

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Presidente Prudente, 28 de Junho de 2019.

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*“*A mente que se abre a uma nova ideia jamais voltará ao seu tamanho original.”

Albert Einstein

RESUMO

Prebióticos melhoram os indicadores de osteoporose em modelo pré-clínico: revisão sistemática com meta-análise

Introdução: A osteoporose é a doença metabólica do tecido ósseo, caracterizada pela perda gradual de massa óssea e deterioração da microarquitetura do tecido ósseo em humanos, tendo como consequência a fragilidade óssea e risco de fraturas. O comprometimento ósseo acarreta aumento de custo na carga econômica nos sistemas de saúde com consultas médicas, hospitalizações e colocações em lares de idosos relacionados a fraturas osteoporóticas, sendo considerado um grave problema de saúde pública. **Objetivo:** Esta revisão sistemática com meta-análise examinou ensaios pré-clínicos para elucidar os efeitos dos alimentos prebióticos na osteoporose induzida em ratas ovariectomizadas. **Material e métodos:** Os dados e artigos em inglês utilizados na meta-análise foram obtidos através de buscas nos bancos eletrônicos de dados científicos disponíveis na internet: “Pub Med”, “ScienceDirect”, “Scielo” , “LILACS”, “Cochrane” e “Bireme” publicados antes de novembro de 2018. Encontrado 813 artigos completos, resumos ou capítulos de livros que citavam as palavras chaves utilizadas na pesquisa bibliográfica, após triagem de títulos e resumos, seis estudos atendiam os critérios de inclusão. Ao todo foram utilizados 116 animais, divididos randomicamente entre grupos controle e tratamento. Foram selecionados trabalhos completos de ensaios experimentais e que atendiam os seguintes critérios: (i) População: murinos (ratas/camundongos) ovariectomizadas; (ii) Intervenção: suplementação na dieta com prebióticos; (iii) Controle: avaliação dos efeitos nos grupos suplementados e não suplementados; (iv) Desfechos: avaliação de parâmetros indicadores de osteoporose. Usamos o software Review Mananger 5 para a realização dos cálculos da meta-análise e avaliação do risco de viés. **Resultados:** Os dados da densidade mineral óssea (DMO), conteúdo mineral ósseo (CMO) e biomecânica óssea dos seis estudos incluídos demonstraram que os prebióticos são capazes de melhorar significativamente estes parâmetros nos animais estudados.
Conclusão: Esta meta-análise forneceu evidências de que a atividade prebiótica esta envolvida nos mecanismos anti-osteoporóticos e sugere que novos estudos em humanos devam ser realizados usando prebióticos como uma alternativa terapêutica ou terapia complementar aos tratamentos convencionais da osteoporose pós-menopausa em humanos.

Palavras-chave: Densidade Mineral Óssea; Conteúdo Mineral Ósseo; Biomecânica óssea; Ratas ovariectomizadas; e Alimentos funcionais

ABSTRACT

Prebiotics improve osteoporosis indicators in preclinical model: systematic review with meta-analysis

Introduction: Osteoporosis is a metabolic disease of bone tissue, characterized by the gradual loss of bone mass and the microarchitectural deterioration of bone tissue in men, resulting in bone fragility and a risk of fractures. Bone impairment leads to an increase in the cost of health services with medical consultations, hospitalizations and hopices related to osteoporotic fractures, being a serious public health problem. **Objective**: This systematic review with meta-analysis aimed to elucidate the effects of prebiotics on induced osteoporosis in ovariectomized rats. **Material and methods:** The data and methods used in the statistical analysis were obtained through searches in the scientific databases available on the Internet: "Pub Med", "ScienceDirect", "SciELO", "LILACS", "Cochrane" and "bireme "2018 before. found 813 complete articles, abstracts or book chapters that cited the key words in the bibliographic research, after screening of titles and abstracts, six studies met the inclusion criteria, in total 116 animals were used, divided randomly between control groups and treatment. The following experimental works were selected: (i) Population: murine (rats / mice) ovariectomized; (ii) Intervention: dietary supplementation with prebiotics; (iii) Control: dosage of supplements and not supplemented; (iv) Outcomes: evaluation of osteoporosis indicators. We used the Risk Analysis software 5 to measure the meta-analysis calculations and bias risk assessment. **Results**: Bone mineral density (BMD), bone mineral content (BMD) and bone biomechanical data from the six included studies demonstrated that serum levels are able to register from them. **Conclusion:** This meta-analysis provided evidence that prebiotic activity is involved in anti-osteoporotic mechanisms and suggests that further studies in humans should be performed using prebiotics as a therapeutic alternative or therapy complementary to conventional postmenopausal osteoporosis treatments in humans.

Keywords: Bone Mineral Density; Bone Mineral Content; Bone biomechanics; Ovariectomized rats; and Functional Food.

LISTA DE SIGLAS

DMO	– Densidade Mineral óssea
CMO	– Conteúdo Mineral ósseo
PRISMA	– Itens de relatório para Revisões Sistemáticas e Meta-análises
LILACS	- Literatura Latino-americana e do Caribe em Ciências da Saúde
SCIELO	– Biblioteca Eletrônica Científica Online
DPD	– Dosagem de Deoxipiridinolina Plasmática
SAEAF	– Extrato Aquoso Padronizado de *Anoectochilus formosanus*
OVX	– Ratas Ovariectomizadas
OCN	- Osteocalcina
FOS	- Frutooligossacarídeo
GOS	- Galactooligossacarídeo
AGCC	- Ácido Graxo de Cadeia Curta
IL-7R	- Receptor de Interleucina 7
mRNA	- RNA mensageiro

SUMÁRIO

Prebióticos Melhoram os Indicadores de Osteoporose em Modelo Pré-Clínico: Revisão Sistemática com Meta-análise

João Alberto Artoni de Carvalho[1]; Leticia Rocha Magalhães[1]; Laryssa Mayara Polastri[1]; Ingrid Eloise Trobine Batista[1]; Marcos Natal Rufino[1]; Hermann Bremer-Neto[1]

Esta revisão sistemática com meta-análise examinou ensaios pré-clínicos para elucidar os efeitos dos alimentos prebióticos na osteoporose induzida em ratas ovariectomizadas. Os dados e artigos utilizados na meta-análise foram obtidos por meio de buscas nos bancos eletrônicos de dados científicos disponíveis na internet e publicados antes de novembro de 2018. Encontramos 813 artigos completos, resumos ou capítulos de livros que citavam as palavras chaves utilizadas na pesquisa bibliográfica, após triagem de títulos e resumos, seis estudos atendiam os critérios de inclusão, ao todo foram utilizados 116 animais, divididos randomicamente entre grupos controle e tratamento. Foram selecionados trabalhos completos de ensaios experimentais e que atendiam os seguintes critérios: (i) População: murinos (ratas/camundongos) ovariectomizadas; (ii) Intervenção: suplementação na dieta com prebióticos; (iii) Controle: avaliação dos efeitos nos grupos suplementados e não suplementados; (iv) Desfechos: avaliação de parâmetros indicadores de osteoporose. Nesta meta-análise, os dados da densidade mineral óssea (DMO), conteúdo mineral ósseo (CMO) e biomecânica óssea dos estudos incluídos, demonstraram que os prebióticos são capazes de melhorar significativamente estes parâmetros nos animais estudados. Em conclusão, essa meta-análise forneceu evidências de que a atividade prebiótica esta envolvida nos mecanismos anti-osteoporóticos e sugere que novos estudos em humanos devam ser realizados usando prebióticos como uma alternativa terapêutica ou terapia complementar aos tratamentos convencionais da osteoporose pós-menopausa em humanos.

