Jpn. J. Meat Sci. Technol., Vol.64, No.2（2023）107-117

【Original Paper（General paper）】

Effects of a mixed diet containing whole ground adult chicken on bone properties in growing rats with low calcium intake

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（Received: 2023.4.13，Accepted: 2023.10.5）

Abstract

This study aimed to examine the effects of a mixed diet containing whole ground adult chicken (WGAC) on bone properties in growing rats with low calcium (L.Ca) intake. Four-week-old male Sprague-Dawley rats were divided into three groups and were fed a standard laboratory diet (CON group), L.Ca diet (L.Ca group), and L.Ca diet mixed with WGAC (L.Ca+WGAC group) for 13 weeks. Cortical bone mass, cortical bone geometry, and bone mechanical strength were significantly lower in the L.Ca group compared with the CON and L.Ca+WGAC groups; the levels were similar in the latter two groups. Our study revealed that L.Ca intake induced bone deterioration, and that Ca supplementation with WGAC improved cortical bone properties in growing bones such that they were comparable to those grown on a standard laboratory diet. Implications for the use of WGAC to improve bone loss under L.Ca conditions are discussed with regard to bone health during childhood. Careful consideration will be needed regarding other factors that might have affected bone properties since nutrition contents were not fixed.

Jpn. J. Meat Sci. Technol., **64**（2）, 107-117, 2023

Key Words：Growing rats, Low calcium diet, Whole ground adult chicken, Bone Properties

Abbreviations：1,25-DHVD3, 1,25-dihydroxyvitamin D3; ALP, alkaline phosphatase; AV, all bone volume; BALP, bone specific alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BUN, blood urea nitrogen; BV, bone volume ; BV/TV, bone volume fraction; BW, body weight; Ca, calcium; CBG, cortical bone geometry; CON, control; Conn.D, connectivity density; Ct.Ar, cortical bone sectional area; Ct.Th, cortical bone thickness; Ct.V, cortical bone volume; Ct.Po, cortical porosity; Ct.V/AV, cortical bone volume fraction; DBW, dry bone weight; EDL, extensor digitorum longus; ELISA, enzyme-linked immunosorbent assay; Ec.Pm, endocortical perimeter; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphate; L.Ca, low calcium; LDL-C, low-density lipoprotein cholesterol; Mg, magnesium; MV, medullary volume; PBM, peak bone mass; PTH, parathyroid hormone; Ps.Pm, periosteal perimeter; ROI, region of interest; TBA, trabecular bone architecture; TBPf, trabecular bone pattern factor; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TC, total cholesterol; TG, Triacylglycerol; TMD, tissue mineral density; TP, total protein; TRACP-5b, tartrate-resistant acid phosphatase 5b; TV, tissue volume; vBMD, volume BMD; WBW, wet bone weight; WGAC, whole ground adult chicken.

1. Introduction

Bone health during growth is deeply influenced by lifestyle. Optimizing peak bone mass (PBM), a predictor of future osteoporosis and fracture risk [1], is important for boys and girls. Lifestyle choices reportedly influence PBM in 20-40% of adult, and calcium (Ca) intake and physical activity in childhood have positive effects on PBM [2]. Impact and loading exercises and a well-balanced diet including Ca are reportedly associated with higher PBM [2,3]. Adequate Ca supplementation has been

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reported to increase bone mineral content (BMC) and bone mineral density (BMD) in childhood [4-6], whereas low dietary Ca intake is associated with lower appendicular BMD in children [7]. Thus, Ca intake is important for both BMD and BMC in children and adolescents.

Previous animal studies have reported that low Ca (L.Ca) intake induces deterioration of bone properties such as bone mass, architecture, strength, and growth [8-12]. While Ca supplementation reportedly improves bone mass [12-17], its effects on bone properties differ by type of Ca supplement [13-17]. Milk consumption increases trabecular and cortical bone structure as well as maximum force of the femur [13], and hake fish bone improves femoral breaking force, tibial density, and Ca/phosphorus content [14] in growing rats with L.Ca intake. In addition, dietary Ca (milk solids) protects bone against Ca depletion in young rats compared with calcium carbonate [15]. Thus, regardless of the source of Ca supply, Ca intake has a beneficial effect on bone properties during growth and potentially improves bone health in L.Ca conditions.

Milk and fish bone are rich in Ca and have been used as Ca sources in Ca supplementation [13-15]. However, no study on bone health has investigated the usefulness of animal bone and flesh as Ca sources. Adult chicken that has a long spawning period or can no longer lay eggs is generally used as a raw material for processed food (e.g., ground chicken), as the meat is tougher compared with broiler chicken meat [18]. Although meat and bone are ground together with a meat-bone separator when processed as ground chicken, whole ground adult chicken (WGAC) is considered to contain beneficial ingredients, such as bone fragments, for bone health. Thus, WGAC is expected to improve bone properties as a source of Ca supplementation, thereby helping to prevent bone deterioration in childhood. However, the effects of WGAC on bone properties have yet to be verified in humans. Accordingly, this pilot study aimed to examine the effects of a mixed diet containing WGAC on bone properties, such as bone mass, structure, and mechanical strength, in growing rats with L.Ca intake as a model of insufficient Ca intake in childhood or adolescence.

