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Taro (*Colocasia esculenta*) flour intake enable bone integrity in male rats

O consumo de farinha de taro (Colocasia esculenta) favorece a integridade óssea em ratos machos

Abstract

Objective: This study aimed to investigate bone composition in male rats treated with diet supplemented with taro (Colocasia esculenta) flour until their 90 days. ***Methods:*** Weanling male rats were divided into control (C, n=11) and experimental group (T, n=12); the latter comprised animals treated with taro flour until their 90 days. Food intake, body mass and length were evaluated on a weekly basis throughout the experimental period. Spine bone dimension, as well as bone mineral density (BMD), mineral content (BMC), total area and biomechanical properties were determined after 90 days. ***Results***: T group recorded higher values for (P<0.05) body mass and length; BMD, BMC and total spinal area; BMD of the fourth lumbar vertebra; femoral mass, distance between epiphysis, medial point of diaphysis width, BMD, maximum strength and osteocalcin concentrations than the control. ***Conclusion:*** Taro flour intake had positive effect on bone health.

Keywords: Colocasia esculenta. Diet. Rats. Femur. Bone development.

Resumo

Objetivo: A proposta deste estudo foi investigar a composição óssea em ratos tratados com dieta suplementada com farinha de taro (Colocasia esculenta) até eles completarem 90 dias de idade. ***Métodos:*** No momento do desmame, os ratos foram divididos em grupo controle (C, n = 11) e experimental (T, n = 12) – composto por animais tratados com farinha de taro até os 90 dias de idade. Ingestão alimentar, massa e comprimento corporal foram avaliados semanalmente ao longo de todo o período experimental. Dimensões ósseas, bem como a densidade mineral óssea (DMO), conteúdo mineral ósseo (CMO), área óssea total e propriedades biomecânicas foram determinadas no final de 90 dias. ***Resultados:*** Grupo T apresentou elevados valores (P<0.05) para massa e comprimento corporal; DMO, CMO e área óssea da coluna vertebral; DMO na quarta vértebra lombar; massa femoral, distância entre as epífises, largura do ponto médio da diáfise, DMO, força máxima e concentrações séricas de osteocalcina, quando comparado ao grupo controle. ***Conclusões:*** A ingestão da farinha de taro apresentou efeito positivo na saúde óssea.

Palavas-chave: Colocasia esculenta. Dieta. Ratos. Fêmur. Desenvolvimento ósseo.

INTRODUCTION

Taro (*Colocasia esculenta*) is a conventional yam consumed in tropical and subtropical countries. It is rich in carbohydrates, vitamins and minerals, as well as in phytochemicals such as saponins, flavonoids and phenolic acids.[1-3]

Scientific literature reports the effect of different yam types on peripheral blood circulation, antigenic activity, cancer cells, glucose, cholesterol and triglycerides concentrations, antioxidant activity, hormone synthesis and hypertension.[3-5]

Saponins play an osteogenic role in bone formation.[6-9] Rego et al.[10] have reported improved bone density in diabetic rats treated with yam (*Dioscorea bulbifera*) flour. They suggested that several nutrients found in cocoyam can contribute to bone formation and development during individuals' early life stages. Despite the potential role played by yam in bone health, the literature lacks studies focused on investigating the performance of *Colocasia esculenta* in this field.

Colocasia esculenta is an important tuber for Brazilian gastronomy and culture. However, there is a gap of knowledge about taro's role in critical stages of life and bone health. This study aimed to understand the effect of taro's rich diet on bone structure of male rats. The experimental protocol investigated a decisive period in bone formation that starts at birth up to 90 days.

METHODS

The study was approved by Ethics Committee on Animal Research of Fluminense Federal University, Niteroi City, Rio de Janeiro State, Brazil (number 669/2015). All procedures were in accordance with the Brazilian Society of Science and Laboratory Animal and the Guide for the Care for the Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication n. 85-23, revised in 1996).

Taro (*Colocasia esculenta*) tubers were purchased in a public local market. Taro's nutritional composition comprised (g/100g): humidity, 9.48; ashes, 3.97; carbohydrates, 79.33; ether extract, 0.43; protein, 6.79. Tubers were washed, skinned, cut (0.5 cm of slices), boiled (60 seconds), frozen and lyophilized for flour preparation. Dried yam was milled, sieved (35 mesh) and stored in plastic bags at -20ºC until the time for use.[10-12]

Experiments were conducted with male Wistar rats, who were kept under controlled room temperature (23±1ºC), and humidity (60±10%) conditions, and subjected to artificial dark-light cycle (lights on from 7am to 7pm).

