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ORIGINAL PAPER

Evaluation of the effects of inulin supplementation in a high-fat diet rich in saturated fatty acids on bone properties in young pigs

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ABSTRACT. This experiment was conducted to evaluate the effects of supplementing a high-fat diet rich in saturated fatty acids (SFA) with inulin on bone health in a pig model. Twenty-eight 50-day-old gilts were randomly assigned to four treatment groups: a control (C) group fed a standard diet and three experimental groups (H, H5, and H10) fed a high-fat diet rich in SFA. Animals in groups H5 and H10 received additional inulin (7% of daily feed intake) for 5 and 10 weeks, respectively. At 120 days of age, the animals were slaughtered, and samples and third metacarpal bone were collected. The morphometric, geometric, densitometric, and mechanical properties of the third metacarpal bone were the lowest in group H, higher in group H5, and the highest in group C, while values in group H10 often approached those in group C (P = 0.001). Osteocalcin (OC) concentrations decreased in the following order: H10>H5>H>C (P = 0.001). The concentration of cross-linked C terminal telopeptide of type I collagen (CTX-I) ranged from 0.162 ng/ml (group C) to 0.272 ng/ml (group H, P = 0.001). The OC/CTX-I ratio decreased in the following order: C,H10>H5>H (P = 0.001). In conclusion, supplementing a high-fat diet rich in SFA with inulin was associated with favourable changes in bone turnover markers and attenuated the adverse effects of the diet on bone properties, with greater benefits observed after longer inulin supplementation.

Introduction

Current dietary habits among children and adolescents, characterised by excessive consumption of energy-dense foods rich in saturated fatty acids (SFA) (the so-called Western diet), lead to obesity in this demographic group (Hu et al., 2023). The prevalence of childhood and adolescent obesity continues to rise, making it an increasingly important global health concern (WHO, 2022). In addition, childhood and adolescent obesity contributes to the development of many chronic diseases in adulthood, e.g., cardiovascular diseases, type 2 diabetes, and cancer, reducing quality of life and life expectancy (WHO, 2022). Obesity also increases the risk of hormonal and metabolic disorders and causes degenerative changes in the musculoskeletal system. Excessive consumption of unhealthy fats may also impair calcium deposition in bones weakening them and increasing the risk of fractures and osteoporosis (WHO, 2022).

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Therefore, in light of the growing obesity epidemic among children and adolescents, there is a need to explore alternative strategies that complement the promotion of healthy lifestyles and nutrition without requiring substantial changes in dietary habits. Because changing individual eating habits across an entire population is very difficult, public health experts focus on improving the food environment and implementing systemic policies rather than relying solely on individual behaviour. One of the main goals of nutritional strategies aimed at maintaining bone health and preventing the occurrence of osteoporosis is to increase calcium absorption, as only about one-third of dietary calcium is absorbed. Maintaining adequate vitamin D status is an established approach to improving calcium absorption (Liu et al., 2023). However, another promising method is the consumption of prebiotics (such as nondigestible carbohydrates, including oligofructose and inulin), which serve as substrates for beneficial bacteria inhabiting the large intestine, thereby stimulating their growth and/or activity. Consequently, prebiotics can modify the composition of the gut microbiota, which may indirectly support intestinal health and exert positive effects on other organs and systems, including the cardiovascular system, skin, and bones (Kansra et al. 2020; de Sire et al., 2022). Some researchers (Nzeusseu et al., 2006; Cieślik et al., 2009) found that inulin had greater beneficial effect on bone properties than oligofructose; however, the animals were fed a standard diet. Prebiotic interventions acting through the gut-bone axis may therefore help prevent deterioration in bone quality associated with high consumption of the Western diet in children and adolescents (Cieślik et al., 2009; Kansra et al., 2020).

Thus, the aim of the present study was to determine the effect of supplementing a high-fat diet rich in SFA (as an equivalent of the Western diet in human nutrition) with inulin on the morphometric, geometric, densitometric, and mechanical properties of the third metacarpal bone and on plasma concentrations of bone turnover markers in young pigs. It was hypothesised that dietary inulin supplementation would reduce the negative impact of such a diet on bone health.

Material and methods

Ethics

According to the 3Rs (Replacement, Reduction, and Refinement) ethical principle, the study design and experimental procedures allowed to minimise the number of animals while maintaining high statistical power. The use of live animals could not be completely replaced by an alternative research model.

All experimental procedures were conducted in accordance with national and local ethical guidelines and were approved by the Third Local Ethics Committee for Animal Experimentation at the Warsaw University of Life Sciences, Poland (protocol No. 1/2013 dated 9 January 2013).

Animals, diets, housing, and treatment

This study was carried out at the Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences using 28 crossbred gilts (♂Duroc × ♀Polish Large White × Danish Landrace) growing from 50 to 120 days of age (10 weeks).