2. Materials and Methods

2.1. Animal Care

Twenty-four 4-week-old male Sprague-Dawley rats (Japan SLC, Inc., Hamamatsu, Japan) were used in this study. Rats were individually housed in cages at 22-24°C temperature and under a 12-hour day-night cycle, and were divided into the following three groups (n=8 each): the control diet (CON) group, L.Ca diet (L.Ca) group, and L.Ca mixed diet (L.Ca+WGAC) groups. Rats in the CON group were fed a standard laboratory diet (CE-2; CLEA Japan, Inc., Tokyo, Japan). Those in the L.Ca and L.Ca+WGAC groups were fed a L.Ca diet (CE-2 low Ca; CLEA Japan, Inc., Tokyo, Japan) and a L.Ca diet mixed with a freeze-dried powder of WGAC, respectively, for 13 weeks. The freeze-dried powder of WGAC was prepared by heat-sterilizing and freeze-drying WGAC and then powdering it. The compounding ratio of the L.Ca diet and freeze-dried powder was 1:1. Raw materials of the standard diet included soybean cake, white fish meal, yeast, embryo, soybean oil, flour, corn, milo, bran, alfalfa meal, salt, multiple vitamins (vitamins A, D3, E, B1, B2, B6, B12, and C, niacin, pantothenic acid, biotin, folic acid, choline chloride, and inositol), and minerals (calcium carbonate, sodium chloride, iron sulfate, manganese

Table 1. Nutrition contents of the experimental diets (per 100g).

	Standard laboratory diet	L.Ca diet	L.Ca diet mixed with WGAC	Freeze-dried powder of WGAC
Moisture (g)	9.0	8.8	4.8	0.8
Protein (g)	25.2	24.0	32.8	37.5
Fat (g)	6.1	6.1	29.7	60.5
Carbohydrates (g)	52.0	57.1	29.2	0.0
Ash (g)	6.9	4.0	3.5	3.3
Calcium (mg)	1099.5	142.3	350.0	660.6
Phosphorus (mg)	1006.0	700.0	641.1	595.3
Iron (mg)	29.6	31.5	17.0	3.7
Energy (kcal)	363.7	379.3	515.3	694.5

L.Ca, low calcium; WGACA, whole ground adult chicken.

sulfate, cobalt sulfate, and folic acid calcium salt). For the L.Ca diet, casein and corn were included instead of white fish meal and calcium carbonate. All rats were allowed to eat their food and drink distilled water ad libitum during the feeding period. The type of diet was powder. Nutrition contents as analytical values are shown in Table 1.

After the 13-week feeding period, blood samples were collected from all rats under isoflurane anesthesia. Rats were sacrificed by bleeding, and muscles, bones, and mesenteric fat were collected. Serum samples, obtained by blood centrifugation at 1000 *g* for 30 minutes, were stored at -80°C until biochemical analysis and enzyme-linked immunosorbent assay (ELISA). Bilateral soleus and extensor digitorum longus (EDL) muscles were harvested and weighed. Bilateral femurs were harvested, soft tissues were removed, and wet bone weight (WBW) and length were measured. Muscle weight and WBW were corrected for body weight (BW). Right and left femurs were stored in saline and 70% ethanol, respectively, until analysis.

This study was approved by the Committee of Research Facilities of Laboratory Animal Science, Kio University (No. R01-06), and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Bone mechanical strength measurement

The maximum load and break point of right femurs were measured by a 3-point bending strength test using the Universal Testing Machine (Autograph AGS; Shimadzu Corp., Kyoto, Japan), and stiffness was calculated as the slope of load-displacement curves. Bone was supported by two fulcrums 5 mm in diameter separated by half the bone length, and pressed downward at the center at a speed of 1 mm/min.

2.3. Trabecular bone architecture (TBA) and cortical bone geometry (CBG) analyses

The left distal femur and mid-shaft of the femur were scanned at 60 kV, 60 μA, with a voxel size of 9.7 μm to analyze TBA and CBG, respectively, using micro-computed tomography (Micro-CT; Yamato Scientific Co., Ltd. Tokyo, Japan). The region of interest (ROI) for TBA of the distal femur was a 2-mm long portion of the femur metaphysis, and the first slice was scanned 0.5 mm proximal of the physeal-metaphyseal demarcation. The ROI for CBG was a 1-mm long portion of the center of the femur diaphysis. Scanned data were transmitted to a personal computer, and TBA (trabecular bone volume, thickness, number, separation, and connectivity in cancellous bone) and CBG (cortical bone volume, thickness, area, and porosity) of the ROI were analyzed using the bone analysis software (TRI/3D-BON; Ratoc System Engineering Co. Ltd., Tokyo, Japan). Bone volume (BV, bone volume of the ROI), bone volume fraction (BV/TV, ratio of BV to tissue volume included in BV and marrow volume (MV) of the ROI), trabecular thickness (Tb.Th, mean thickness of trabeculae), trabecular number (Tb.N, measure of the average number of trabeculae per unit length), trabecular separation (Tb.Sp, mean distance between trabeculae), connectivity density (Conn.D, measure of the degree of connectivity of trabeculae normalized to TV), and trabecular bone pattern factor (TBPf, ratio of variation in trabecular surface area to change in trabecular volume of neighboring trabecular surface) were assessed as TBA parameters [19,21] in the femur metaphysis. Cortical bone volume (Ct.V, bone volume of the ROI), MV (marrow volume of the ROI), cortical bone volume fraction (Ct.V/AV, ratio of Ct.V to all bone volume (AV) of the ROI), cortical bone thickness (Ct.Th, average cortical thickness), cortical bone sectional area (Ct.Ar, cortical bone area=Ct.V/number of slices*slice thickness), periosteal perimeter (Ps.Pm), endocortical perimeter (Ec.Pm), and cortical porosity (Ct.Po, volume of pores/Ct.V in a given cortical region) were assessed as CBG parameters [19, 21] in the femur diaphysis. A BMD phantom was simultaneously scanned under the same scanning conditions to obtain tissue mineral density (TMD; BMC/BV), BMC, and volume BMD (vBMD; BMC/TV) of the femoral trabecular and cortical bone [19, 21].

