

# Effects of dietary brown algae (*Ascophyllum nodosum*) on mineral digestibility and femoral bone properties in growing piglets

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**ABSTRACT.** This study evaluated the effects of dietary supplementation with brown macroalgae (*Ascophyllum nodosum*) at two inclusion levels (0.6% and 1.0%) on mineral digestibility, serum mineral concentrations, and femoral bone characteristics in piglets. A total of 210 piglets were randomly assigned to control (0%) or supplemented diets (0.6% or 1% macroalgae) from day 18 to day 64. Supplementation at the 1.0% level significantly improved digestibility and serum concentrations of essential minerals including calcium, phosphorus, magnesium, copper, and iron. Meanwhile, the 0.6% supplementation led to greater improvements in femoral bone mechanical properties, such as fracture load, elastic work, and work to fracture, as well as in material properties, including fracture and yield stress. These findings indicate that moderate supplementation with *A. nodosum* (0.6%) is most effective in optimising bone mechanical strength, and may represent a practical dietary strategy for improving skeletal development in fast-growing piglets.

## Introduction

In recent years, significant efforts have been directed toward identifying novel nutritional and bioactive compounds capable of enriching animal diets with health-promoting substances. Marine algae have emerged as a promising candidate due to their rich content of bioactive components, including vitamins, polyunsaturated fatty acids, polysaccharides, and pigments, which exert anti-inflammatory and immunomodulatory effects (Prabu et al., 2025). Among these, alginates act as prebiotics, stimulating the growth of beneficial gut microbiota (*Bifidobac-*

*terium bifidum*, *B. longum*), and increasing concentrations of acetic acid, propionic acid, and several short-chain fatty acids (SCFAs) (Afonso et al., 2019). Additionally, fucoidan, a sulphated polysaccharide found in the cell wall of brown algae, possesses broad biological activity, enhancing antioxidant defence mechanisms through the upregulation of redox enzymes such as glutathione, superoxide dismutase, and catalase (Afonso et al., 2019).

Moreover, algae are valuable sources of minerals, primarily due to their unique cell wall composition characterised by high levels of fucoidans and alginates combined with hydroxyperoxidases

(Holdt and Kraan, 2011). Species-specific differences among algae, particularly regarding the type and proportions of soluble and insoluble polysaccharides, may influence the content and bioavailability of minerals. Certain algal species contain calcium levels up to 10-fold higher than those found in milk (Haq et al., 2019), alongside significant quantities of magnesium, phosphorus, and other trace minerals exceeding levels in terrestrial meadow plants by 10- to 100-fold (Circuncisão et al., 2018). The presence of minerals critical for bone formation, together with key vitamins for bone health (vitamins K and D) (Siddiqui et al., 2024), suggests their potential in promoting skeletal health, osteogenesis, and preventing fractures.

Proper bone mineralisation is particularly important in rapidly growing young animals, whose intensive growth requires a high and consistent supply of essential trace elements. Deficiencies in key minerals involved in bone formation can impair skeletal development, potentially leading to lameness, a major concern in animal husbandry (Williams et al., 2024). Consequently, the assessment of bone mineralisation and structure is an essential diagnostic and preventive measure in veterinary medicine, facilitating early detection and correction of mineral deficiencies essential for animal health and welfare. Studies examining the influence of various algae species on bone health using laboratory animal models have shown promising results. For instance, dietary supplementation with spirulina (a blue-green microalga) in rats has been shown to improve bone mineral density and prevent bone loss (Carson and Clarke, 2018). Similarly, another rat-based study reported increased bone mineral density and mechanical strength following supplementation with brown algae (*Sargassum horneri*) (Chen et al., 2020).

However, limited literature is available regarding the impact of dietary algae supplementation on mineral bioavailability and bone structural and mechanical parameters in piglets (Siddiqui et al., 2024). Given the positive outcomes reported in studies involving laboratory animals and human trials, it is justified to investigate the effects of algae supplementation in livestock diets, particularly in young piglets. In these animals, insufficient dietary mineral intake can lead to rickets, skeletal fragility, locomotor impairment, and general physical deterioration, ultimately compromising production efficiency.

Therefore, this study aimed to analyse the effects of varying levels of macroalgae supplementation in piglet diets on mineral digestibility and

femoral bone characteristics. It was hypothesised that algae supplementation would improve the digestibility of essential minerals and trace elements, thereby improving bone mineralisation, structural properties, and mechanical strength.