At 50 days of age, the animals were randomly assigned to four groups (n = 7 per group): the control (C) group was fed a standard diet balanced according to the nutrient requirements of swine (NRC, 2012), while the three experimental groups (H, H_5, and H_{10}) were fed a high-fat diet rich in SFA. The high-fat diet, containing 14.1% fat, was produced according to our formulation at an animal feed factory (Ferm-Pasz sp.j., Baboszewo, Poland). Pigs in groups H_5 and H_{10} received additional inulin (7% of daily feed intake) extracted from chicory root (100% inulin content, 0% sweetness level) with an average degree of polymerisation of at least 23 (Inulin Orafti®HPX; BENEO GmbH, Mannheim, Germany) for the last 5 weeks of the experimental period (group H_5) or throughout experimental period (10 weeks, group H_{10}). The inulin dose was selected based on literature data (Estrada et al., 2001; Demigné et al., 2008). This issue was discussed in more detail in our previous publication (Sobol et al., 2018). Beef tallow (Ferm-Pasz sp.j., Baboszewo, Poland), a source of SFA, was included at 13% in the high-fat diet to increase its energy content. The mineral content of the standard and high-fat diets was identical and met the nutrient requirements of swine (NRC, 2012). The amino acid content of the high-fat diet was reduced compared to the standardised diet (while maintaining the proportions of essential amino acids), as the study aimed to assess the effects of an unbalanced lysine-to-metabolizable energy ratio and increased SFA intake on bone health. The feeding regimen was designed to resemble the so-called Western diet. The composition and nutritional value of diets are given in Table 1. The fatty acid contents of the control and high-fat diets were as follows: total SFA 23.67 and 31.30%; total monounsaturated fatty acids 31.17 and 30.16%; total polyunsaturated fatty acids 45.16 and 38.54%; linoleic acid 41.35 and 35.69%; linolenic acid

Table 1. Ingredients, chemical composition and nutritive value of diets, g/kg DM

Indices	Standard diet	High-fat diet
Ingredients		
barley	227.27	226.24
wheat	420.45	135.75
maize	255.68	407.24
rapeseed meal	-	90.50
soyabean meal	170.45	90.50
beef tallow	-	147.06
rapeseed oil	22.73	-
premix A*	39.77	-
premix B*	-	39.59
Chemical composition		
ash	28.98	27.26
organic matter	971.59	972.85
crude protein	172.73	152.71
ether extract	49.66	159.50
crude fibre	40.45	39.03
starch	543.18	463.80
sugar	38.64	32.81
Fat content, %	4.37	14.10
Nutritive value (determined)		
digestive crude protein	140.91	124.43
Apparent ileal digestible		
lysine	11.36	7.92
methionine	3.41	2.38
threonine	7.05	5.09
thryptophan	2.27	1.58
P, digestible	3.41	3.39
Ca	8.86	8.82
Na	1.70	1.70
Metabolizable energy, MJ/kg DM	15.34	17.42
Lysine/metabolizable energy, g/MJ	0.741	0.454

* provided per kg diet: µg: vitamin A 4500, vit. D_3 50, vit. E 100; mg: Fe 240, Zn 150, Cu 25, Mn 20, I 0.30, Se 0.30, vit. K_3 3.5, vit. B_1 3.5, vit. B_2 7.0, vit. B_6 5.0, vit. B_{12} 0.40, biotin 0.20, folic acid 3.0, nicotinic acid 30, calcium D-pantothenate 15, choline chloride 400; g: Ca 6.8 (premix A) and 6.6 (premix B), P 2.9 (premix A) and 2.8 (premix B); essential amino acids: g: lysine 4.51 (premix A) and 1.94 (premix B), methionine 0.90 (premix A), threonine 2.10 (premix A) and 0.25 (premix B)

3.37 and 2.46% (analysed values). To fully control nutrient intake, pigs were fed *semi ad libitum* (95% of *ad libitum* intake) twice daily (8:00 and 14:00). Pigs were housed individually in pens (3.3 m^2) with concrete floors, without straw, and equipped with nipple drinkers. They were maintained on a 12 h light–12 h dark cycle, with lights on from 06:00 to 18:00. The environmental conditions in the piggery, controlled by a Fancom ventilation system (model ISM0.12; Fancom BV, NK Panningen, The Netherlands), were as follows: air temperature (18–20 °C), relative humidity (60–70%), and air flow (0.2–0.4 m/s). These conditions complied with national regulations (Regulation of the Ministry of Agriculture and Rural Development, 2003).

Slaughter and sample collection

Animals were slaughtered at 120 days of age, following a 16-h fast, by exsanguination at the Institute's experimental slaughterhouse. During slaughter, blood samples were collected into tubes containing an anticoagulant to determine the concentrations of bone turnover markers, i.e., osteocalcin (OC) and cross-linked C-terminal telopeptide of type I collagen (CTXI). Directly after collection, the blood samples were centrifuged for 15 min at 1 000 *g* at 4 °C. Plasma samples were stored at −80 °C until analysis. After slaughter, the third metacarpal bone was manually dissected from the right side of each carcass. The excised bones were cleaned of any remaining soft tissue, weighed, and stored at −20 °C until analysis.

Determination of OC and CTX-I

Plasma concentrations of OC and CTX-I were determined using commercial ELISA kits (MBS456492 and MBS261253, respectively; MyBioSource, Inc., San Diego, CA, USA). The intra-assay coefficients of variation (CVs) were 10 and 8% for OC and CTX-I, respectively, and the inter-assay CV was 12% for both markers. The detection range was 0.781–50 ng/ml for OC and 0.156–10 ng/ml for CTX-I. Plate incubation and absorbance measurements were performed at a wavelength of 450 nm using the VersaMax microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA).