2.4. Dry bone weight (DBW) and ash weight measurements

Following TBA and CBG analyses, the femur was dehydrated in ethanol for 48 hours and then dried at 100°C for 24 hours with a drying machine (Yamato Scientific Co., Ltd., Tokyo, Japan) to measure DBW. Finally, bones were burned to ash at 600°C for 24 hours with an electric furnace (Nitto Kagaku Co., Ltd., Nagoya, Japan) and ash weight was measured. DBW and ash weight were corrected to WBW and DBW (%ash), respectively.

2.5. Blood biochemical analysis

Serum concentrations of Ca, inorganic phosphate (IP), magnesium (Mg), albumin, blood urea nitrogen (BUN),

creatinine, alkaline phosphatase (ALP), total protein (TP), total cholesterol (TC), triacylglycerol (TG), and high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) were determined. These biochemical analyses were consigned to Nagahama Life Science Laboratory (Oriental Yeast Co., Ltd., Nagahama, Japan). In addition, serum levels of osteocalcin and bone specific alkaline phosphatase (BALP) as bone formation markers, tartrate-resistant acid phosphatase 5b (TRACP-5b) as a bone resorption marker, homocysteine, parathyroid hormone (PTH), and 1,25-dihydroxyvitamin D3 (DHVD3) were measured with an osteocalcin EIA kit, TRACP-5b ELISA kit (Immunodiagnostic Systems, Ltd., Boldon, UK), homocysteine ELISA kit (CUSABIO, Hua Mei Biotech Co., Ltd., Wuhan, China), BALP, PTH, and DHVD3 ELISA kit (MyBioSource.com. San Diego, USA), respectively.

2.6. Statistical analysis

Values of all indices are expressed as mean and standard deviation. The Kruskal-Wallis test was performed to examine overall differences among three groups, and the Steel-Dwass test was used to determine the significance between two groups. In addition, Spearman's rank correlation coefficients were determined to examine the relationships between BW gain, nutritional intake (Ca, carbohydrate, protein, and fat) and TBA/CBG parameters and bone mechanical strength. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using the Excel system for personal computers with Excel Statistics software (BellCurve for Excel version 4.04 for Windows; Social Survey Research Information Co. Ltd., Tokyo, Japan).

3. Results

3.1. Final BW, food intake, muscle weight, and bone sizes (Table 2)

Final BW was significantly higher in the L.Ca+WGAC group compared with the CON and L.Ca groups. Food intake was significantly lower in the L.Ca+WGAC group compared with the CON and L.Ca groups. BW gain corrected for food intake and BW gain corrected for calorie intake were significantly higher in the L.Ca+WGAC group compared with the CON and L.Ca groups (Table 2). Carbohydrate intake was significantly lower in the L.Ca+WGAC group compared with the CON and L.Ca groups. Protein intake was significantly lower in the L.

Table 2. Body weight, food intake, muscle weight and bone size in femurs.