Palavras-chave: Densidade mineral óssea, Conteúdo mineral ósseo, Biomecânica óssea, Ratas ovariectomizadas, Alimento funcional.

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INTRODUÇÃO

A Organização Mundial de Saúde (OMS) define osteoporose como uma doença esquelética sistêmica com diminuição da massa óssea e deterioração microarquitetural do tecido ósseo, tendo como consequência a fragilidade óssea e o risco de fratura, considerada um grave problema de saúde pública. Estima-se que atualmente mais de 200 milhões de pessoas no mundo sofram desta enfermidade, (WRIGHT et al., 2014) sendo mais comum em caucasianos, mulheres e pessoas idosas.

Essa doença crônica silenciosa permanece não diagnosticada até se manifestar como fraturas, dor, problemas de saúde secundários e até a morte. (SOZEN; OZISIK; CALIK BASARAN, 2017) O diagnóstico de osteoporose é determinado principalmente pela baixa densidade mineral óssea (DMO), redução do conteúdo mineral ósseo (CMO) e comprometimento da microarquitetura / mineralização assim como a diminuição da resistência óssea. O comprometimento ósseo relacionados a fraturas osteoporóticas acarreta o aumento nas consultas médicas, hospitalizações e colocações em lares de idosos e que contribuirão para uma maior carga econômica nos sistemas de saúde. [4]

Nos últimos anos, os principais avanços terapêuticos em humanos no tratamento da osteoporose foram feitos na medida em que os cientistas obtinham uma maior compreensão da morfologia óssea e dos mecanismos subjacentes que causam a doença. (TU et al., 2018) Ratas ovariectomizadas têm sido utilizadas como modelo experimental para osteoporose e demonstraram que alimentos funcionais com atividade prebiótica, isoladamente ou associados a fito-hormônios, estão envolvidos em mecanismos benéficos anti-osteoporóticos e possível potencial para o tratamento da osteoporose pós-menopausa. (DEVAREDDY et al., 2006; LEGETTE et al., 2011, 2012; MATHEY et al., 2004; MCCABE; BRITTON; PARAMESWARAN, 2015; TOUSEN et al., 2016; YANG et al., 2013)

Esta revisão sistemática com meta-análise examinou ensaios pré-clínicos para elucidar os efeitos dos alimentos prebióticos na osteoporose induzida em ratas ovariectomizadas e, assim, encorajar ensaios clínicos randomizados para avaliar a eficácia dos prebióticos na melhora dos indicadores da osteoporose em humanos.

MATERIAL E MÉTODOS

Esta meta-análise foi conduzida de acordo com as recomendações do Cochrane Handbook for Systematic Reviews of Interventions, (HIGGINS; GREEN, 2011) porém, o protocolo seguido não foi registrado. Para o relato, seguimos as diretrizes

descritas na Preferred Reporting Items for Systematic Reviews and Meta-analyses: The PRISMA statement. (MOHER et al., 2015; SHAMSEER et al., 2015)

Estratégia de Pesquisa

Os dados e artigos utilizados na meta-análise foram obtidos durante o mês de novembro de 2017. Foram realizadas buscas nos bancos eletrônicos de dados científicos disponíveis na internet: "Pub Med", "ScienceDirect", "Scielo", "LILACS", "Cochrane" e "Bireme". As palavras chaves pesquisadas foram "Osteoporose" e "Prebióticos", usadas em conjunto. Para tornar as buscas mais abrangentes, os trabalhos de interesse foram buscados sem restrições de datas de publicação, e as palavras chaves foram escritas nos idiomas português e inglês. Nesta fase, todos os resultados obtidos foram analisados sem restrição de idioma de publicação e categoria de trabalhos publicados, incluindo-se artigos completos, resumos e capítulos de livro. A estratégia de busca foi repetida no mês de novembro de 2018.

Critérios de Inclusão e Exclusão para Seleção de estudos

Os trabalhos foram preliminarmente selecionados a partir dos títulos e resumos. Os trabalhos elegíveis foram analisados de maneira independente por dois pesquisadores do grupo (Carvalho, J. A. A. e Batista, I. E. T.) e em caso de discordância um terceiro autor foi consultado. Registros duplicados e trabalhos que não eram relevantes para se alcançar os objetivos propostos para esta meta-análise foram removidos. Foram selecionados trabalhos completos de ensaios experimentais e que atendiam os seguintes critérios: (i) População: murinos (ratas/camundongos) ovariectomizadas; (ii) Intervenção: suplementação na dieta com prebióticos; (iii) Controle: avaliação dos efeitos nos grupos suplementados e não suplementados; (iv) Desfechos: avaliação de parâmetros indicadores de osteoporose.

Para avaliação dos estudos selecionados, utilizamos as determinações do Protocolo CONSORT. (SCHULZ; ALTMAN; MOHER, 2010) Foram incluídos estudos com pontuação ≥ 5 (pontuação máxima 7), conforme descrito no Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. (HIGGINS; GREEN, 2011) Foram excluídos capítulos de livros, resumos, ensaios clínicos com pacientes humanos, estudos pilotos, artigos de revisão, ensaios clínicos em animais e estudos que não contemplaram parâmetros relevantes para o objetivo desta meta-análise.

Extração de dados

Foi elaborada uma planilha com dados de cada estudo selecionado na etapa anterior: autor(s)/ano de publicação, prebiótico utilizado, tempo de duração do experimento, espécie e número de animais utilizados e parâmetros analisados.

Parâmetros de Interesse

Definimos a Densidade Mineral Óssea (DMO), a Concentração Mineral Óssea (CMO) e a Avaliação da Resistência Óssea através do Teste de Flexão de Três Pontos como parâmetros de relevância primária para os objetivos desta meta-análise.

O peso do intestino, dosagem de osteocalcina, dosagem de deoxipiridinolina plasmática (DPD), avaliação da estrutura óssea por Micro CT, dosagem do Cálcio (Ca), dosagem do Magnésio (Mg), a formação de ácidos graxos de cadeia curta (AGCC), a mudança da microbiota e dosagem de β-glicosidase foram classificados como parâmetros secundários de avaliação, e foram considerados para este estudo quando os resultados destes parâmetros foram expressos de forma numérica e discutidos.

Avaliação do risco de viés

A avaliação do risco de viés foi realizada utilizando o software Review Mananger 5 (RevMan 5) A classificação dos estudos foi feita por dois autores (Carvalho, J. A. A. e Batista, I. E. T.), que analisaram os seguintes riscos de viés: viés de seleção dos animais, viés de alocação, viés de desempenho (cegamento do pessoal envolvido no tratamento dos animais), viés de detecção dos resultados (cegamento dos avaliadores) e viés de publicação. Os riscos de viés foram classificados em alto ou baixo, e ainda risco indefinido, quando a avaliação do nível do risco deixava dúvidas.