Determination of the third metacarpal bone properties

The third metacarpal bone was analysed using dual-energy X-ray absorptiometry (DEXA) with a Norland XR-800™ densitometer in Research Scan mode (Norland, CooperSurgical, Fort Atkinson, WI, USA). Scans were obtained and analysed according to the manufacturer's instructions. Daily quality assurance scans were performed to verify calibration stability and measurement precision. The daily calibration procedure was performed using the Quality Control Phantom and Quality Assurance Calibration Standard (Norland, CooperSurgical). Specimens for scanning were thawed at room temperature (23 °C) for 12 h before use. During scanning, the third metacarpal bone was positioned horizontally with the head facing upwards and the condyles downwards and was scanned from the distal to the proximal end. Each specimen was scanned three times by the same operator, and the mean value was used for statistical analysis. Bone mineral content (BMC, g), bone mineral density (BMD, g/cm^2) and bone area (cm^2) were recorded.

After DEXA scanning, bone mechanical properties were determined by a three-point bending test using a TA-HDi Texture Analyser (Stable Micro Systems Ltd., Godalming, UK). The distance between the bone supports was set at 40% of the bone length, and the measuring head loaded the bone samples at the midshaft at a constant speed of 50 mm/min. Elastic bone strength (N) and maximum bone strength (N) were recorded.

The geometric properties of the third metacarpal bone were determined from measurements of the internal and external horizontal and vertical diameters taken after sectioning the bone. Measurements were made using an electronic calliper. The values of cross-sectional area (mm^2), cortical wall thickness (mm), and cortical index (%) were determined using the following equations:

$$cross_sectional\ area = \frac{[\pi(HV-hv)]}{4},$$

$$cortical\ wall\ thickness = \frac{[(V+H)-(v+h)]}{4},$$

$$cortical\ index = \left\{\frac{[(\frac{H-h}{H})+\frac{V-v}{V}]}{2}\right\}100.$$

where: V – vertical external diameter (mm), H – horizontal external diameter (mm), v – vertical internal diameter (mm), and h – horizontal internal diameter (mm) (Ferretti et al., 1993).

Chemical analysis

Dry matter, ash, crude protein, crude fibre, ether extract, simple sugar and starch contents in the control and high-fat diets were determined according to AOAC International (2011) methods. Metabolizable energy and standardised ileal digestible amino acid contents in the diets were calculated according to the Nutrient Requirements of Swine (NRC, 2012).

Lipids from the diets were saponified with potassium hydroxide according to the method described by Folch et al. (1957). Fatty acid methyl esters were then prepared by esterification with thionyl chloride (4% in methanol) and extracted with n-heptane. Fatty acid methyl esters were analysed using a GC-2010AF Shimadzu Gas Chromatograph (Shimadzu Europa GmbH, Duisburg, Germany) equipped with a flame ionisation detector. The derivatives were separated on a capillary column (BPX70, 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness; Phenomenex, Torrance, CA, USA). The operating conditions were as follows: carrier gas – helium; split ratio 1:100; injector temperature 260 °C; detector temperature 260 °C. The initial column temperature was maintained at 110 °C for 5 min, increased to 200 °C at 3.5 °C/min and held for 2.5 min, then increased to 205 °C at 0.3°C/min and to 215 °C at 1.5 °C/min, where it was held for 3 min. Individual fatty acid peaks were identified by comparison with the commercial standard Supelco 37 Component FAME Mix (SUPELCO, Bellefonte, USA).

Statistical analysis

All analyses were performed using Statistica, version 13.3 (StatSoft, Tulsa, OK, USA). The effects of diets on the concentrations of plasma bone turnover markers and the morphometric, geometric, densitometric, and mechanical properties of the third metacarpal bone were analysed using one-way analysis of variance (ANOVA). Data normality was assessed using the Shapiro Wilk test, and homogeneity of variance was assessed using Levene's test. When the ANOVA F-test was significant, Tukey's honestly significant difference post hoc test was applied. Relationships between inulin intake, plasma bone turnover marker concentrations and third metacarpal bone properties were analysed using Pearson's correlation coefficient. Statistical significance was set at $P < 0.05$.

Results

Initial body weight did not differ between the groups (mean 12.80 kg; Table 2). Total weight gain was higher in groups H_5 and H_{10} than in groups C and H (average 62.25 vs. 58.69 kg, $P = 0.001$). Total feed intake differed between groups and ranged from 111.19 kg (group H_5) to 124.04 kg (group C) ($P = 0.001$). Total inulin intake in groups H_5 and H_{10} amounted to 4.94 and 8.01 kg ($P = 0.001$).