	CON	L.Ca	L.Ca+WGAC
Final BW (g)	546.8 ± 49.3^{b}	525.4 ± 43.9^{b}	647.8 ± 75.3^{a}
Food intake (g/day)	26.0 ± 2.3^{a}	24.3 ± 2.9^{a}	18.5 ± 1.4^{b}
BW gain (g)	425.0 ± 38.3^{b}	404.2 ± 40.0^{b}	527.5 ± 70.8^{a}
BW gain - food intake ratio (BW/g)	0.181 ± 0.007^{b}	0.183 ± 0.017^{b}	0.314 ± 0.016^{a}
BW gain - calorie intake ratio ($BW*10^3$/kcal)	49.7 ± 1.9^{b}	48.3 ± 4.5^{b}	61.0 ± 3.1^{a}
Calculated intake of nutrients from the diets			
Carbohydrates (g/day)	13.5 ± 1.2^{a}	13.9 ± 1.6^{a}	5.4 ± 0.4^{b}
Protein (g/day)	6.6 ± 0.6^{a}	5.8 ± 0.7^{b}	6.1 ± 0.5^{b}
Fat (g/day)	1.6 ± 0.1^{b}	1.5 ± 0.2^{b}	5.5 ± 0.4^{a}
Calcium (mg/day)	286.1 ± 25.7^{a}	34.5 ± 4.1^{c}	64.7 ± 4.9^{b}
Phosphorus (mg/day)	261.8 ± 23.5^{a}	169.9 ± 20.1^{b}	118.5 ± 9.0^{c}
Energy (kcal/day)	94.6 ± 7.5	92.1 ± 10.6	95.2 ± 9.0
Mesenteric fat-BW ratio (g/BW)	1.47 ± 0.36^{b}	1.25 ± 0.22^{b}	2.58 ± 0.57^{a}
Soleus-BW ratio (mg/BW)	0.343 ± 0.023	0.356 ± 0.021	0.338 ± 0.029
EDL-BW ratio (mg/BW)	0.439 ± 0.024^{a}	0.418 ± 0.033^{a}	0.371 ± 0.025^{b}
Bone length (mm)	40.6 ± 1.1	40.9 ± 0.9	40.8 ± 0.7
WBW-BW ratio (mg/BW)	2.36 ± 0.11^{a}	2.19 ± 0.08^{b}	2.01 ± 0.12^{c}
DBW-WBW ratio (mg/WBW)	65.9 ± 0.6^{a}	60.3 ± 1.2^{b}	65.4 ± 0.9^{a}
%ash	62.7 ± 0.5^{a}	54.9 ± 1.3^{c}	60.9 ± 1.6^{b}

All values are expressed as mean ± SD.
Different letters represent significance by Steel-Dwass test ($p<0.05$).
BW, body weight; EDL, extensor digitorum longus; WBW, wet bone weight; DBW, dry bone weight.

Ca and L.Ca+WGAC groups compared with the CON group. Fat intake was significantly higher in the L.Ca+WGAC group compared with the CON and L.Ca groups. Ca intake was significantly lower in the L.Ca and L.Ca+WGAC groups compared with the CON group. Ca intake was significantly higher in the L.Ca+WGAC

group compared with the L.Ca group. Mesenteric fat-BW ratio was significantly higher in the L.Ca+WGAC group compared with the CON and L.Ca groups. EDL-BW ratio was significantly lower in the L.Ca+WGAC group compared with the CON and L.Ca groups. WBW-BW ratio was significantly lower in the L.Ca and L.Ca+WGAC groups compared with the CON group. DBW-WBW ratio and %ash were significantly lower in the L.Ca group compared with the CON and L.Ca+WGAC groups, and %ash was significantly lower in the L.Ca+WGAC group compared with the CON group.

3.2. TBA and CBG parameters (Table 3)

Trabecular TMD and vBMD were significantly lower in the L.Ca and L.Ca+WGAC groups compared with the CON group. Trabecular TMD was significantly higher in the L.Ca+WGAC group compared with the L.Ca group. BV/TV and Tb.Th were significantly lower in the L.Ca and L.Ca+WGAC groups compared with the CON group. Tb.Th was significantly lower in the L.Ca and L.Ca+WGAC groups compared with the CON group, and Tb.Th was higher in the L.Ca+WGAC group compared with the L.Ca group. TBPf was significantly higher in the L.Ca group compared with the CON group. Cortical TMD and BMC were significantly lower in the L.Ca group compared with the CON and L.Ca+WGAC groups. Ct.V, CtV/AV, Ct.Th, and Ct.Ar were significantly lower in the L.Ca group compared with the CON and L.Ca+WGAC groups. MV and Ec.Pm were significantly higher in the L.Ca group compared with the CON and L.Ca+WGAC groups.

3.3. Bone mechanical strength

Femoral maximum load, break point, and stiffness were significantly lower in the L.Ca (111.9±8.8 N, 105.0

Table 3. Trabecular bone microstructure and cortical bone geometry in tibias.