## Material and methods

The experiment was approved by the Local Ethics Committee for Animal Experiments at the University of Life Sciences in Lublin (Approval No. 67/2017).

The study included 210 piglets (Landrace × Yorkshire Hybrid [LY] × Duroc × Pietrain Hybrid [DP]), born to 15 sows after their second or third lactation. To ensure uniform experimental conditions, piglets were evenly distributed among the sows and ear-tagged for clear identification and replicate assignment. Each sow was allocated 14 piglets (7 gilts and 7 barrows) and randomly assigned to one of three groups (five sows per group). From day 18 post-birth (10 days before weaning), piglets received experimental pre-starter diets at the litter level. The experimental factor was dietary supplementation with brown macroalgae (*Asco-phyllum nodosum*; European Protein, Bække, Denmark) at inclusion levels of either 0.6% (Alg0.6) or 1.0% (Alg1.0) of feed dry matter. The control group (Alg0) received a standard pre-starter feed without macroalgae (Table 1). All diets were formulated to be isoenergetic and isoproteic. Detailed chemical composition of the macroalgae were described previously (Czech et al., 2024). At weaning (day 28), 10 piglets from each sow (5 gilts and 5 barrows) were transferred to respective pens, with a total of 50 piglets per group and 150 pigs overall. The experimental feeding period continued until day 64 when piglets reached approximately 15 kg body weight (Satessa et al., 2020).

For faecal collection and digestibility assessment, six piglets per treatment group (three gilts and three barrows) were selected, including one piglet from each of the five replicate litters (identified by ear tags), plus one randomly selected piglet to maintain sex balance. The selected piglets were housed individually in separate pens and fed experimental diets containing 2 g/kg silicon dioxide (SiO<sub>2</sub>) as an inert marker, incorporated prior to pelleting. Following a six-day adaptation period, faeces were collected daily at the same time for four consecutive days. Samples were weighed, pooled per individual, dried, homogenised, and analysed for mineral content (phosphorus, calcium, magnesium, copper,

**Table 1.** Ingredients and chemical compositions of the experimental diets (Satessa et al., 2020)

Item	Diets		
	Alg0	Alg0.6	Alg1
Ingredients, %			
macroalgae	0	0.6	1
barley	35	35	35
wheat	27.89	27.35	26.92
fermented rapeseed meal	10	10	10
soybean meal	2.7	2.6	2.6
soy protein	2.5	2.5	2.5
potato protein	2.5	2.5	2.5
fishmeal	3	3	3
whey powder	4.25	4.25	4.25
whole milk powder	5	5	5
vegetable oil	2.69	2.78	2.84
L-Lys (98.5%)	0.76	0.75	0.75
DL-Met (99%)	0.21	0.21	0.21
L-Thr (98.5%)	0.29	0.29	0.29
L-Trp (98%)	0.15	0.15	0.15
monocalcium phosphate	0.98	0.99	0.99
sodium chloride	0.36	0.31	0.28
calcium formate	0.7	0.7	0.7
iron fumarate 31%	0.25	0.25	0.25
Pigor® flavouring aroma	0.2	0.2	0.2
mineral-vitamin premix <sup>1</sup>	0.5	0.5	0.5
sucram	0.07	0.07	0.07
Calculated values			
ME, MJ/kg	14.0	14.1	14.2
calcium, g/kg	7.41	7.54	7.62
phosphorus, g/kg	6.60	6.61	6.60
Determined values, g/kg			
dry matter	88.03	88.10	88.07
crude ash	5.85	5.93	5.90
crude protein	18.75	18.60	18.55
crude fat	6.92	7.01	7.05
crude fibre	3.49	3.50	3.49

ME – metabolizable energy; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; <sup>1</sup> mineral-vitamin premix (mg/kg of diet) for both pre-starter and starter diets: IU: vit. A (retinyl acetate) 13000, vit. D<sub>3</sub> (cholecalciferol) 2000; mg: vit. E (tocopheryl acetate) 165, vit. B<sub>1</sub> (thiamine mononitrate) 2.5, vit. B<sub>2</sub> (riboflavin) 7.0, vit. B<sub>6</sub> (pyridoxine hydrochloride) 4.0, vit. B<sub>12</sub> (cyanocobalamin) 0.05, vit. C (ascorbic acid) 100, vit. K<sub>3</sub> (MSB) 3, biotin 0.2, niacin (nicotinic acid) 35, folic acid 1.5, pantothenic acid 21.7, iron (sulphate monohydrate) 180, zinc (oxide) 150, manganese (oxide) 55, selenium (sodium selenite) 0.40, iodine (calcium iodate) 0.60