Análises Estatísticas

Usamos o software RevMan 5 para a realização dos cálculos da meta-análise. Para a análise do inverso da variância utilizamos as médias e desvios padrões dos resultados de cada estudo para comparar os dados encontrados. A heterogeneidade foi calculada usando o teste do Q^2 e I^2, a significância foi definida em $p<0,10$ ou $I^2>50\%$. Foram analisados os efeitos fixos dos tratamentos, porem quando observamos heterogeneidade significativa entre os estudos, analisamos os efeitos randômicos. Os valores da DMO foram padronizados usando o método de ajuste de Hedges e foi implementado pelo RevMan. O valor de $P<0,05$ foi adotado como estatisticamente significativo.

RESULTADOS

Encontramos 813 artigos completos, resumos ou capítulos de livros que citavam as palavras chaves utilizadas na pesquisa bibliográfica. Destes, após triagem de títulos e resumos, 23 estudos foram potencialmente elegidos para avaliação posterior. (BRITTON et al., 2014; CHIANG; LIAO; PAN, 2012; CHIANG; PAN, 2011; CHONAN; MATSUMOTO; WATANUKI, 1995; DEVAREDDY et al., 2006; ERFANIAN; RASTI; MANAP, 2017; HOOSHMAND; JUMA; ARJMANDI, 2010; LEGETTE et al., 2011, 2012; LI et al., 2013; LU et al., 2008; MATHEY et al., 2004, 2007; MATSUBARA et al., 2010; MCCABE et al., 2013; PARVANEH et al., 2015; POULSEN et al., 2009; RODRIGUES et al., 2012; SHIM et al., 2013; TOUSEN et al., 2016; VELASCO et al., 2011; YANG et al., 2013; ZHANG et al., 2015) Ao final, seis estudos experimentais atendiam os critérios de inclusão, conforme representado no fluxograma da declaração PRISMA (Fig. 1).

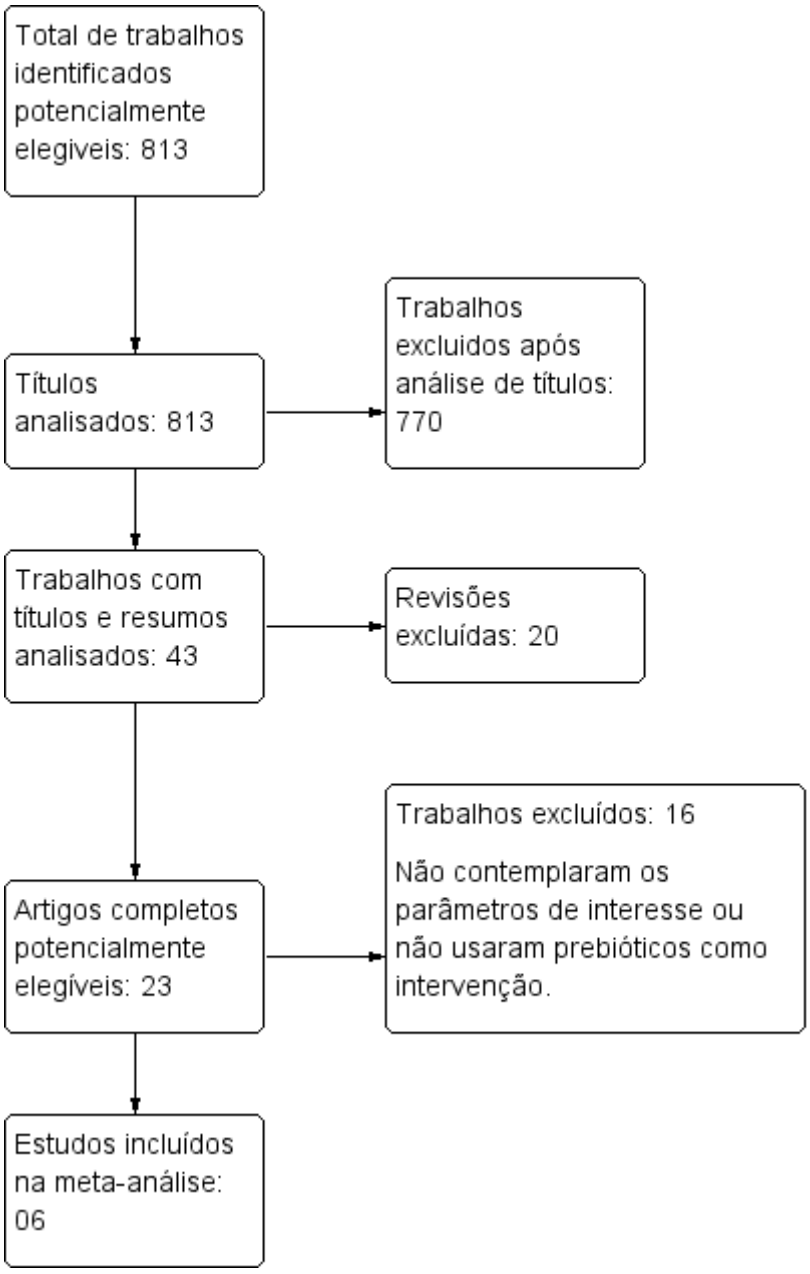

Fig. 1 Diagrama de fluxo PRISMA mostrando o processo de seleção de literatura, seleção de estudos e motivos de exclusão.

Características dos estudos incluídos

Os seis trabalhos selecionados estudaram os efeitos da suplementação com diferentes prebióticos: Frutoligossacarídeos de cadeia curta (SC-FOS), (MATHEY et al., 2004) Frutoligossacarídeos, (DEVAREDDY et al., 2006) Synergy®, (LEGETTE et al., 2011) Synergy1®; Polydextrose; Fruitafit HD® ,(LEGETTE et al., 2012) Standardised Aqueous Extract of *Anoectochilus formosanus* (SAEAF); Inulina, (YANG et al., 2013)

158 Amido Resistente. (TOUSEN et al., 2016) As doses utilizadas e a composição de cada
159 prebiótico são apresentadas na Tabela 1.

160 A Tabela 1 também demonstra que Legette et al (2012) incluiu no mesmo trabalho
161 comparações entre o grupo controle e grupos suplementados por três prebióticos
162 diferentes (Synergy1®; Polydextrose; Fruitafit HD®), e que Yang et al (2013) incluiu
163 três grupos de tratamentos que receberam os prebióticos SAEAF em duas dosagens
164 diferentes (200 ou 400 mg/Kg de ração/dia) e Inulina, respectivamente, para as
165 comparações com o grupo controle. Todas as análises foram consideradas para esta
166 meta-análise.

167 Dentre os estudos selecionados, Mathey et al (2004) avaliou a DMO e biomecânica
168 (Flexão de Três Pontos), Tousen et al (2016) avaliou a DMO, e os demais [Devareddy
169 et al (2006), Legette et al (2011), Legette et al (2012) e Yang et al (2013)] descreveram
170 os resultados da avaliação da DMO, CMO e biomecânica (Tabela 2).