	CON	L.Ca	L.Ca+WGAC
Trabecular bone mass			
TMD (mg/cm^3)	963.7 ± 10.1^a	834.0 ± 19.5^c	914.1 ± 25.5^b
BMC (mg)	4.60 ± 1.35	3.26 ± 0.61	3.19 ± 1.25
vBMD (mg/cm^3)	193.1 ± 39.0^a	111.6 ± 16.5^b	125.2 ± 40.9^b
TBA parameters			
Trabecular bone volume fraction (BV/TV, %)	20.2 ± 4.0^a	13.3 ± 1.9^b	13.7 ± 4.2^b
Trabecular thickness (Tb.Th, μm)	99.8 ± 3.9^a	73.5 ± 3.7^c	90.0 ± 3.6^b
Trabecular number (Tb.N, mm^{-1})	1.44 ± 0.22	1.34 ± 0.18	1.15 ± 0.27
Trabecular separation (Tb.Sp, μm)	208.4 ± 24.4	236.1 ± 24.5	233.2 ± 23.1
Connectivity density (Conn.D, mm^{-3})	40.6 ± 9.5	36.1 ± 6.6	29.0 ± 9.7
Trabecular bone pattern factor (TBPf, mm)	7.4 ± 1.0	9.6 ± 2.0^a	9.9 ± 2.1
Cortical bone mass			
TMD (mg/cm^3)	1366.2 ± 11.2^a	1265.7 ± 29.0^b	1343.2 ± 24.9^a
BMC (mg)	10.95 ± 0.63^a	7.37 ± 0.31^b	10.69 ± 0.97^a
CBG parameters			
Cortical bone volume (Ct.V, mm^3)	8.01 ± 0.49^a	5.81 ± 0.30^b	7.94 ± 0.65^a
Medullary volume (MV, mm^3)	4.44 ± 0.69^b	6.35 ± 1.14^a	5.06 ± 0.47^b
Cortical bone volume fraction (Ct.V/AV, %)	64.5 ± 3.2^a	48.0 ± 3.5^b	61.1 ± 2.9^a
Cortical bone thickness (Ct.Th, μm)	779.6 ± 40.5^a	513.7 ± 31.0^b	742.7 ± 55.9^a
Cortical bone sectional area (Ct.Ar, mm^2)	8.02 ± 0.50^a	5.82 ± 0.29^b	7.93 ± 0.65^a
Periosteal perimeter (Ps.Pm, mm)	13.2 ± 0.6	13.1 ± 0.7	13.4 ± 0.4
Endocortical perimeter (Ec.Pm, mm)	8.1 ± 0.6^b	10.2 ± 1.0^a	8.7 ± 0.6^b
Cortical porosity (Ct.Po, %)	0.08 ± 0.04	0.26 ± 0.19	0.06 ± 0.04

All values are expressed as mean ± SD.
Different letters represent significance by Steel-Dwass test ($p<0.05$).
TMD, tissue mineral density; BMC, bone mineral content; vBMD, volume bone mineral density.

±9.7 N, 374.2±44.8 N/mm) group compared with the CON (154.3±18.3 N, 138.0±18.2 N, 548.8±59.5 N/mm) and L.Ca+WGAC (168.2±19.1 N, 160.9±19.9 N, 546.0±75.5 N/mm) groups.

3.4. Blood biochemical analysis (Table 4 and Fig. 1)

Serum IP and Mg concentrations were significantly higher in the L.Ca group compared with the CON group. Serum Mg concentration was significantly lower in the L.Ca+WGAC group compared with the L.Ca group. Serum BUN concentration was significantly higher in the L.Ca+WGAC group compared with the L.Ca group, and serum creatinine and LDL-C concentrations were significantly higher in the L.Ca+WGAC group compared with the CON and L.Ca groups. Serum TC concentration was

Table 4. Biochemical data in all groups.

	CON	L.Ca	L.Ca+WGAC
Calcium (mg/dL)	10.4 ± 0.4	10.3 ± 0.3	10.5 ± 0.3
IP (mg/dL)	5.4 ± 0.6^{b}	6.0 ± 0.3^{a}	5.7 ± 0.3^{ab}
Mg (mg/dL)	2.01 ± 0.06^{b}	2.24 ± 0.05^{a}	2.06 ± 0.13^{b}
Albumin (g/dL)	4.3 ± 0.2	4.3 ± 0.1	4.3 ± 0.2
BUN (mg/dL)	24.6 ± 2.4^{ab}	24.4 ± 2.4^{b}	26.9 ± 1.8^{a}
Creatine (mg/dL)	0.33 ± 0.02^{b}	0.31 ± 0.01^{b}	0.44 ± 0.06^{a}
ALP (IU/L)	754.1 ± 232.4	724.6 ± 156.1	795.3 ± 170.9
TP (g/dL)	6.6 ± 0.5	6.8 ± 0.4	7.3 ± 0.9
TC (mg/dL)	67.5 ± 14.5^{b}	86.6 ± 16.6^{ab}	97.1 ± 21.5^{a}
TG (mg/dL)	172.9 ± 79.3	176.6 ± 81.2	221.3 ± 121.5
LDL-C (mg/dL)	5.9 ± 1.5^{b}	7.3 ± 1.9^{b}	13.4 ± 3.6^{a}
HDL-C (mg/dL)	30.8 ± 4.3	31.3 ± 2.9	33.0 ± 3.6

All values are expressed as mean ± SD.
Different letters represent significance by Steel-Dwass test ($p<0.05$).
IP, inorganic phosphate; Mg, magnesium; BUN, blood urea nitrogen; ALP, alkaline phosphatase; TP, total protein; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