iron, and zinc) according to AOAC methods (Latimer and AOAC International, 2016). The mineral and SiO<sub>2</sub> content in the feed were analysed concurrently. Apparent total tract digestibility (ATTD) was calculated using the standard formula:

$$\text{ATTD} = (1 - (\text{Nf} \times \text{Md}) / (\text{Nd} \times \text{Mf})) \times 100\%,$$

where: Nd – nutrient concentration in diet, Ni – nutrient concentration in faeces, Md – marker (SiO<sub>2</sub>) concentration in diet, and Mi – marker concentration in faeces (all values expressed as g/kg DM) (Czech et al., 2025).

On day 64, selected piglets from the digestibility trial (n = 6 per group) were euthanised by electrical stunning followed by exsanguination. Whole blood was collected into heparinised tubes, centrifuged at 1400 g for 10 min at 4 °C to obtain plasma, which was subsequently aliquoted into polypropylene tubes, and stored at –20 °C until analysis. Plasma concentrations of calcium, zinc, copper, and iron were determined using atomic absorption spectrometry, while phosphorus content was measured colorimetrically.

After euthanasia, both femora were dissected, individually sealed in zip-lock bags, and stored at –20 °C until analysis. Bone mineral content (BMC) and bone mineral density (BMD) of the right femur were determined using dual-energy X-ray absorptiometry (DXA) with a Lunar iDXA densitometer (GE, Madison, WI, USA). Subsequently, bones were sectioned with an MBS 240/E diamond bandsaw (Proxxon, Föhren, Germany). External and internal mid-diaphyseal diameters were measured in transverse and anteroposterior directions using a digital calliper. These measurements were used to calculate mean relative wall thickness (MRWT), cortical index (CI), cross-sectional area (CSA), and moment of inertia (Ix) (Osiak-Wicha et al., 2023). Mid-diaphyseal bone samples were then washed, defatted, dried overnight at 105 °C and analysed for major and trace mineral contents (calcium, phosphorus, copper, magnesium, iron, and zinc). All analyses of macro- and micronutrients in feed, faeces, blood serum and bone samples were performed at the Central Analytical Laboratory, University of Sciences in Lublin, Lublin, Poland, accredited according to ISO/IEC 17025:2017 by the Polish Accreditation Centre (Certificate No. AB 1375).

Left femora were subjected to mechanical testing. Bone length and weight were measured using a digital precision scale, and the Seedor index (bone weight/bone length) was calculated. Mechanical properties were assessed using a quasi-static three-point bending test on a ZwickRoell 005 universal testing machine (ZwickRoell GmbH & Co., Ulm, Germany) with a support span of 36 mm (representing 40% of the average bone length) at a loading rate of 10 mm/min. Load-deformation curves were analysed using Origin software (Origin-Lab Co., Northampton, MA, USA) to determine

structural parameters, including yield load, elastic work, fracture load, work to fracture, and stiffness. These properties, together with geometrical bone measurements, were used to calculate material properties such as Young's modulus, elastic and fracture strain, and elastic and fracture stress (Osiak-Wicha et al., 2023).

Statistical analysis included verification of data normality (Shapiro-Wilk test) and variance homogeneity (Levene's test). Treatment effects were assessed by one-way ANOVA with dietary treatment as the fixed factor and individual piglets as experimental units ( $n = 6$ ). Post-hoc comparisons were performed using Tukey's HSD test, with statistical significance set at  $P < 0.05$ . All analyses were performed using Statistica software (v.13.3; TIBCO Software Inc., Paolo Alto, CA, USA).

## Results

Piglets supplemented with algae at 1% (Alg1 group) had significantly higher ATTD coefficients for phosphorus, calcium, magnesium, copper, and iron compared to the control group (Table 2). No statistically significant differences in digestibility were observed between the Alg0.6 and control groups. Zinc digestibility was not affected by dietary treatments.