Table 2. Performance of pigs during the study, kg

Item	Diet				SEM	*P*-value
	C	H	H_5	H_{10}		
Initial body weight	12.71	12.34	12.99	13.15	0.128	0.161
Total weight gain	58.57^{A}	58.81^{A}	62.93^{B}	61.57^{B}	0.431	0.001
Total feed ntake	124.04^{C}	115.28^{B}	111.19^{A}	114.25^{AB}	0.994	0.001
Total inulin supply	-	-	4.94^{A}	8.04^{B}	0.616	0.001

C – control diet, H – high-fat diet without inulin supply, H_5 – high-fat diet and extra inulin supplement during the last 5 weeks of the experimental period, H10 – high-fat diet and extra inulin supplement during the whole experimental period (10 weeks); SEM – standard error of the mean; ^{ABC} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Concentration of bone turnover markers (osteocalcin (OC) and cross-linked C-telopeptide of type I collagen (CTX-I) and the OC/CTX-I ratio in pig plasma

Item	Diet				SEM	*P*-value
	C	H	H_5	H_{10}		
OC, ng/ml	21.06^{A}	23.91^{B}	26.31^{C}	31.56^{D}	0.5896	0.001
CTX-I, ng/ml	0.162^{A}	0.272^{D}	0.229^{B}	0.243^{C}	0.0049	0.001
OC/CTX-I	130.19^{C}	87.77^{A}	115.12^{B}	129.51^{C}	0.7465	0.001

C – control diet, H – high-fat diet without inulin supply, H_5 – high-fat diet and extra inulin supplement during the last 5 weeks of the experimental period, H10 – high-fat diet and extra inulin supplement during the whole experimental period (10 weeks); OC – osteocalcin; CTX-I – cross linked C-telopeptide of type I collagen; OC-CTX-I – the ratio of osteocalcin to cross-linked C-telopeptide of type I collagen; SEM – standard error of the mean; ^{ABC} – means within a row with different superscripts are significantly different at $P < 0.05$

Plasma bone turnover marker concentrations are presented in Table 3. OC concentrations differed between the groups and decreased in the following order: H_{10} (31.56 ng/ml) > H_5 (26.31 ng/ml) > H (23.91 ng/ml) > C (21.06 ng/ml) ($P = 0.001$). CTX-I concentrations differed between the groups and ranged from 0.162 ng/ml (group C) to 0.272 ng/ml (group H) ($P = 0.001$). The OC/CTX-I ratio was the highest in groups C and H_{10} (average 129.85), lower in group H_5 (115.12), and the lowest in group H (87.77) ($P = 0.001$).

The diets affected all measured third metacarpal bone parameters except bone area (Table 4). The mass and length of the third metacarpal bone ranged from 22.46 g and 7.10 cm in group H to 23.18 g and 7.36 cm in group C ($P = 0.001$).

BMC was the lowest in group H (6.000 g), higher in group H_5 (6.400 g), and the highest in groups H_{10} and C (average 6.634 g) ($P = 0.001$). BMD, elastic bone strength, and maximum bone strength were the highest in group C, followed by groups H_{10} and H_5, and the lowest in group H ($P = 0.001$). Cross-sectional area was the highest in group H (163.25 mm^2), followed by H_5 (148.72 mm^2), C (130.24 mm^2) and H_{10} (120.53 mm^2) ($P = 0.001$). Cortical wall thickness was the highest in group H (4.042 mm), lower in group H5 (3.513 mm), and the lowest in groups C and H_{10} (average 3.207 mm) ($P = 0.001$), whereas the cortical index (%) was higher in group H (47.79) compared to the remaining groups (average 39.18) ($P = 0.001$).

Pearson's correlation coefficients for the relationships between inulin intake, bone turnover marker concentrations and third metacarpal bone properties are presented in Table 5. Inulin intake was positively correlated with OC concentration (r = 0.8063, $P = 0.001$), bone mass (r = 0.7256, $P = 0.001$) and length (r = 0.5270, $P = 0.008$), BMC (r = 0.9119, $P = 0.001$), BMD (r = 0.9327, $P = 0.001$), elastic bone strength (r = 0.9552, $P = 0.001$) and maximum bone strength (r = 0.9653, $P = 0.001$). The positive correlation between inulin intake and third metacarpal bone area was not statistically significant (r = 0.3940, $P = 0.057$). Inulin intake was negatively correlated with CTX-I concentration (r = −0.5555, $P = 0.005$), cross-sectional area (r = −0.8844, $P = 0.001$), cortical wall thickness (r = −0.8709, $P = 0.001$) and cortical index (r = −0.7442, $P = 0.001$).

Table 4. Morphometric, geometric, densitometric, and mechanical properties of the 3rd metacarpal bone

Item	Diet				SEM	*P*-value
	C	H	H_5	H_{10}		
Mass, g	23.18^{C}	22.46^{A}	22.85^{B}	23.11^{BC}	0.1023	0.001
Length, cm	7.36^{C}	7.10^{A}	7.20^{AB}	7.26^{BC}	0.0411	0.001
Area, cm^2	13.44	13.24	13.35	13.45	0.0908	0.378
Bone mineral content, g	6.697^{C}	6.000^{A}	6.400^{B}	6.570^{C}	0.0458	0.001
Bone mineral density, g/cm^2	0.498^{D}	0.453^{A}	0.479^{B}	0.489^{C}	0.0021	0.001
Elastic bone strength, N	914.48^{D}	763.12^{A}	813.58^{B}	872.60^{C}	24.6961	0.001
Maximum bone strength, N	1255.95^{D}	1076.12^{A}	1157.38^{B}	1216.81^{C}	34.7853	0.001
Cross-section area, mm^2	130.24^{B}	163.25^{D}	148.72^{C}	120.53^{A}	2.7057	0.001
Cortical wall thickness, mm	3.209^{A}	4.042^{C}	3.513^{B}	3.205^{A}	0.0666	0.001
Cortical index, %	38.41^{A}	47.79^{B}	39.30^{A}	39.82^{A}	0.8953	0.001