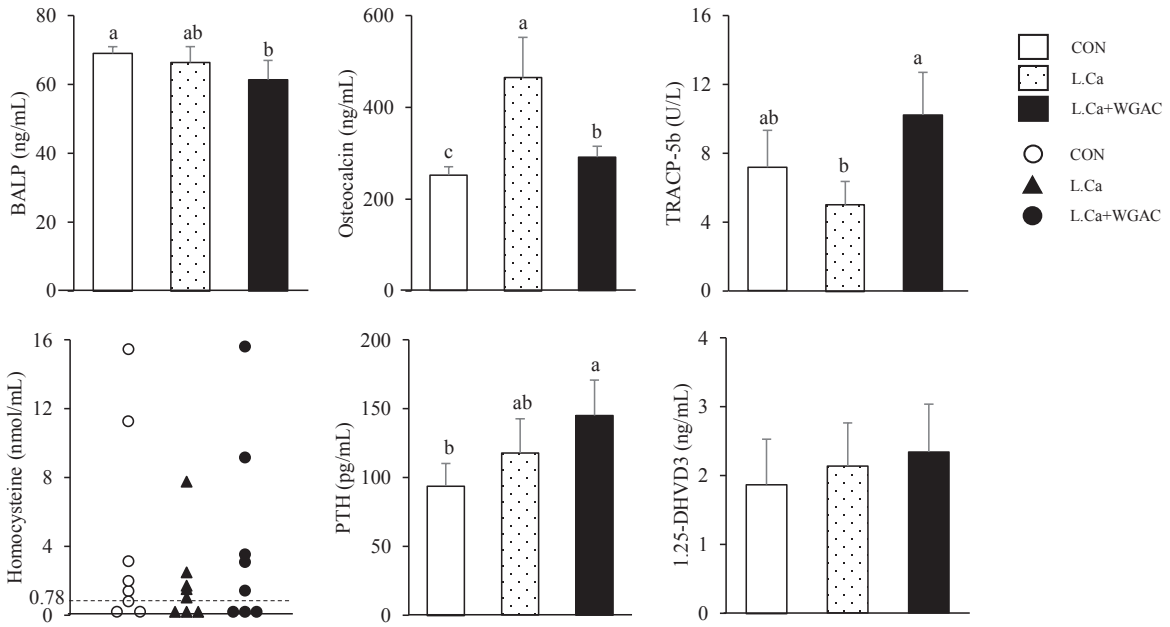

Fig. 1. Serum levels of bone markers in all groups.

Different letters represent significance by Steel-Dwass test ($p<0.05$). Bar means SD.
BALP, Bone specific alkaline phosphatase; TRACP-5b, Tartrate-resistant acid phosphatase-5b; PTH, Parathyroid hormone; 1,25-DHVD3, 1,25-dihydroxy vitamin D_3.

Table 5. Correlations between body weight gain, nutrition intake and femoral bone properties.

	BW gain	Calcium	Carbohydrates	Protein	Fat
Bone mass parameters					
Trabecular TMD	——	0.911	——	0.514	——
Trabecular BMC	——	0.530	0.444	0.610	——
vBMD	——	0.677	——	0.514	——
Cortical TMD	——	0.817	——	0.424	——
Cortical BMC	0.574	0.854	——	0.670	0.577
Trabecular bone architecture parameters					
Bone volume fraction (BV/TV)	——	0.644	——	0.489	——
Trabecular thickness (Tb.Th)	——	0.911	——	0.528	——
Trabecular separation (Tb.Sp)	——	-0.425	——	——	——
Trabecular bone pattern factor (TBPf)	——	-0.502	——	-0.405	——
Cortical bone geometry parameters					
Cortical bone volume (Ct.V)	0.655	0.817	——	0.645	0.645
Medullary volume (MV)	——	-0.631	——	——	——
Cortical volume fraction (CV/AV)	——	0.799	——	——	——
Cortical bone thickness (Ct.Th)	——	0.818	——	0.427	——
Cortical bone area (Ct.Ar)	0.655	0.817	——	0.645	0.645
Periosteal perimeter (Ps.Pm)	0.804	0.406	——	0.624	0.624
Endocortical perimeter (Ec.Pm)	——	-0.645	——	——	——
Cortical porosity (Ct.Po)	——	——	0.405	——	——
Mechanical strength parameters					
Maximum load	0.763	0.677	——	0.524	0.765
Break point	0.730	0.617	——	0.458	0.764
Stiffness	0.644	0.790	——	0.525	0.560

Values are Spearman's correlation coefficients ($p<0.05$).
BW, body weight; TMD, tissue mineral density; BMC, bone mineral content; vBMD, volume bone mineral density.

significantly higher in the L.Ca+WGAC group compared with the CON group.

Serum BALP levels were significantly lower, and serum osteocalcin and PTH levels were significantly higher, in the L.Ca+WGAC group compared with the CON group. Serum osteocalcin levels were significantly lower, and serum TRACP-5b levels were significantly higher, in the L.Ca+WGAC group compared with the L.Ca group. Serum osteocalcin levels were significantly higher in the L.Ca group compared with the CON group.

3.5. Relationships between BW gain, nutrition intake and trabecular and cortical bone properties

Table 5 shows significant rank correlation coefficients ($p<0.05$). Strong correlations ($r>0.7$) were observed mainly between Ca intake and cortical bone mass and CBG parameters. BW gain and fat intake were correlated with bone mechanical strength.

4. Discussions

In the present study, trabecular and cortical bone mass (TMD, BMC), TBA, CBG, and femoral mechanical strength were significantly deteriorated in the L.Ca group compared with the CON group. L.Ca intake has been reported to induce deterioration of bone quantity, quality, and mechanical properties [8-13], and our results are in line with this. However, cortical bone mass (TMD, BMC), CBG, and bone mechanical strength were significantly higher in the L.Ca+WGAC group compared with the L.Ca group and were at the same levels as those in the CON group, despite that the Ca content of the L.Ca+WGAC diet was one third of that of the standard diet, and that food intake was significantly lower in the L.Ca+WGAC group compared with the CON and L.Ca groups.