**Table 2.** Apparent total tract digestibility (%) coefficients of minerals in piglets fed diets with different levels of macroalgae supplementation

Item	Diets			SEM	P-value
	Alg0	Alg0.6	Alg1		
Phosphorus	47.6 <sup>b</sup>	49.4 <sup>ab</sup>	51.8 <sup>a</sup>	0.61	0.0104
Calcium	38.4 <sup>b</sup>	40.3 <sup>ab</sup>	42.6 <sup>a</sup>	0.58	0.0047
Magnesium	63.8 <sup>b</sup>	66.4 <sup>ab</sup>	67.7 <sup>a</sup>	0.66	0.0384
Copper	44.0 <sup>b</sup>	43.7 <sup>b</sup>	46.1 <sup>a</sup>	0.41	0.0183
Iron	22.5 <sup>b</sup>	22.8 <sup>b</sup>	25.0 <sup>a</sup>	0.43	0.0249
Zinc	35.1	34.7	36.2	0.28	0.0613

SEM – standard error of the mean; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; data are presented as means ( $n = 6$ ); <sup>ab</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$  based on Tukey's HSD *post hoc* test

Serum analysis revealed that algae supplementation significantly increased calcium and phosphorus concentrations, and additionally elevated serum zinc levels (Table 3). The Alg0.6 group had higher serum copper concentrations, whereas magnesium and iron levels did not differ significantly between groups.

**Table 3.** Mineral contents in the blood plasma of piglets fed diets with different levels of macroalgae supplementation

Item	Diets			SEM	P-value
	Alg0	Alg0.6	Alg1		
Phosphorus, mmol/l	2.51 <sup>b</sup>	2.69 <sup>a</sup>	2.62 <sup>a</sup>	0.030	0.045
Calcium, mmol/l	2.62 <sup>b</sup>	2.88 <sup>a</sup>	2.81 <sup>a</sup>	0.038	0.001
Magnesium, mmol/l	1.46	1.38	1.36	0.026	0.279
Copper, $\mu\text{mol/l}$	20.1 <sup>b</sup>	22.4 <sup>a</sup>	19.4 <sup>b</sup>	0.41	0.001
Iron, $\mu\text{mol/l}$	31.5	31.4	33.4	0.76	0.515
Zinc, $\mu\text{mol/l}$	10.2 <sup>b</sup>	11.6 <sup>a</sup>	11.6 <sup>a</sup>	0.25	0.013

SEM – standard error of the mean; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; the data are presented as means ( $n = 6$ ); <sup>ab</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$  based on Tukey's HSD *post hoc* test

Similar to serum results, algae supplementation, regardless of dosage, significantly increased bone calcium and phosphorus concentrations without altering the overall bone Ca:P ratio (Table 4). Treatment did not affect bone magnesium, iron, or zinc levels; however, the higher algae dose was associated with reduced bone copper content.

**Table 4.** Mineral contents in the femora of piglets fed diets with different levels of macroalgae supplementation (dry matter basis)

Item	Diets			SEM	P-value
	Alg0	Alg0.6	Alg1		
Phosphorus, g/kg	77.1 <sup>b</sup>	79.6 <sup>a</sup>	79.0 <sup>a</sup>	0.31	0.0301
Calcium, g/kg	171 <sup>b</sup>	176 <sup>a</sup>	175 <sup>a</sup>	0.5	0.0482
Magnesium, g/kg	6.61	6.64	6.62	0.012	0.5378
Copper, mg/kg	0.37 <sup>a</sup>	0.37 <sup>a</sup>	0.34 <sup>b</sup>	0.005	0.0014
Iron, mg/kg	17.0	17.2	16.6	0.12	0.1103
Zinc, mg/kg	120	119	119	0.4	0.7586
Ca:P	2.20	2.22	2.24	0.011	0.1307

SEM – standard error of the mean; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; the data are presented as means ( $n = 6$ ); <sup>ab</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$  based on Tukey's HSD *post hoc* test

Bone geometry and density were unaffected by treatment in terms of weight, length, Seedor index, or density parameters (BMC and BMC). However, mid-diaphyseal cross-sectional geometry parameters, including mean relative wall thickness (MRWT) and cortical index (CI), were significantly higher in piglets fed algae-supplemented diets compared to the control. No other changes were observed.