171 Todas as avaliações foram realizadas em ratas ovariectomizadas suplementadas ou
172 não com prebióticos. Ao todo foram utilizados 116 animais, divididos randomicamente
173 entre grupos controle e tratamento. Os estudos foram realizados na América do Norte,
174 Europa e Asia, entre os anos de 2004 e 2016 e tiveram duração entre 30 e 90 dias. O
175 resumo das caracteristicas dos trabalhos selecionados está descrito na Tabela 3.

176 **Tabela 1** Composição dos prebióticos e doses utilizadas nos estudos

Autor/Ano de Publicação	Prebiótico	Dose	Composição
Mathey et al. (2004)	Frutoligossacarídeos de cadeia curta (SC-FOS)	2,250g/dia	SC-FOS, 95 ± 2% FOS e 5 ± 2% de glicose, frutose e sacarose
Devareddy et al. (2006)	Frutoligossacarídeos (FOS)	1,500g/dia	Frutoligosscarídeos 5%
Legette et al. (2011)	Synergy®	1,500g/dia	Inulina + Oligofrutose
Legette et al. (2012)	Synergy 1®	1,500g/dia	Mistura aproximada de 50:50 de FOS de cadeia curta e longa
Legette et al. (2012)	Polydextrose	1,500g/dia	Polímeros não digeríveis de glicose
Legette et al. (2012)	Fruitafit HD®	1,500g/dia	Inulina 98% + 2% Oligossacarídeos
Yang et al. (2013)	Standardised Aqueous Extract of *Anoectochilus formosanus* (SAEAF)	0,006g/dia	Standardised Aqueous Extract of *Anoectochilus formosanus*
Yang et al. (2013)	Standardised Aqueous Extract of *Anoectochilus formosanus* (SAEAF)	0,012g/dia	Standardised Aqueous Extract of *Anoectochilus formosanus*
Yang et al. (2013)	Inulina	0,012g/dia	Inulina
Tousen et al. (2016)	Amido Resistente	4,500g/dia	Amido resistente

177

178

Tabela 2 Trabalhos incluidos e parâmetros primários

Autor/ano	DMO	CMO	Biomecânica
Mathey et al. (2004)	X		X
Devareddy et al. (2006)	X	X	X
Legette et al. (2011)	X	X	X
Legette et al. (2012)	X	X	X
Yang et al. (2013)	X	X	X
Tousen et al. (2016)	X		

Resultado da eficácia da suplementação com prebióticos sobre os parâmetros primários

Os resultados dos parâmetros primários eleitos para serem analisados nesta meta-análise (DMO, CMO e Teste de Flexão de Três Pontos) foram expressos com clareza, de tal forma que permitiram extrair com segurança os valores para serem meta-análisados, porém os parâmetros CMO e Teste de Flexão de Três Pontos não foram avaliados em todos os estudos incluídos na meta-análise. (Tabela 2).

A meta-análise dos resultados da DMO demonstra que os prebióticos são capazes de melhorar este parâmetro significativamente ($P<0,05$), quando comparado aos grupos controle, apesar de, individualmente os resultados de Legette et al. (2011) usando Synergy®, e de Yang et al. (2013) usando SAEAF em duas dosagens diferentes (200 ou 400 mg/Kg de ração/dia) não revelaram diferença significativa ($P<0,05$) quando comparados com os grupos controle (Figura 2).

193 **Tabela 3** Características dos estudos incluídos na meta-análise

Autor (s)/ ano de publicação	Prebiótico utilizado	Duração experimento	Nº de ratos por grupo	Parâmetros analisados*
Mathey et al. (2004)	Frutoligossacarídeos de cadeia curta (SC-FOS)	90 dias	8	**Densidade Mineral Ósseo; Teste de Flexão de três Pontos**; Fitoestrógenos (Genisteína, Daidzeina e Equol); Osteocalcina; Deoxipiridinolina.
Devareddy et al. (2006)	Frutoligosscarídeos	90 dias	13	**Densidade Mineral Óssea; Conteúdo Mineral Ósseo; Teste de Flexão de Três Pontos;** Peso Corpóreo e do Intestino; Micro CT.
Legette et al. (2011)	Synergy®.	2 meses	16	**Densidade Mineral Óssea; Conteúdo Mineral Ósseo; Teste de Flexão de três Pontos**; Peso corpóreo e do ceco; Micro CT; Cálcio; Colesterol total e frações; AGCC.
Legette et al. (2012)	Synergy1®; Polydextrose; Fruitafit HD®.	12 semanas	8	**Densidade Mineral Óssea; Conteúdo Mineral Ósseo; Teste de Flexão de três Pontos**; Peso corpóreo e do ceco; Micro CT; Cálcio; Magnésio; AGCC.
Yang et al. (2013)	Standardised Aqueous Extract of A. formosanus (SAEAF); Inulina.	12 semanas	8	**Densidade Mineral Óssea; Conteúdo Mineral Ósseo; Teste de Flexão de três Pontos**; Osteocalcina; Micro CT; Cálcio; AGCC; Microbiota.
Tousen et al. (2016)	Amido Resistente	42 dias	7	**Densidade Mineral Óssea;** Peso corpóreo e do ceco; Fitoestrógenos (Genisteina, Daidzeina e Equol); Micro CT; AGCC; Microbiota; β-glicosidase.

194 *Os parâmetros escritos em negrito foram considerados primários.

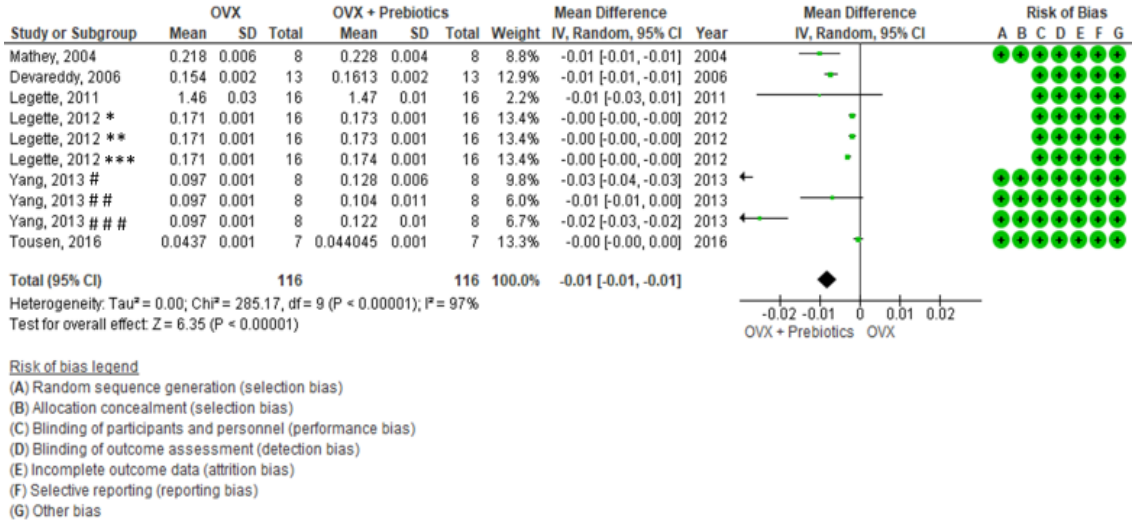

Fig. 2: Comparação da DMO dos grupos Controle (OVX) e Tratamento (OVX + Prebiótico). * Synergy1®, ** Polydextrose,*** Fruitafit HD®, # Inulina, ## SAEAF 200 mg/Kg de dieta, ### SAEAF 400 mg/Kg de dieta.