Twenty-one-day-old male pups were weaned and divided into two groups: control group, which comprised animals treated with control diet (C, n=11); and experimental group (T, n=12), which comprised animals treated with a taro-supplemented diet (25g of taro flour, 27.95g of cornstarch, 20g of casein and 7g of soybean oil/100g). Both diets had the same amounts of sucrose, fiber, mineral mix and vitamin mix, L-cystine and choline bitartrate (table 1).[10-12]

Table 1. Composition of experimental diets. Niterói-RJ, 2020

Ingredients (g/100g)	C	T
Casein	20.00	20.00
Cornstarch	52.95	27.95
Cocoyam Flour	0	25.00
Sucrose	10.00	10.00
Soybean oil	7.00	7.00
Fiber	5.00	5.00

Table 1. Composition of experimental diets. Niterói-RJ, 2020 (Continues)

Ingredients (g/100g)	C	T
AIN-93G Mineral Mix	3.50	3.50
AIN-93G Vitamin Mix	1.00	1.00
L-Cystine	0.30	0.30
Choline Bitartrate	0.25	0.25
Carbohydrate (kcal)	212	177.30
Lipid (kcal)	63	64
Protein (kcal)	80	86.80
Total (kcal)	355	328.10

C, control diet; T, experimental with taro flour; AIN, American Institute of Nutrition. Casein, mineral and vitamin mix, l-cystine and choline bitartrate: Pragsoluções®; cornstarch and fiber: FARMOS®; Soybean oil: Liza®; commercial sucrose: Qualitá®; cocoyam: local market. Formulated on recommendations of AIN-93G for rodent diets. Taro's nutritional composition comprised (g/100g): humidity, 9.48; ashes, 3.97; carbohydrates, 79.33; ether extract, 0.43; protein, 6.79.

Food intake (g) was evaluated on a weekly basis throughout the experimental period. Body mass (g) and length (cm) of 90-day rats subjected to 6-hour fasting, were evaluated. Subsequently, animals were anesthetized with Thiopentax ® (Sodium thiopental, 0.1mg/100g) and subjected to dual-energy X-ray absorptiometry (DXA) analysis in Lunar DXA 200368 GE instrument (Lunar with software encore 2008, version 12.20, GE Healthcare, Madison, Wisconsin).[13,14] Bone information described by DXA comprised total and spine bone mineral density (BMD, g/cm²), bone mineral content (BMC, g) and bone area (cm²).

After DXA analysis was over, animals were euthanized through exsanguinations via cardiac puncture.[10-12] Collected blood was centrifuged and serum was stored at -80°C for further osteocalcin (ng/mL) analysis carried out with multiplex assay kits (Millipore rat bone panel RBN1MAG-31K-01, Billerica, MA, USA).

Right femur and fourth lumbar vertebra (LV4) were collected, cleaned of soft tissue and preserved at -80ºC. Bone pieces were weighted (g) and BMD was measured through DXA.[14,15] The distance between epiphysis and the medial point of diaphysis width (mm, respectively) in the femur were evaluated with the aid of calipers, at readability of 0.01mm.[15]

Biomechanical properties of the right femur were measured with three-point universal (D L 2000, EMIC, São José dos Pinhais, SP, Brazil), at load cell capacity of 200 kgf. Bones were supported on two rollers (3 mm in diameter) at distance of 21.70 mm. Maximum force (N), breaking strength (N) and rigidity (MPa) were loaded by the software.[16]

Statistical analyses were carried out in the Graph Pad Prism Statistical package (Version 5.0, GraphPad Software, San Diego, CA, USA). Results were analyzed through Student's *t* test and expressed as means ± SEM, at significance level of 0.05.

RESULTS

Both groups recorded similar body mass and length at the 21st day of life (table 2). Food intake and energy consumption were similar between groups throughout the experimental period. T group recorded higher ($P< 0.05$) body mass (+20.56%) and length (+2.87%) than C group at 90-day.

Table 2. Food intake, body mass, length, bone analysis and serum osteocalcin at 90 days. Niterói-RJ, 2020

	C (n11)		T (n12)	
	Mean	SEM	Mean	SEM
Food intake (g)	54.80	3.58	63.98	3.65
Energy (kcal)	194,5	12,73	209,8	11,99
Body mass at 21 days (g)	58.43	1.66	61.38	1.29
Body mass at 90 days (g)	383.20	13.67	462.00*	9.78
Body length at 21 days (cm)	19.36	0.30	20.13	0.35
Body length at 90 days (cm)	41.72	0.33	42.92*	0.25
Total BMD (g/cm²)	0.145	0.002	0.156*	0.001
Total BMC (g)	9.10	0.28	11.13*	0.28
Total bone area (cm)	63.18	1.39	71.17*	1.58
Spine BMD (g/cm²)	0.138	0.003	0.155*	0.002
Spine BMC (g)	2.01	0.09	2.30	0.16
Spine bone area (cm)	14.40	0.70	15.50	1.11
LV4 Mass (g)	0.36	0.01	0.38	0.01
LV4 BMD (g/cm²)	0.137	0.005	0.148*	0.002
Femur mass (g)	0.97	0.03	1.11*	0.02
Distance between epiphysis (mm)	37.46	0.39	38.55*	0.15
Width of the diaphysis (mm)	5.53	0.06	5.88*	0.08
Femur BMD (g/cm²)	0.158	0.002	0.171*	0.001
Maximum force (N)	143.50	5.74	158.80*	4.43
Breaking strength (N)	143.10	5.74	152.00	3.89
Rigidity (MPa)	737100	28670	746000	45590
Osteocalcin (pg/mL)	166.20	15.90	279.80*	26.16

C, group fed with control diet. T, group fed with experimental diet, containing taro flour; both from 21 until 90 days; SEM, standard error of the mean; BMD, bone mineral density; BMC, bone mineral content. *Significantly different (Student *t* test, P<0.05).