Supplementation with 0.6% algae significantly improved several femoral structural parameters, increasing fracture load, elastic work, and work

**Table 5.** Osteometric, geometric, and densitometric characteristics of the femora of piglets fed diets with different levels of macroalgae supplementation

Item	Diets			SEM	P-value
	Alg0	Alg0.6	Alg1		
Bone weight, g	39.9	35.5	38.9	1.13	0.2584
Bone length, mm	92.8	89.9	91.9	1.03	0.5318
Seedor index, g/mm	0.43	0.39	0.42	0.009	0.1737
MRWT	0.75 <sup>c</sup>	0.98 <sup>a</sup>	0.91 <sup>b</sup>	0.039	0.0327
CI, %	42.9 <sup>b</sup>	49.5 <sup>a</sup>	46.6 <sup>a</sup>	1.17	0.0476
CSA, mm <sup>2</sup>	80.4	89.7	84.9	2.67	0.3914
Ix, mm <sup>4</sup>	1101	1149	1091	60.7	0.9268
BMD, g/cm <sup>2</sup>	0.46	0.44	0.42	0.009	0.1463
BMC, g	7.22	6.61	6.52	0.322	0.1227

MRWT – mid-diaphysis mean relative wall thickness, CI – cortical index, CSA – cross-sectional area, Ix – cross-sectional moment of inertia, BMD – bone mineral density, BMC – bone mineral content, SEM – standard error of the mean; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; data are presented as means (n = 6); <sup>abc</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$  based on Tukey's HSD *post hoc* test

to fracture, as well as material properties such as higher elastic and fracture stress and lower fracture strain, compared to the control group (Table 6). Meanwhile, the higher algae dose (1%) only significantly reduced fracture strain.

**Table 6.** Mechanical characteristics of the femora of piglets fed diets with different levels of macroalgae supplementation

Item	Diets			SEM	P-value
	Alg0	Alg0.6	Alg1		
Yield load, N	638.6	818.9	651.4	36.75	0.074
Elastic work, J	0.43 <sup>b</sup>	0.70 <sup>a</sup>	0.49 <sup>b</sup>	0.034	0.001
Fracture load, N	859 <sup>ab</sup>	1052.3 <sup>a</sup>	734.1 <sup>b</sup>	43.73	0.005
Work to fracture, J	1.42 <sup>a</sup>	1.40 <sup>a</sup>	0.88 <sup>b</sup>	0.065	0.001
Stiffness, N/mm	536.2	571.6	557.9	15.44	0.669
Young's modulus, GPa	0.511	0.671	0.562	0.037	0.195
Elastic strain, %	7.08	7.08	6.14	0.318	0.405
Elastic stress, MPa	34.9 <sup>b</sup>	43.1 <sup>a</sup>	35.3 <sup>b</sup>	1.76	0.047
Fracture strain, %	13.9 <sup>a</sup>	9.3 <sup>c</sup>	11.7 <sup>b</sup>	0.65	0.006
Fracture stress, MPa	47.21 <sup>ab</sup>	56.21 <sup>a</sup>	41.08 <sup>b</sup>	2.554	0.024

SEM – standard error of the mean; Alg0 – group fed a standard diet without macroalgae addition, Alg0.6 – group fed a diet with 0.6% macroalgae, Alg1 – group fed a diet with 1% macroalgae; data are presented as means (n = 6); <sup>abc</sup> – means within a row with different superscripts are significantly different at  $P < 0.05$  based on Tukey's HSD *post hoc* test

## Discussion

The present research demonstrates the potential benefits and rationale behind dietary supplementation with macroalgae. Incorporating macroalgae at

a concentration of 1% significantly improved the digestibility of minerals essential for bone growth, such as calcium, phosphorus, magnesium, copper, and iron, compared to the control group, consistent with previous studies on *Ascophyllum nodosum* (Cabrita et al., 2016). The improved bioavailability of these minerals is likely due to the presence of non-starch polysaccharides, such as laminarin, fucoidan, and alginate in brown algae that facilitate mineral binding and absorption. These polysaccharides, comprising 30% to 75% of seaweed dry matter, show prebiotic properties (Gotteland et al., 2020), modulating gut microbiota by promoting beneficial bacteria (*Lactobacillus*, *Bifidobacterium*) development while inhibiting pathogens (*Escherichia coli*) (Michiels et al., 2012; Corino et al., 2019; Gotteland et al., 2020). Macroalgae supplementation has also been associated with increased concentrations of short-chain fatty acids (SCFAs), particularly butyric acid, which directly maintain and stabilise gut barrier and function (Michiels et al., 2012; Corino et al., 2019). Additionally, fucoidan promotes the growth of *Lactobacillus*, which produces lactic acid, lowering gastrointestinal pH, thereby optimising mineral solubility and uptake (Smith et al., 2011).