Para o parâmetro CMO, a meta-análise dos resultados dos estudos incluídos demonstrou que os prebióticos melhoram esta condição significativamente (P<0,05) quando comparado ao grupo controle. Estudo usando Synergy1® e Fruitafit HD®, evidenciou que estes prebióticos, nas condições experimentais do seu estudo, não diferem significativamente (P>0,05) do grupo controle. Nesse mesmo estudo, a suplementação da Polidestrose demonstrou diminuição da CMO, quando comparado com o grupo controle. Yang et al (2013), utilizando SAEAF na concentração de 200 mg/Kg de dieta não observou diferença significativa.

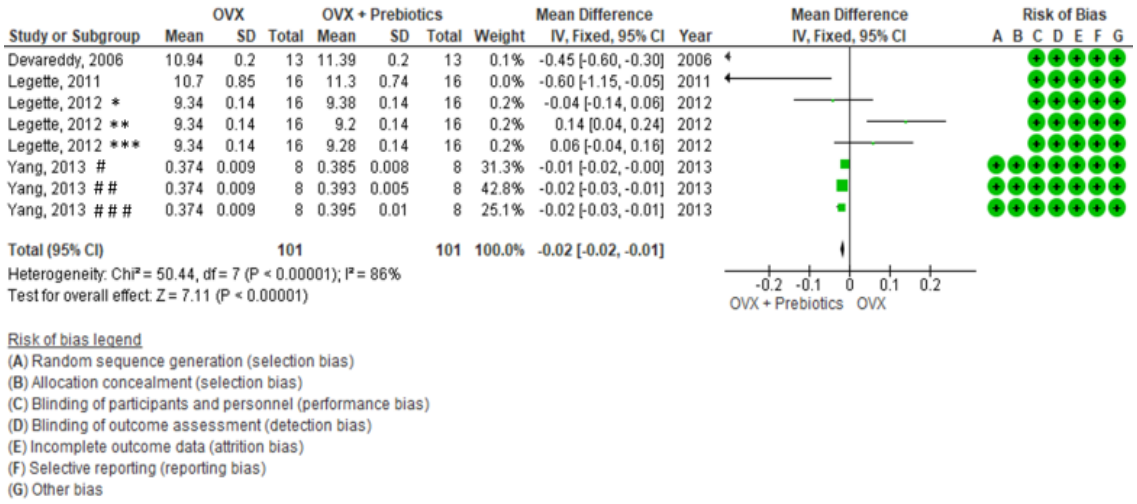

Fig. 3 Comparação da CMO dos grupos Controle (OVX) e Tratamento (OVX+Prebiótico). * Synergy1®, ** Polydextrose, *** Fruitafit HD®, # Inulina, ## SAEAF 200 mg/Kg de dieta, ### SAEAF 400 mg/Kg de dieta.

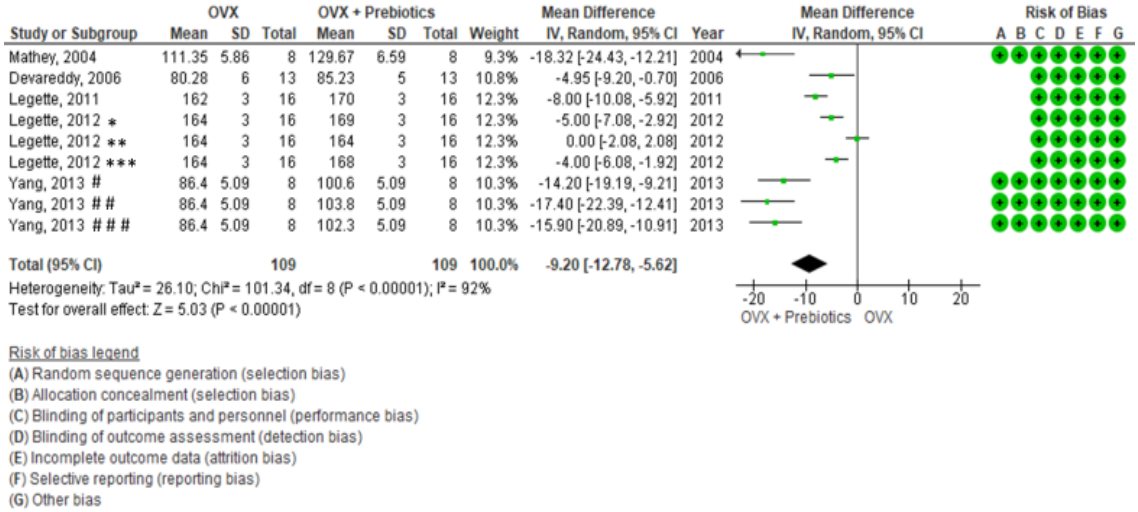

Fig. 4 Comparação do Teste de Flexão de Três Pontos dos grupos Controle (OVX) e Tratamento (OVX+Prebiótico). * Synergy1®, ** Polydextrose,*** Fruitafit HD®, # Inulina, ## SAEAF 200 mg/Kg de dieta, ### SAEAF 400 mg/Kg de dieta.

A meta-análise dos resultados dos testes biomecânicos (Flexão de Três Pontos) evidenciou que os prebióticos melhoraram este parâmetro. Porém, individualmente o resultado de Legette et al. (2012) usando Synergy 1® não demonstrou melhora.

Resultado da eficácia da suplementação com prebióticos sobre os parâmetros secundários

Na tabela 4 demostramos os parâmetros secundários alterados, o peso corpóreo final dos ratos foi descrito em três estudos (DEVAREDDY et al., 2006; LEGETTE et al., 2012; MATHEY et al., 2004) que não observaram diferença significativa ($P<0,05$) da média dos resultados dos grupos suplementados com prebióticos e dos grupos controle. O peso médio do intestino dos animais no final do período experimental foi informado em quatro estudos (LEGETTE et al., 2011, 2012; TOUSEN et al., 2016; YANG et al., 2013) e mostrou aumento significativo nos grupos de animais tratados com prebióticos.

A concentração de osteocalcina sérica foi dosada por dois autores: Mathey et al. (2004) e Yang et al. (2013) relataram não ter sido observada diferença significativa entre os grupos prebiótico e controle.

Dois estudos, Mathey et al. (2004) e Tousen et al. (2016), dosaram a concentração plasmática de fitoestrógenos (ginestein, daidzein e equol) e os resultados revelaram aumento significativo somente do hormônio daidzein nos grupos suplementados com prebiótico. No estudo de Mathey et al. (2004), os autores observaram que no grupo suplementado com prebióticos a concentração plasmática dos fitoestrógenos aumentou, porém não significativamente. O mesmo autor relata também aumento não significativo de deoxipiridinolina plasmática (DPD).