C – control diet, H – high-fat diet without inulin supply, H_5 – high-fat diet and extra inulin supplement during the last 5 weeks of the experimental period, H10 – high-fat diet and extra inulin supplement during the whole experimental period (10 weeks); SEM – standard error of the mean; ^{ABC} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 5. The Pearson correlation coefficients between the inulin intake and the concentration of osteocalcin (OC) and C-terminal telopeptide of type I collagen (CTX-I) in pig plasma, as well as the examined properties of the 3rd metacarpal bone

Item	Inulin intake, kg	*P*-value
OC, ng/ml	0.8063	0.001
CTX-I, ng/ml	-0.5555	0.005
Bone mass, g	0.7256	0.001
Bone length, cm	0.5270	0.008
Bone area, cm^2	0.3940	0.057
Bone mineral content, g	0.9119	0.001
Bone mineral density, g/cm^2	0.9327	0.001
Elastic bone strength, N	0.9552	0.001
Maximum bone strength, N	0.9653	0.001
Cross-sectional area, mm^2	-0.8844	0.001
Cortical wall thickness, mm	-0.8709	0.001
Cortical index, %	-0.7442	0.001

Discussion

Improving calcium absorption is an important nutritional strategy for maintaining bone health and preventing metabolic bone disorders, including osteoporosis. Calcium metabolism may be improved by increasing dietary calcium intake or its bioavailability. Bone mineral measurements and biochemical markers of bone formation and resorption are commonly used to assess bone metabolism.

Pig diet composition

The present study examined the effects of an unbalanced lysine-to-metabolizable energy ratio, resulting from increased SFA content in the diet and a simultaneous reduction in amino acid content, while maintaining the relative proportions of essential amino acids, to mimic the so-called Western diet, together with inulin supplementation on bone turnover marker concentrations and bone properties in a pig model. To our knowledge, this combination of dietary interventions has not previously been studied in pigs. However, experiments in rodents (Roberfroid et al., 2002; Demigné et al., 2008; Cieślik et al., 2009; Lobo et al., 2009; Legette et al., 2012; Bueno-Vargas et al., 2016), pigs (Estrada et al., 2001; Grela et al., 2013; Herosimczyk et al., 2025), and humans (Holloway et al., 2007; de Souza et al., 2010; Drabińska et al., 2019) have shown that inulin added to a standard diet may show anti-inflammatory and immunomodulatory properties and modify lipid oxidation, mineral absorption, and osteoblast formation, potentially increasing BMD.

Performance data

Pigs receiving inulin (throughout the experiment or during its second half) had an approximately 6% greater total weight gain than animals that were not administered the prebiotic (i.e., those fed the standard or unsupplemented high-fat diet). The absence of a reduction in growth rates of animals fed the experimental diets was presumably due to its higher energy content. However, the composition of the weight gain may have been less favourable, although this was not analysed in the present study. Animals fed such diets generally deposit more fat and less protein during growth, as indicated by our previous studies (Fandrejewski et al., 2001; Skiba et al., 2001). Moreover, animals in the H_5 and H_{10} groups received inulin supplementation. Thus, both the higher metabolizable energy content of the diet and inulin supplementation may have contributed to the greater weight gain in these groups. Literature data (e.g., Świątkiewicz and Świątkiewicz, 2008) indicate that the physiological effects of inulin are associated with its prebiotic properties, including beneficial changes in the gut microbiota, increased production of short-chain fatty acids through fermentation, expansion of the intestinal absorptive surface area, improvements in lipid and mineral metabolism, and immunomodulatory effects. These factors may contribute to improved feed utilisation and greater weight gain, as observed by Xu et al. (2002) and Chen et al. (2005). Differences in body weight gain and total feed intake between the experimental groups may also have influenced some of the bone parameters, particularly bone mass, dimensions, BMC, and mechanical properties. Moreover, despite the same mineral concentration in the diets, variations in feed intake may have led to differences in total calcium and phosphorus intake. Other studies have also reported increased daily weight gain in pigs supplemented with inulin, although these animals were fed standard diets (Estrada et al., 2001; Grela et al., 2013). Similarly, a meta-analysis by Zybert et al. (2026) indicated that inulin supplementation can have a beneficial effect on average daily gain in pigs, although the magnitude of this effect depends on the degree of inulin polymerisation and the production phase. The authors suggested that the favourable effect of inulin on growth rate may be related to the stimulation of beneficial intestinal bacteria, particularly lactobacilli and bifidobacteria, which inhibit the growth of pathogenic bacteria that impair nutrient digestion and absorption. In contrast, Kumar et al. (2016) showed that the addition of inulin to a high-fat diet reduced gain in rats compared with controls. The differences between these findings and our results may reflect variation in inulin intake, the source and amount of dietary fat, and species-specific tolerance to high-fat diets.

Rodents can tolerate diets containing a higher proportion of fat than pigs, with fat accounting for up to 60% of the diet (Bastías-Pérez et al., 2020).