Higher Ca intake increases bone properties [8, 9], and Ca supplementation has been reported to improve bone

mass, architecture, and mechanical strength in Ca deficiency rats [12-14]. A previous study reported that the intake of normal and high Ca diets for 15 days improved whole body BMD, BV/TV, and Tb.N of the femur following 15 days of L.Ca diet intake compared with 30 days of L.Ca diet intake in growing rats [12]. The intake of milk and fish bone as Ca supplementation also improved bone mechanical strength [13, 14]. Thus, Ca supplementation is a factor that improves cortical bone properties under the condition of dietary Ca deficiency. In the present study, the L.Ca diet mixed with WGAC increased cortical bone mass, CBG, and bone mechanical strength. These improvements are considered to depend on Ca supplementation under L.Ca condition. However, the Ca content of the L.Ca+WGAC diet was twice that of the L.Ca diet, and one third of that of the standard diet. Moreover, daily Ca intake calculated for the L.Ca+WGAC group was 1.9 times more than that of the L.Ca group and less than a quarter of that of the CON group. Thus, factors other than Ca intake might have contributed to the improvement of cortical bone properties. One possible factor is BW gain with higher fat intake, which was related to bone mechanical strength, whereas Ca intake was related to all measured bone parameters in the present study. Obesity is associated with bone mass and is protective against osteoporosis [21]. Some studies reported that a high-fat diet (HFD) helped achieve PBM during early growth and increased bone mass, TBA, and CBG in young rats with higher BW [22, 23]. Meanwhile, other studies observed loss of bone mass and mechanical strength due to HFD via altered lipid metabolism [24, 25]. A high-carbohydrate high-fat diet decreased trabecular (BV/TV, Tb.Th, Tb.N, and Conn.D) and cortical (Ct./AV and Ct.Ar) bone parameters and bending strength of the femur, and resulted in significantly higher TC, TG, and LDL-C compared with the CON group [26, 27], with decreased bone formation markers and increased bone resorption markers [27]. In adult male rats, a low-carbohydrate HFD decreased BV/TV and bone formation compared with the control group, but no differences were observed in cortical bone parameters [28]. The HFD reportedly had no effects on cortical bone mass, although it decreased trabecular bone mass in young mice [29]. A high-fructose diet resulted in stronger bones with enhanced microarchitecture than a high-glucose diet did [30]. As mentioned above, HFD had conflicting effects on bone properties. In the present study, daily fat intake was higher, and daily carbohydrate intake lower, in the L.Ca+WGAC group compared with the L.Ca and CON groups. In addition, BW gain was significantly higher in the L.Ca+WGAC group compared with other groups despite that there was no difference in daily energy intake. Although the results regarding bone properties in the present study are similar to those reported by the study on a low-carbohydrate HFD [28], beneficial effects of L.Ca+WGAC on cortical bone may depend on the stage of diet intake (early growth) [22, 23]. In addition, the HFD with Ca supplementation is suggested to improve TBA to a greater extent than Ca supplementation alone in growing rats [31]. The impacts of HFD on bone properties vary depending on the content of protein, carbohydrates, and Ca. In the present study, cortical bone parameters were improved in the L.Ca+WGAC group to the same levels as those in the CON group, even though the L.Ca+WGAC diet was high in fat and low in carbohydrates. BW gain is considered to contribute to CBG and mechanical strength. Lecka-Czernik et al. [23] suggested a two-phase process in acquired bone mass in obesity: the beneficial effect of fat expansion to increase bone mass is observed in the first phase, either by increased mechanical loading or increased production of bone anabolic adipokines or possibly due to the nutritional effect of fatty acids. In addition, a positive correlation between BW and lumber BMD has been observed in high-fat sucrose diet-fed rats [32]. In fact, the present study found that a higher BW gain was associated with higher cortical BMC, CBG, and mechanical bone strength, suggesting that BW gain may contribute to improved cortical bone properties by mechanical loading. The negative effect, observed in the second phase, is caused by decreased bone formation and bone turnover resulting from the development of metabolic impairment. The HFD has been reported to induce high TG, TC, and LDL-C [33], and LDL-C reportedly has a negative causal association with BMD [34]. High serum TC, LDL-C, creatinine, and PTH concentrations in the L.Ca+WGAC group may have affected TBA parameters, but not cortical bone properties, in the present study. In addition, serum BALP levels were significantly lower, but serum osteocalcin levels significantly higher, in the L.Ca+WGAC group compared with the CON group. Serum BALP and osteocalcin levels reflect immature and mature osteoblast function, respectively. It was thus con-

sidered that bone formation in the L.Ca+WGAC group was maintained at the same level as that in the CON group. The effects of HFD on TC, TG, and LDL-C were observed in the early stage, whereas its effects on receptor activator for nuclear factor-kappa B and its ligand appeared later [33]. That there were no differences in serum TRACP-5b and 1.25-DHVD3 levels between the L.Ca+WGAC and CON groups may be related to the findings that CBG and mechanical strength were maintained, with inhibited bone resorption. Normal to high protein intake has been reported to increase tibial BMC, BMD, TBA, CBG, and bone mechanical strength compared with low dietary protein intake with the same level of Ca intake in growing rats [35, 36], and low dietary protein intake reportedly delays skeletal growth and reduces bone mass [37]. Meanwhile, a low Ca/P diet containing 5% casein, but not a normal Ca/P diet containing 5% casein, attenuated decreases in BMD and bone mechanical strength in the tibia resulting from low protein intake [36]. The effects of protein intake on bones likely vary according to Ca and P intake levels. The present study may provide some evidence of some correlation between protein intake and bone properties.