The increased availability and absorption of essential minerals from diets supplemented with *Ascophyllum nodosum* were reflected in significantly elevated serum concentrations of calcium, phosphorus, and zinc in piglets, with copper levels also increased in the group administered 0.6% algae in the diet. These findings differ from studies using dried green algae (*Enteromorpha* sp.), which showed no improvements in serum mineral element concentrations (Michalak et al., 2015), although the specific supplementation rate was not specified. In contrast, supplementation with red algae (*Pyropia tenera*) and brown algae (*Undaria pinnatifida*) in rats led to reduced digestibility of calcium, zinc, magnesium, and iron compared to controls (Urbano and Goñi, 2002). Such differences may stem from variations in amino acid profiles in individual algal species. Additionally, polyphenolic compounds and peptides present in algae may contribute to decreased digestibility and mineral retention in intestinal contents (Urbano and Goñi, 2002). On the other hand, laminarin, a polysaccharide found in brown algae, may indirectly stimulate mineral absorption by modifying the resident gut microbiota and promoting mucin synthesis and secretion, both of which play a crucial role in metal cation absorption (Smith et al., 2011).

Dietary supplementation with algae *Ascophyllum nodosum* at 1% and 0.6% significantly increased

calcium and phosphorus content in piglet femora, maintaining an optimal Ca:P ratio crucial for bone health (Lautrou et al., 2020). Both minerals are key components of hydroxyapatite, the main crystal-line structure in bone matrix that determines bone density, mechanical strength, and overall skeletal integrity. Deficiencies in these minerals during early life stages increases the risk of fractures later in piglet growth (Grela et al., 2020; Sobol et al., 2024). Algae are a particularly rich source of bio-available calcium, thus they can be applied as a dietary calcium source in animal nutrition (Cabrita et al., 2016; Karimidastjerd et al., 2024). Although increased calcium and phosphorus supply did not significantly alter bone mineral content (BMC), algae supplementation improved cortical bone parameters, as indicated by higher cortical index (CI) and mean relative wall thickness (MRWT). From a mechanical perspective, bone strength largely depends on material distribution, specifically the spatial arrangement of cortical bone (Adams et al., 2016). These improvements indicate that, despite similar bone length and mass compared to control animals, the bones of supplemented piglets were more structurally mature and better adapted to withstand greater mechanical loads an advantage for rapidly growing animals, whose increasing body weight puts considerable strain on their skeletal system.

Interestingly, a decrease in copper content in compact bone was observed in piglets receiving the higher algae supplementation dose (1%). Copper is essential for bone health, particularly due to its role in collagen synthesis and cross-linking, supporting bone marrow function and the trabecular bone integrity (Gaffney-Stomberg, 2019). As copper levels were measured only in the compact bone of the mid-diaphysis, the lower concentrations observed in the Alg1 group compared to the Alg0.6 group could partly explain the limited improvement in mechanical properties, despite the elevated calcium and phosphorus content relative to controls. In contrast, the Alg0.6 group, where copper levels remained stable, showed more pronounced increases in bone strength. Femoral bones from piglets supplemented with 1% algae showed only significantly reduced fracture strain compared to controls, indicating greater rigidity likely resulting from altered cortical bone geometry (Currey, 1999).

Piglets supplemented with the lower algae dose (0.6%) demonstrated broader improvements in bone mechanical properties, including reduced

fracture strain as well as many enhancements in structural and material parameters. Specifically, increases were observed in fracture load, elastic work, and work to fracture, indicating that bones became more resistant to deformation and capable of absorbing higher energy before deformation or fracturing. These changes imply that the bones of piglets supplemented with 0.6% algae could withstand significantly higher stress levels, both within the elastic region (yield stress) and at the point of fracture (fracture stress). These mechanical improvements may have resulted from modifications in hydroxyapatite crystal organisation and size, which could affect mineral phase stress distribution and load-sharing capacity (Almer and Stock, 2005). This requires further investigation, particularly in light of previous reports concerning changes in hydroxyapatite crystal size during pig development (Tomaszewska et al., 2019), as such crystallographic alterations may influence mechanical properties more significantly than bone mineral density alone (Ruppel et al., 2008).

## Conclusions

This study demonstrates the practical benefits of incorporating macroalgae into piglet diets. Although the 1% inclusion level significantly improved mineral absorption, piglets receiving 0.6% algae supplementation showed marked improvements in structural parameters, such as fracture load, and in material properties, including fracture and yield stress. Taken together, these findings suggest that the lower supplementation dose (0.6%) appears more optimal for improving skeletal development in piglets.

## Conflict of interest

The Authors declare that there is no conflict of interest.

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