A microarquitetura óssea foi analisada por micro CT computadorizada em cinco estudos, em dois estudos observaram melhora significativa nos grupos suplementados com prebióticos, quando comparados com os grupos controle (LEGETTE et al., 2011; YANG et al., 2013), contrastando com os estudos de Devareddy et al. (2006), Legette et al. (2012) e Tousen et al. (2016). Yang et al. (2013) descreve diferença significativa entre os grupos tratados com os prebióticos SAEAF na concentração de 400 mg/Kg de dieta e inulina em relação aos grupos controle.

254 **Tabela 4** Tabela com os parâmetros secundários avaliados

Estudos	Peso Corporal	Peso do Intestino	Osteocalcina	Ginestesin	Didazein	Equol	DPD	Micro CT	Ca	Mg	AGCC	Microbiota	B-glicosidase
Mathey et al. (2004))	X		X	X	X	X	X						
Devareddy et al. (2006)	X							X					
Legette et al (2011)		X						X	X		X		
Legette et al (2012)	X	X						X	X	X	X		
Yang et al (2013)		X	X					X	X		X	X	
Tousen et al (2016)	X	X		X	X	X		X				X	X

Os níveis séricos de cálcio foram dosados em três estudos (LEGETTE et al., 2011, 2012; YANG et al., 2013) e foi relatado aumento significativo somente nos de Legette et al. (2012) e Yang et al. (2013) somente na concentração de 400 mg/Kg de prebiótico SAEAF na dieta. A concentração sérica de magnésio foi dosada somente por Legette et al. (2012) e apresentou aumento significativo.

Os prebióticos também se mostraram capazes de aumentar significativamente a concentração de AGCC (LEGETTE et al., 2011, 2012), modificar beneficamente a composição da microbiota intestinal (TOUSEN et al., 2016; YANG et al., 2013) e elevar os níveis de β-glicosidase plasmático dos animais. (TOUSEN et al., 2016)

DISCUSSÃO

A osteoporose primária está associada à deficiência de hormônios sexuais e a idade, sendo consequência da deterioração contínua das trabéculas ósseas. Em mulheres na pós-menopausa, o declínio da produção de estrogênio provoca significativa perda óssea, elevando o risco de fraturas. (RAISZ, 2005)

A melhor compreensão da fisiopatologia da osteoporose levou a um aumento no número de estudos com animais, observação clínica ou reaproveitamento oportunista de compostos existentes, evoluindo para o desenvolvimento de diversos medicamentos. Porém, a criação de novos tratamentos não reduziu a carga de fraturas na população. Isso ocorreu devido o ao tratamento inadequado realizado pelos pacientes, com receio dos efeitos colaterais e dúvidas em relação à eficácia em longo prazo dos medicamentos. (KHOSLA; HOFBAUER, 2017)

Estudos experimentais demonstraram que bifosfonatos são capazes de promover lesão gastrointestinal alta, especialmente no contexto de irritação esofágica preexistente a exposição diária repetida e dispepsia. (ARANTES; SILVA; LAZARETTI-CASTRO, 2010) Efeito colateral de medicamento biológico, anticorpo monoclonal (denosumabe) foi evidenciado, podendo causar a diminuição do nível de cálcio e não pode ser prescrito para paciente com hipocalcemia. (BINELLO et al., 2012) Medicamentos de reposição hormonal apresentaram também efeitos adversos, como a indução de câncer de mama e uterino. (BERAL et al., 2005) Com o mesmo intuito, dietas com altas doses de cálcio aumentam a oferta deste elemento, no entanto, apresentam limitações importantes, como por exemplo, o aumento do risco para doenças cardíacas e renais. (HAMILTON; TERENTYEV, 2019; TU et al., 2018)

Para estudar a fisiopatologia da osteoporose pós-menopáusica humana, utiliza-se ratas ovariectomizadas (OVX), como modelo experimental validado. (KALU, 1991) Observou-se

redução da DMO diafisária e comprometimento das propriedades biomecânicas, quando comparadas com animais controle. (MATHEY et al., 2004) Nesse sentido, ratas OVX vêm sendo utilizadas como modelo animal na avaliação dos efeitos da suplementação de substratos prebióticos, como terapia alternativa as tradicionalmente utilizadas para a manutenção da saúde óssea. (ARJMANDI et al., 1996; DEVAREDDY et al., 2006; LEGETTE et al., 2012; TOUSEN et al., 2016; YANG et al., 2013) Esses alimentos funcionais são utilizados seletivamente por microrganismos hospedeiros, conferindo benefícios para a saúde. (GIBSON et al., 2017)

Os resultados benéficos observados nos estudos utilizando os prebióticos se devem a capacidade desses suplementos alimentares maximizarem a absorção de minerais da dieta, (LEGETTE et al., 2012; OHTA et al., 1995) estimulam seletivamente o crescimento e/ou atividade de bactérias benéficas nativas, (GIBSON et al., 2017) acarretando o aumento da produção de ácidos graxos de cadeia curta (AGCC) e consequente diminuição do pH luminal e aumento da solubilidade dos minerais, principalmente cálcio e magnésio. (SCHOLZ-AHRENS et al., 2007, 2016) Além disso, podem influenciar a função intestinal, por meio da proliferação de enterócitos induzida por AGCC, levando ao aumento da área de superfície absortiva e modulando a expressão de proteínas de ligação ao cálcio e fatores de crescimento. (RUFINO et al., 2018)

Parâmetros Primários: DMO, CMO e biomecânica óssea

Nesta revisão sistemática, a meta-análise dos dados dos parâmetros primários (DMO, CMO e biomecânica óssea) dos estudos incluídos demonstrou que os prebióticos são capazes de melhorar significativamente estes parâmetros nos animais estudados. Em ratos e humanos, a DMO e a CMO estão relacionados ao risco de fratura óssea (LEGETTE et al., 2012) e a redução destes parâmetros e a deterioração da microarquitetura óssea, comprometem as propriedades biomecânicas do osso (por exemplo: a resistência mecânica femoral a carga). (MATHEY et al., 2007)

Esses efeitos benéficos são decorrentes da fermentação dos prebióticos no cólon dos mamíferos pela microbiota nativa, aumentando a produção de AGCC, acarretando redução do pH luminal, aumento da solubilidade e concentração de minerais ionizados e facilitando a difusão de minerais nas partes distais do intestino. (JONES; MULLE; PACIFICI, 2018; MATHEY et al., 2004) O aumento da absorção de minerais, principalmente cálcio e magnésio, observado com a suplementação de prebiótico na dieta, compensam as perdas diárias desse mineral, fazendo com que a absorção exceda a excreção. Como consequência a

concentração adequada de cálcio reduz a remodelação excessiva comum em mulheres na pós-menopausa e em muitos osteoporóticos. (TU et al., 2018) O alto remodelamento é um fator de risco para fratura provavelmente tão importante quanto à baixa massa óssea. (HEANEY, 2006) Isso é demonstrado, por exemplo, pelo fato de que a taxa de remodelação prediz a fratura (TU et al., 2018) e, em ensaios de tratamento com bifosfonatos ou cálcio e vitamina D em humanos, a taxa de fratura cai imediatamente após o início do tratamento, antes que a massa óssea possa ter mudado sensivelmente. (HEANEY; WEAVER, 2005) A melhora da utilização de cálcio pelo osso nas dietas suplementadas com prebiótico em ratos, conforme determinado pela abordagem de modelagem cinética, atua beneficamente na saúde óssea. (DEVAREDDY et al., 2006; HEANEY, 2006; LEGETTE et al., 2011, 2012; YANG et al., 2013)

A resistência óssea depende dos minerais, mas também de outros fatores, como a microarquitetura óssea e o teor de proteína da matriz. (DEVAREDDY et al., 2006) Em ratos e camundongos suplementados com prebióticos, FOS e GOS, (RIZZOLI, 2019) observou aumento do volume e a organização das trabéculas ósseas, melhorando a resistência, assim como aumento da força necessária para a ruptura do fêmur distal.