Bone turnover

Bone development can be determined by the concentration of markers of bone turnover, which are proteins or protein-derived biomarkers released by osteoblasts or osteoclasts during bone remodelling (Greenblatt et al., 2017). Changes in biochemical markers may precede measurable changes in bone properties. Therefore, these markers can be used to assess the effects of dietary factors on bone metabolism over relatively short intervention periods (de Souza et al., 2010). Plasma OC and bone alkaline phosphatase concentrations are commonly used markers of bone formation, while CTX-I and N-terminal telopeptide of type I collagen (NTX-I) are most often used as markers of bone resorption processes. Together, these markers provide information on bone remodelling and allow monitoring changes in bone metabolism (Greenblatt et al., 2017). In scientific research, the OC/CTX-I ratio is often used as an auxiliary, exploratory measure derived from two bone-turnover markers (Seibel, 2005; van der Merwe et al., 2025). While separately, these parameters reflect bone formation or resorption, their ratio provides an indirect indication of the relative balance between these processes. A high OC/CTX-I ratio (indicating a relatively high OC concentration compared with CTX-I) may suggest a predominance of bone formation over resorption, as may occur during periods of rapid growth. Conversely, a low OC/CTX-I ratio (reflecting a relatively high CTX-I concentration compared with OC) may suggest greater bone resorption relative to formation. Studies have shown that this ratio is lower in patients with osteoporosis than in healthy controls (e.g., van der Merwe et al., 2025), suggesting a shift towards greater bone resorption relative to formation. Although individual bone turnover markers remain the standard clinical measures, the OC/CTX-I ratio provides additional information on relative changes in bone formation and resorption under specific experimental conditions. Therefore, in the present study, OC and CTX-I concentrations were measured and their ratio calculated to assess bone turnover in pigs fed a high-fat diet rich in SFA.

The results of the present study showed that the addition of inulin to the SFA-rich highfat diet enhanced bone formation (as reflected by higher plasma OC levels) with simultaneous reduction in bone resorption (lower plasma CTX-I levels). Inulin supplementation was also associated with a higher OC/CTX-I ratio, which, after 10 weeks, was similar to that in the control group. However, this ratio should be interpreted cautiously because it is an exploratory measure calculated from two distinct markers rather than a direct measure of bone formation and resorption balance. Drabińska et al. (2019) found that three months of oligofructoseenriched inulin supplementation in children with coeliac disease increased plasma OC concentrations and reduced plasma NTX-I levels. They suggested that such supplementation might help improve bone metabolism in these patients. Holloway et al. (2007) likewise reported higher serum OC concentrations in postmenopausal women after six weeks of oligofructose-enriched inulin supplementation compared to a three-week intervention. In female athletes, Ishizu et al. (2021) found that 12 weeks of consumption of a prebiotic food fortified with inulin and lactulose reduced concentrations of tartrate-resistant acid phosphatase 5b, a marker of bone resorption. They also observed an increase in the proportion of *Bifidobacterium* in the intestinal microbiota and suggested that the prebiotic intervention could benefit both bone health and the intestinal microbial environment. In rats fed a diet enriched with short-chain fructo-oligosaccharides, Porwal et al. (2020) reported increased concentrations of the bone formation marker procollagen type I N-terminal propeptide, with no change in CTX-I levels. Together, these findings suggest that inulin and related fructo-oligosaccharides may influence bone turnover by stimulating bone formation processes.

In postmenopausal women, Kruger et al. (2016) found that milk fortified with calcium, vitamin D and inulin reduced CTX-I and procollagen type I N-terminal propeptide concentrations more effectively than regular milk. In girls with low calcium intake, de Souza et al. (2010) reported that oligofructose-enriched inulin supplementation increased plasma calcium and bone alkaline phosphatase concentrations and reduced parathyroid hormone levels, suggesting a possible improvement in bone formation. These effects were not observed with calcium supplementation alone. The authors proposed that inulin increased intestinal calcium absorption, thereby raising plasma calcium concentrations. Topolska et al. (2020) also reported favourable changes in bone formation markers, including serum OC and alkaline phosphatase, following fructan supplementation in rats fed a low-calcium diet. In addition, strawberry sorbet enriched with yacon, or Jerusalem artichoke, reduced serum CTX-I concentrations. However, unlike in our study, urinary deoxypyridinoline, another marker of bone resorption, also

increased significantly. The authors noted that the bone turnover markers did not respond consistently to increased mineral absorption, although one resorption marker was reduced over the short term. Discrepancies between their findings and ours may reflect the use of other resorption markers, with different sensitivities to intervention, as well as various sample types (plasma, serum and urine) and measurement units. These methodological differences also prevent direct comparison of ratios between bone formation and resorption markers.

Bone properties

The SFA-rich high-fat diet adversely affected several characteristics of the third metacarpal bone, whereas inulin supplementation mitigated these changes. The effects observed in pigs fed the experimental diet may be partly related to alterations in bone lipid metabolism and adipogenic differentiation, as suggested by previous studies (Cao, 2011; Tencerova et al., 2018). However, bone fat content was not measured in the present study. One possible explanation involves the differentiation of multipotent mesenchymal stem cells into either osteoblasts, which synthesise and mineralise the bone matrix, or adipocytes (fat cells) (Cao, 2011). Excess dietary fat, particularly a high proportion of SFA such as palmitic and stearic acids, may favour differentiation into adipocytes rather than osteoblasts. These adipocytes accumulate in bone marrow, particularly in metabolically active trabecular bone (Cao, 2011; Tencerova et al., 2018). The resulting increase in marrow adipose tissue at the expense of new bone formation may impair bone mineralisation (BMC and BMD) and, consequently, mechanical strength (Cao, 2011; Tencerova et al., 2018). Tencerova et al. (2018) reported a nearly 200% increase in bone marrow adipose tissue in mice fed a high-fat diet, accompanied by adverse skeletal changes. Sørensen et al. (2018) also observed increased bone fat content associated with reduced calcium and phosphorus deposition. Studies in rats have shown that inulin and other fructans, including oligofructose and oligofructose-enriched inulin, may reduce tissue fat accumulation (Cani et al., 2004; Jamieson et al., 2008). This effect may be linked to their rapid fermentation in the proximal colon and the modulation of hormones involved in appetite and carbohydrate and lipid metabolism, including glucagon-like peptide-1 and ghrelin (Cani et al., 2004). The latter authors also suggested that the response to inulin may depend on sex and its degree of polymerisation.