The present study has some limitations. First, since nutrition contents and composition differed among the diets, the contents of nutrients related to bone differed among the diets as well. Second, nutrition contents outside of Ca were not fixed. Third, Ca absorption and retention were not measured, although Ca intake could be calculated from daily food intake. The effects of the L.Ca+WGAC diet on bone properties might have been similar to those of similar diets used in previous studies (e.g., low-carbohydrate HFD); however, their effects would differ in quality as well as quantity depending on nutrition contents. Moreover, the effects of vitamin D on bone properties may also differ, although vitamin D content in chicken is low and thus has limited effects [38]. Therefore, the extent to which the intake of Ca and other nutrients including vitamins influenced bone properties in the L.Ca+WGAC group remains unclear. A future study will be necessary to control for other nutrition contents such as fat, protein, carbohydrates, and vitamins to clarify the effects of Ca supplementation on bone properties. Nevertheless, the results of the present pilot study suggest that L.Ca intake causes bone deterioration, which provides an insight into the effects of Ca supplementation on bone properties under L.Ca conditions.

The present study showed that L.Ca intake causes bone deterioration, and that a L.Ca diet mixed with whole ground adult chicken can improve bone properties, especially cortical bone properties, to the same levels as those achieved with a normal Ca diet in growing rats. Our findings suggest the possible use of whole ground adult chicken, which contains bone fragments, for Ca supplementation to improve bone loss under L.Ca conditions, which may help improve bone health in childhood. Careful consideration will be needed regarding nutrients or factors associated with diets that may affect bone properties, given that factors other than Ca supplementation have potential effects on bone metabolism and properties. Nonetheless, our results may at least open up a new route to utilize adult chicken effectively. Further investigation will be needed to clarify the effects of Ca supplementation on bone properties without the influence of other nutrients.

Conflicts of interest

This study was supported by Marudai Food Co., Ltd. Co-authors Tamao Nishiura, Satoshi Kotoura, and Masato Nakane belong to Marudai Food Co., Ltd.

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低カルシウム飼料摂取成長期ラットにおける成鶏ミンチの骨特性への影響

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要　約

本研究は低カルシウム（L.Ca）飼料摂取成長期ラットにおいて，成鶏ミンチ混合飼料が骨特性（骨量，骨微細構造，機械的骨強度）へ与える影響を調査することを目的とした。4 週齢 SD ラットを 3 群に分け，それぞれに標準飼料（対照群），L.Ca 飼料（L.Ca 群），L.Ca+ 成鶏ミンチ混合飼料（混合飼料群）を 13 週間与えた。L.Ca 群では海綿骨，皮質骨ともに骨特性の劣化を来たしたが，混合飼料群では特に皮質骨の骨特性の劣化が抑制された。本研究の結果は，骨成長期の骨の健康において L.Ca 状態による骨量減少を改善する一助になると考えられる。但し，骨特性に影響をおよぼす栄養素を含めた他の要因についても考慮，検討する必要がる。

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キーワード：Growing rats, Low calcium diet, Whole ground adult chicken, Bone properties

Abbreviations: 1,25-DHVD3, 1,25-dihydroxyvitamin D3; ALP, alkaline phosphatase; AV, all bone volume; BALP, bone specific alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BUN, blood urea nitrogen; BV, bone volume ; BV/TV, bone volume fraction; BW, body weight; Ca, calcium; CBG, cortical bone geometry; CON, control; Conn.D, connectivity density; Ct.Ar, cortical bone sectional area; Ct.Th, cortical bone thickness; Ct.V, cortical bone volume; Ct.Po, cortical porosity; Ct.V/AV, cortical bone volume fraction; DBW, dry bone weight; EDL, extensor digitorum longus; ELISA, enzyme-linked immunosorbent assay; Ec.Pm, endocortical perimeter; HDL-C, high-density lipoprotein cholesterol; IP, inorganic phosphate; L.Ca, low calcium; LDL-C, low-density lipoprotein cholesterol; Mg, magnesium; MV, medullary volume; PBM, peak bone mass; PTH, parathyroid hormone; Ps.Pm, periosteal perimeter; ROI, region of interest; TBA, trabecular bone architecture; TBPf, trabecular bone pattern factor; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TC, total cholesterol; TG, Triacylglycerol; TMD, tissue mineral density; TP, total protein; TRACP-5b, tartrate-resistant acid phosphatase 5b; TV, tissue volume; vBMD, volume BMD; WBW, wet bone weight; WGAC, whole ground adult chicken.