Indiretamente os prebióticos melhoram a digestão e a utilização de proteínas, por meio de modulação benéfica das bactérias intestinais relacionadas à proteólise, que podem induzir atividade protease digestivas e peptidase do hospedeiro, e liberar exoenzimas envolvidas na digestão de proteínas, auxiliando beneficamente na absorção de pequenos peptídeos e aminoácidos, na capacidade de absorção e transporte do epitélio e adicionalmente reduzindo a fermentação proteica prejudicial e diminuição da toxicidade dos metabólitos. (WANG; JI, 2018) Além disso, participam da diminuição da expressão e formação de genes inflamatórios e relacionados com inflamação na medula óssea. Efeito benéfico do prebiótico amido-resistente foi evidenciado pela inibição do aumento da expressão de mRNA de IL-7R induzida por OVX em camundongos, demonstrando a capacidade de diminuir o estado de inflamação na medula óssea, resultando na atenuação da perda óssea. (TOUSEN et al., 2016)

Parâmetros secundários

Nos estudos incluídos nesta revisão os prebióticos não foram capazes de reduzir os ganhos induzidos pela ovariectomia no peso corporal. Esse aumento no peso corporal deveu-se, em parte, à maior gordura corporal. (DEVAREDDY et al., 2006) Isoladamente, o FOS não é capaz de reduzir os ganhos induzidos pela ovariectomia no peso corporal. (DEVAREDDY et al., 2006; MATHEY et al., 2004) Essa heterogeneidade dos resultados dos estudos utilizando

prebióticos suplementados na dieta, em humanos e animais, pode ser decorrente das diferentes composições das dietas, tipos e doses do prebiótico, tempo de duração dos estudos e diferentes composição da microbiota nativa de humanos ou animais. (BINNS, 2013; JOHN et al., 2018; MARKOWIAK; SLIZEWSKA, 2017; MATHEY et al., 2007)

Foi relatado aumento significativo do peso médio das paredes do intestino dos animais tratados com prebióticos, e este aumento demonstrou ser dose dependente. Aumentos na produção de AGCC e no peso da parede cecal são consequências do consumo de fibras prebióticas, que modificam a fermentação bacteriana intestinal e estão associadas a benefícios para a saúde. (LEGETTE et al., 2012)

A suplementação na dieta do prebiótico SAEAF suprimiu significativamente os aumentos nos níveis plasmáticos de OCN e g-GT urinário em ratos OVX. A osteocalcina (OCN) plasmática é um marcador sensível de renovação óssea e esses resultados indicaram que, além de aumentar a absorção de cálcio, o SAEAF inibiu o turnover ósseo. A análise por microtomografia mostrou que o tratamento com SAEAF reduziu significativamente os sinais de perda óssea em ratas OVX tratados com prebióticos. (YANG et al., 2013) Além disso, a suplementação de prebiótico resultou no aumento da absorção e retenção de magnésio. Isto pode ser valioso, uma vez que o magnésio influencia a regulação hormonal da modelagem e mineralização óssea, reduzindo o tamanho dos cristais ósseos e evitando fragilidade óssea, desempenhando, portanto, um papel importante em todos os aspectos do metabolismo ósseo. (LEGETTE et al., 2012)

Os prebióticos demonstraram ser eficientes por alteraram a composição da microbiota para uma estrutura de comunidade mais benéfica. Este processo resulta da estimulação de gêneros potencialmente promotores da saúde do hospedeiro, mas não de grupos nocivos. (TOUSEN et al., 2016; YANG et al., 2013) Os prebióticos fornecem carboidratos não digeríveis, oligossacarídeos, que as bactérias benéficas do cólon são capazes de fermentar, levando à produção de ácido lático, ácidos graxos de cadeia curta e gases. Consequentemente, há redução do pH do lúmen e estimulação da proliferação de células epiteliais do cólon. Além disso, sua atividade media principalmente o enriquecimento de espécies de *Lactobacillus* e/ou de *Bifidobacterium* e possivelmente a modulação do metabolismo de outros microrganismos benéficos. (FERRARESE et al., 2018; SAAD, 2006; YANG et al., 2013)

A suplementação com prebiótico, amido-resistente, aumenta significativamente a atividade da β-glicosidase no conteúdo cecal, que é um indicador da atividade de enzimas intestinais que hidrolisam a ligação glicosídica e aumentam tanto o metabolismo dos conjugados ISO, quanto à aglicona para equol da daidzeína. (TOUSEN et al., 2016)

Avaliação do viés

Nesta revisão sistemática com meta-análise, após rigoroso processo de seleção, os estudos foram incluídos por apresentarem metodologia rigorosa e bem descrita. A randomização foi considerada um requisito metodológico importante, no entanto, os estudos que não citaram a sequência aleatória de randomização e a ocultação de alocação foram incluídos quando entendemos que os animais utilizados nos experimentos apresentavam homogeneidade de peso, sexo, tempo de estabilização após OVX e via de administração do suplemento. A consequência desta ponderação foi à possibilidade de inclusão de resultados conflitantes, que em nossa consideração, eleva o nível de confiabilidade do resultado final.

Resultados heterogêneos entre estudos podem ser devidos a vários fatores, dentre eles a diferenças na duração do tratamento, e a duração da estabilização após ovariectomia, método de administração e idade no início da suplementação. (LEGETTE et al., 2012)

CONCLUSÃO

Em conclusão, os resultados deste estudo demonstram que a administração de prebióticos melhora a DMO, CMO e propriedades biomecânicas dos ossos de ratas ovariectomizadas. Como esses são parâmetros validados como indicadores de osteoporose, mostra claramente que a atividade prebiótica pode estar envolvida nos mecanismos anti-osteoporóticos.

Dada à moderada evidência encontrada, esta meta-análise sugere novos estudos usando prebióticos como uma alternativa terapêutica ou terapia complementar aos tratamentos convencionais da osteoporose pós-menopausa em humanos.

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CONFLITO DE INTERESSE

Não há conflito de interesse

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565

ANEXOS

ANEXO A- PARECER FINAL

UNOESTE - Universidade do Oeste Paulista

PRO-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PROBIC - Programa de Bolsas de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"AVALIAÇÃO DA INFLUÊNCIA DOS ALIMENTOS FUNCIONAIS NA OSTEOPOROSE: UMA REVISÃO SISTEMÁTICA COM META-ANÁLISE"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **4321** e tendo como participante(s) **MARCOS NATAL RUFINO (discente), JOAO ALBERTO ARTONI DE CARVALHO (discente), LARYSSA MAYARA POLASTRI (discente), LETICIA ROCHA MAGALHAES (discente), HERMANN BREMER NETO (orientador responsável)**, foi avaliado e **APR. COM RECOMENDAÇÃO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Presidente Prudente, 27 de Fevereiro de 2018.