In the present study, adding inulin to the SFA-rich high-fat diet improved several bone parameters, particularly after the longer supplementation period. After 10 weeks, some values approached those recorded in the control group. Previous studies generally supported beneficial effects of prolonged supplementation with inulin or its derivatives on bone densitometric (Nzeusseu et al., 2006; Demigné et al., 2008) and mechanical properties (Demigné et al., 2008) in rats, although they employed used standard diets. In our earlier study, 10 weeks of inulin supplementation reduced the adverse effects of an SFA-rich high-fat diet on whole-body BMC and BMD in growing pigs; neither measure differed significantly from that of animals fed a standard diet (Sobol et al., 2018). Roberfroid et al. (2002) found that 18 weeks of inulin supplementation in rats fed diets with different calcium contents increased calcium absorption and whole-body BMC and BMD. Similarly, Abrams et al. (2005) reported higher BMC and BMD in adolescents receiving oligofructose-enriched inulin for one year compared to those receiving placebo. They estimated that supplementation with 8 g/day led to an additional 15 g of calcium deposition in the skeleton annually, equivalent to approximately 10–15% of annual skeletal calcium accumulation. In ovariectomised rats, Porwal et al. (2020) demonstrated that 12 weeks of supplementation with short-chain fructooligosaccharides improved BMC and BMD, increased peak bone mass and protected against bone loss induced by oestrogen deficiency by selectively promoting new bone formation through a butyratemediated mechanism.

Bueno-Vargas et al. (2016) investigated the effects of inulin on foetal programming in pregnant rats. Foetal programming refers to the lasting effects of environmental conditions during pregnancy on offspring organ development, metabolism and health in adulthood, and its underlying mechanisms are still being investigated (e.g., Mlyczyńska et al., 2021; Musillo et al., 2025). At birth, offspring of females fed a diet supplemented with oligofructose-enriched inulin had a significantly greater trabecular number in the femur and fourth lumbar vertebra than offspring of females fed a control diet (Bueno-Vargas et al., 2016). Exposure to oligofructose-enriched inulin during pregnancy and lactation, through the placenta and breast milk respectively, lasted a total of 33 days and was also associated with improved bone characteristics at 100 days of age. These included BMC, the bone surface-to-volume ratio, trabecular thickness and cortical

BMD, with significant differences observed in the fourth lumbar vertebra.

Ohta et al. (1998a) reported that four weeks of fructooligosaccharide supplementation reduced, but did not fully prevent, the decline in femoral and tibial BMD in gastrectomised rats, while extending supplementation to six weeks completely prevented this decline (Ohta et al., 1998b). In another six-week study, Takahara et al. (2000) observed that a fructooligosaccharide-enriched diet significantly increased femoral trabecular bone volume in male rats. They also reported significant correlations between the absorption of calcium and magnesium and their respective concentrations in the femur ($r = 0.72$ for both minerals). These findings suggest that fructooligosaccharides, including inulin, may promote intestinal mineral absorption and deposition in bone, thereby helping to preserve bone properties. However, not all studies have achieved improvements in bone mineralisation. Zhao et al. (2024) investigated the effects of six weeks of inulin supplementation in milk- and yoghurt-based diets. Inulin in yoghurt had a greater effect on intestinal fermentation and increased plasma acetate concentrations compared to inulin in milk, but neither treatment affected bone mineralisation or structure. Similarly, Topolska et al. (2020) found no effect of fructans or their derivatives on femoral BMC or BMD in rats fed a low-calcium diet for eight weeks. Legette et al. (2012) reported that four weeks of supplementation with inulin-based fibre improved calcium and magnesium metabolism in rats, as well as increased short-chain fatty acid production and caecal wall mass, without affecting total or trabecular BMD. The authors attributed the limited skeletal response partly to the stable postmenopausal state represented by the rodent model and the short study duration. Jamieson et al. (2008) likewise found no effect of eight weeks of inulin supplementation on femoral or spinal BMC and BMD in growing female rats. However, they reported different findings for male rats in a comparable experiment (unpublished data), suggesting a possible role for hormonal factors. Differences between our results and those of previous studies may therefore be partly explained by the duration of supplementation. Some interventions lasting approximately four to six weeks may have been too short to produce measurable changes in bone structure or densitometric characteristics.