DXA analysis has shown higher (P< 0.05) total BMD (+7.58%), BMC (+22.30%) and bone area (+12.64%) in the T group, which recorded higher (P<0.05) spine BMD (+12.31%) than the control group, as well as similar BMC and spinal bone area.

T and C groups presented similar LV4 mass; however, LV4 BMD (+8.02%) was higher (P< 0.05) in T than in C. Based on femur analysis, T group recorded higher (P<0.05) femoral mass (+14.43%), distance between epiphysis (+2.90%), diaphysis width (+6.32%), BMD (+8.22%) and maximum force (+10.66%) than the control group. Both groups recorded similar bone breaking strength and rigidity values.

Serum osteocalcin was higher (P<0.05) in T group (+68.35%) than in C group.

DISCUSSION

Taro, also known as cocoyam, poi and dasheen, is a popular food in tropical and subtropical countries – its beneficial effects have already been reported in Eastern cultures. Therefore, this tuber, which belongs to family *Araceae,* is a functional food[4,5] that can also be consumed in its flour form. In addition, it has positive effect on patients with diabetes, menopause, cancer, intestinal disorders, arterial hypertension, dyslipidemia, among others.[3,7,10-12,17]

Food presenting functional properties in Brazil are defined based on "the metabolic or physiological role played by them in human body's growth, development, maintenance and/or normal functions".[18] There herein adopted taro (*Colocasia esculenta*) flour-supplemented diet did not affect animals' food intake, but it contributed to body mass and length increase. This outcome suggests that *Colocasia esculenta* enables body development in early life stages; therefore, it can be seen as a food with functional properties.

T group recorded higher total bone, femoral and LV4 parameters than C group in the current study. Given the scarcity of studies about the association between *Colocasia esculenta* and bone structure, this interaction remains poorly understood. However, studies have shown that antioxidants are capable of improving bone health and, consequently, help preventing osteoporosis.[19,20] Flavonoids and saponins found in *Colocasia esculenta* have antioxidant effect on bone tissues. Flavonoid intake has positive effect on the mineral density of children's spinal, hip and femoral bones.[19] Saponins found in *Dioscorea villosa* have anti-osteoporotic effects, since they reduce osteoclast differentiation and negatively affects bone reabsorption.[6] Based on these reports, and on the present study, *Colocasia esculenta* has nutrients, mainly phytochemicals, capable of influencing bone formation, which is essential to children and adolescents' growth.

T group has shown maximum strength in comparison to C group in the biomechanical test. Other parameters did not present significant difference between groups; however, T group recorded higher maximum strength (+10%) and breaking strength (+6%) than C group, at 90 days of life. Bone strength preservation is influenced by adequate calcium and vitamin D intake. However, other nutrients such as potassium, magnesium, as well as vitamins B, K and C, play a key role in bone health.[18,19] *Colocasia esculenta* is rich in nutrients capable of influencing bone formation,[19-22] such as calcium, which plays a key role in peak bone mass and strength acquisition processes, as well as magnesium, essential in bone growth and health.[19,20] Accordingly, it can be suggested that minerals and vitamins found in *Colocasia esculenta* can help improving bone quality. However, further studies should be conducted to investigate the effects of *Colocasia esculenta* on bone metabolic pathways.

Osteocalcin acts in gene expression to enable osteoblast formation; consequently, this hormone supports bone formation. Rats affected by bone diseases, which were treated with phytochemicals found in taro and yam, recorded increased osteocalcin (bone formation marker) concentrations.[23,24] Those results were similar to the ones recorded in the current study, according to which saponins were associated with other elements found in cocoyam or acted in other metabolic pathways and helped improve bone integrity.

Previous study conducted by Ribeiro et al.[12] has reported that taro flour (*Colocacia esculenta*) increased rats testosterone levels, as consequence, this hormone enable muscle and bone development in this experimental model. These actions may be explained by the presence of polyphenols, flavonoids, alkaloids and saponins in taro. Thus, further studies focused on investigating the effects of *Colocasia esculenta* on bone metabolic pathways should be conducted.

CONCLUSION

Taro (*Colocasia esculenta*) flour has shown functional properties capable of enabling body development and bone formation in rat model. Therefore, it is worth conducting further studies focused on investigating other effects this tuber.

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Contributors

Pessoa RL, Abreu MDC, Gracio GR and Boueri BFC participated in animal care, analyzes relevant to body and bone parameters, in addition to serological analyzes. Silva ME contributed with the biomechanics technique, interpretation of results and graph. Boaventura GT and Costa CAS contributed to the study design, interpretation of results and supervision. All authors helped in the preparation of the manuscript and agree with the final version provided.

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