Prof. Dr. José Rodrigues Garcia Jr.
Coordenador Científico do CPDI

ANEXO B- NORMAS DE PUBLICAÇÃO

Bone Research

Guide for Authors

ABOUT THE JOURNAL

Aims and Scope

Bone Research is an Open Access, fully peer-reviewed journal publishing the foremost progress and novel understanding of all aspects of bone science. The journal highlights the breakthrough discoveries in basic and clinical aspects of bone biology, pathophysiology and regeneration, as well as other significant findings related to bone.

Covering all aspects of bone science including its subspecialties, *Bone Research* publishes original, high-quality, peer-reviewed papers including research articles, reviews, correspondence and comments.

Original research articles will be published under, but not limited to, the following headings:

- Morphogenesis of bone and cartilage;
- Musculoskeletal diseases;
- Metabolic bone diseases;
- Bone regeneration and bone tissue engineering;
- Bone related biomaterials;
- Clinical studies.

Journal Details

Editor-in-Chief:
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Executive Editors-in-Chief:
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Yun-feng Lin (China)

Founding Editor
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ARTICLE TYPES

Article Description	Abstract	Word Limit	Tables/ Figures	References guideline
Article A complete, comprehensive report of original research. An Article usually has a fairly complex narrative that is based on multiple techniques and/or approaches.	Unstructured abstract; max word limit: **250**	**4,000-6,000** words excluding abstract, references, figures and tables	More than 5	**No less than 40**
Correspondence The Editors will occasionally consider the publication of correspondence developing the debate relating to a particular journal article that has already been published in the journal. These would usually be published alongside a reply from the authors of the original article.	None	**800** words excluding, references, figures and tables	None	No limits
Comment Comments are an analysis of recently published papers of particular interest. This is a commission-only section.	None	**1,500** words excluding references	No Limits	No limits

Editorial The editors invite editorials to discuss a topical issue or a paper published in the journal and set the problems addressed by the paper in the wider context of the field. These are usually commissioned, but unsolicited editorial submissions are considered for publication.	None	**2,000** words excluding references	No Limits	No limits
Review Article A comprehensive synthesis and/or analysis of specific topics. A short Introduction giving the rationale for the review should be followed by sections with appropriate subheadings, followed by a conclusions section at the end. The standard footer headings (Acknowledgements, Contributions, Competing Interests, Funding) are required. All invited reviews will undergo peer review prior to acceptance.	Unstructured abstract; max word limit: **250**	**No less than 8,000** words excluding abstract, references, figures and tables	No less than 5	No less **than 100**

Word limit

Word limits are provided for guidance only. The Editors will consider submissions that exceed the recommended limit, subject to feedback received during peer review.

PREPARATION OF ARTICLES

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Each manuscript must be accompanied by a cover letter including statements that:

- Highlight of the current manuscript should be limited to no more than 2 short sentences;
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For first submissions (i.e. not revised manuscripts), authors may choose to incorporate the manuscript text and figures into a single file (Microsoft Word or TeX/LaTeX) up to 30 MB in size — the figures may be inserted within the text at the appropriate positions. **Article should include continuous line number**. Supplementary Information should be combined and supplied as a separate file, preferably in Word format.

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All textual content should be provided in a single file, prepared using either Microsoft Word or TeX/LaTeX; figures should be provided in individual files. The manuscript text file should include the following parts, in order: a title page with author affiliations and contact information (the corresponding author should be identified with an asterisk); the sections required for each content type (see information for different content types) then References, Acknowledgements (optional), Author Contributions (Articles only), Competing Financial Interests statement, Figure Legends and Tables. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

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- Title page
- Abstract
- Introduction
- Results
- Discussion
- Materials and methods
- Acknowledgements
- Conflict of interests
- Contributions
- References
- Figure legends
- Tables
- Figures

(i) Title page

The title page should include a succinct title (less than 200 characters); a concise running title (which should normally not exceed 50 characters); the full names of all authors including their given names; the affiliations (including city, state, country and zip code) of all authors; the official email addresses of all authors, and the full contact details of the

Editorial The editors invite editorials to discuss a topical issue or a paper published in the journal and set the problems addressed by the paper in the wider context of the field. These are usually commissioned, but unsolicited editorial submissions are considered for publication.	None	**2,000** words excluding references	No Limits	No limits
Review Article A comprehensive synthesis and/or analysis of specific topics. A short Introduction giving the rationale for the review should be followed by sections with appropriate subheadings, followed by a conclusions section at the end. The standard footer headings (Acknowledgements, Contributions, Competing Interests, Funding) are required. All invited reviews will undergo peer review prior to acceptance.	Unstructured abstract; max word limit: **250**	**No less than 8,000** words excluding abstract, references, figures and tables	No less than 5	No less **than 100**

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- Discussion
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- Contributions
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- Figure legends
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(i) Title page

The title page should include a succinct title (less than 200 characters); a concise running title (which should normally not exceed 50 characters); the full names of all authors including their given names; the affiliations (including city, state, country and zip code) of all authors; the official email addresses of all authors, and the full contact details of the

permission from the individual concerned to quote his/her unpublished work.

Examples:
Journal article, up to five authors:
Belkaid, Y. & Rouse, B. T. Natural regulatory T cells in infectious disease. *Nat. Immunol.* **6**, 353–360 (2005).

Journal article, e-pub ahead of print:
Bonin, M. *et al*. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. *Bone Marrow Transplant*. http://dx.doi.org/10.1038/sj.bmt.1705565 (2007).

Journal article, in press:
Gallardo, R. L., Juneja, H. S. & Gardner, F. H. Normal human marrow stromal cells induce clonal growth of human malignant T-lymphoblasts. *Int. J Cell Cloning* (in the press).

Complete book:
Atkinson, K. *et al*. (eds) Clinical Bone Marrow and Blood Stem Cell Transplantation (Cambridge Univ. Press, 2004).

Chapter in book:
Harley, N. H. & Vivian, L. in Mechanisms of Disease 4th edn, Vol. 2 (eds Sodeman, W. A. & Smith, A.) Ch. 3 (Saunders, 1974).

Abstract:
Feig, S. A. et al. Bone marrow transplantation for neuroblastoma. *Exp. Hematol.* **13**, abstr. 102 (1985).

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Starrfelt, J. & Liow, L.H. How many dinosaur species were there? Fossil bias and true richness estimated using a Poisson sampling model (TRiPS). Preprint at http://biorxiv.org/content/early/2015/12/04/025940 (2015).

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Hao, Z., AghaKouchak, A., Nakhjiri, N. & Farahmand, A. Global Integrated Drought Monitoring and Prediction System (GIDMaPS) Data sets. figshare. http://dx.doi.org/10.6084/m9.figshare.853801 (2014).

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