Few studies have examined the effects of inulin supplementation on bone mechanical and geometric characteristics, which generally require direct testing of excised bones. Lobo et al. (2009) found that oligofructose-enriched inulin increased tibial peak load, yield load and stiffness in young male rats fed a soybean oil-rich diet. Some of these values were similar to those in rats fed a fish oil-rich diet, with or without the supplement. In contrast, inulin-type fructans in rats receiving fish oil improved only bone stiffness. Fish oil is rich in long-chain omega-3 polyunsaturated fatty acids, which have been associated with beneficial effects on bone health (Sobol et al., 2019; Thanabalan and Kiarie, 2021; Skiba et al., 2021). The mechanical improvements reported by Lobo et al. (2009) may have been related to higher BMC and BMD, although these parameters were not assessed.

Overall, our findings and those of previous studies suggest that inulin and related fructans may improve the physical, mineral, geometric and mechanical characteristics of bone and help counteract the adverse effects of an SFA-rich high-fat diet. These benefits may be linked to increased intestinal absorption of minerals, including calcium, and their subsequent deposition in the skeleton. Differences between studies may partly reflect supplementation duration, as longer interventions may be needed for changes in bone mineralisation to become apparent. Age, sex and hormonal status, including sexual maturity, may also influence the response.

Relationships between inulin intake, bone turnover markers and bone properties

Inulin intake was significantly associated with OC and CTX-I concentrations, as well as with the geometric, densitometric (except bone area) and mechanical properties of the third metacarpal bone. However, the correlations with geometric parameters were unexpectedly negative. The biological basis of these relationships remains unclear. Changes in lipid deposition in bone tissue may offer one explanation, although lipid deposition was not assessed in this study.

Previous works have suggested links between inulin supplementation, the gut microbiota, gastrointestinal pH, mineral absorption and bone characteristics. He et al. (2022) reported strong positive correlations between the abundance of *Lactobacillus* and *Streptococcus* and spinal BMC in rats fed an inulin-enriched diet. They also associated the inulin-induced reduction in gastrointestinal pH with a rapid increase in the abundance of *Bifidobacterium* and *Allobaculum*, which promote short-chain fatty acid production, and increased expression of *Trpv6*, a gene encoding a calcium transport channel. In young ducks fed an inulin-supplemented diet, Li et al. (2025) found strong positive correlations between the abundance of *Alistipes*, *Ligilactobacillus* and *Streptococcus* and

tibial BMC and BMD, as well as measures of intestinal health, including villus height in the ileum and jejunum.

Possible mechanisms of action of inulin on the skeletal system

These potential mechanisms are based on findings from previous studies and were not directly investigated in the present experiment:

1) Fermentation of fructans, including inulin, by microorganisms in the large intestine generates short-chain fatty acids, such as acetate, propionate and butyrate, and reduces intestinal pH (Nath et al., 2018; He et al., 2022; de Sire et al., 2022). The resulting increase in mineral solubility may enhance mineral absorption in the large intestine (Nath et al., 2018). Minerals, particularly calcium, are essential for bone matrix formation, while certain minerals also act as cofactors for enzymes involved in collagen synthesis (Nath et al., 2018; Porwal et al., 2020).
2) Short-chain fatty acids may influence the expression of calbindin D9K, an intracellular calcium-binding protein (Ohta et al., 1998b), as well as regulate bone homeostasis and osteoclast metabolism (Zaiss et al., 2019), potentially favouring bone formation relative to resorption.
3) Short-chain fatty acids may enhance passive mineral transport through the spaces between the intercellular junctions of enterocytes of the intestinal epithelial cells (Roberfroid and Delzenne, 1998), thereby increasing mineral absorption and potentially promoting mineral deposition in bone.
4) Short-chain fatty acids may stimulate the expression of the *Trpv6* gene, which encodes a calcium channel involved in active calcium absorption in the caecum (He et al., 2022).
5) Fructans, including inulin, may improve mineral absorption by increasing the intestinal absorptive surface area (Raschka and Daniel, 2005; Li et al., 2025).
6) Inulin-associated increases in the abundance of *Alistipes*, *Ligilactobacillus* and *Streptococcus* may alter the profile of metabolites, including histamine, uric acid, prostaglandin E1, corticosterone, cholesterol sulphate, cholic acid and 7-ketocholesterol. These changes may involve taurine and hypotaurine metabolism, purine and amino acid metabolism (including lysine degradation), and the biosynthesis of fatty acids, primary bile acids and steroid hormones, potentially improving intestinal barrier function and bone properties (Li et al., 2025).
7) Following inulin consumption, *Bifidobacterium* and *Lactobacillus* may contribute to the production of metabolites that support bone health, for example by converting daidzein into equol, a compound with potential bone-forming effects (Jackson et al., 2011);
8) The intestinal microbiota may influence osteoclast activity through changes in circulating IGF-1 concentrations and may also affect intestinal calcium absorption (de Sire et al., 2022).

Conclusions

Inulin supplementation of an saturated fatty acid (SFA)-rich high-fat diet was associated with favourable changes in bone turnover markers. In young pigs aged four months, 10 weeks of inulin supplementation, compared with 5 weeks, resulted in greater improvements in the mineralisation of the third metacarpal bone (bone mineral content and density) and its mechanical properties (elastic and maximum strength). Further research is needed to establish the optimal dose and duration of inulin supplementation and to assess its potential to counteract the adverse effects of an SFA-rich high-fat diet on the whole skeletal system.

Conflict of interest

The Authors declare that there is no conflict of interest.

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