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FUNCTIONAL PROPERTIES OF NATURAL CALCIUM FROM SEAWEEDS

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Abstract

As dietary intakes levels of Calcium decrease below those recommended by health authorities the incidence of Osteoporosis is set to continue to rise. To address this issue it is becoming necessary to fortify regularly consumed foods such as cereals, biscuits etc. with ingredients such as AquaCal.

AquaCal is a natural, organic source of calcium that is produced from calcified seaweed, *Phymatolithon* and *Lithothamnion* and can be utilised for Calcium and magnesium supplementation in a broad range of foods and beverages. AquaCal presents a great potential over other calcium sources because of its porous structure and it composition associating key minerals as calcium, magnesium and boron in addition to being neutral in taste in applications. Once consumed the efficacy of AquaCal must then be verified therefore a bioavailability a comparative feeding study to assess effects of AquaCal on bone density was carried out. There was no significant difference in bioavailability based on calcium absorption between AquaCal and Limestone however the weights of the femurs of the animals fed AquaCal did significantly increase over the group fed Limestone. As a natural and organic mineral AquaCal has many different fields of application.

Key Words: Calcium, Minerals, Bone Density, Osteoporosis, Fortified Foods

INTRODUCTION

Osteoporosis is a debilitating disease characterised by decreased bone mass and increased vulnerability to fractures which has been identified as a global public health problem with serious health and economic consequences. 1 in 8 European citizens greater than 50 years has a fracture of the spine. This figure is further aggravated by hip fractures that will affect 1 in 3 women and 1 in 9 men over the age of 80 (European Commission Report, 1998). Hip fractures alone are estimated to cost in the region of Euro 3,500 million annually. In the U.S.A. this is estimated to be approx. U.S.$13.8 billion (1999). Intake of Calcium and other minerals are essential for healthy bones however global dietary calcium intakes are not satisfactory and fall well below recommended levels.

As dietary change by individuals is very difficult to achieve the best way forward for increasing Calcium intakes includes the fortification of routinuely consumed foods and beverages with an ideal source being the natural seaweed Calcium and mineral source, AquaCal.

AquaCal is natural, organic calcium source which is produced from calcified seaweed, *Phymatolithon* and *Lithothamnion* which is harvested from the seabed off the Southwest coast of Ireland in clean Atlantic waters. Once harvested AquaCal is washed, dried and finely dry milled to highly controlled parameters which produces a high quality food ingredient typically comprising 32% Calcium, 3.2% Magnesium and additional trace elements essential for bone health.

Addition of minerals to food requires that the Calcium source used does not effect the food appearance, texture, shelf life and mouthfeel. The following examples illustrate some of the key points that make AquaCal a unique calcium source and how it can be applied contributing unique properties including taste & texture profiles and functional properties to food applications.

Structure:

AquaCal has a porous structure resulting in a high specific surface: 9.4 m^2/gm vs. 1.7m^2/gm for calcium carbonate. The micro-roughness associated with AquaCal compared with calcium carbonate can be illustrated by viewing attached Scanning Electron Micrographs at the same magnification.

Mouthfeel:

Once hydrated by saliva, the AquaCal structure collapses to yield a smooth texture with a neutral taste (non chalky or sandy).

Buffering capacity:

The buffeirng capacity measures the ability of a salt to control pH variation; for instance the ability to counteract acidification. This could be the case with starchy and sweet products

Table 1 AquaCal Mineral Profile

Calcium	34%	Cobalt	6ppm
Magnesium	3.2%	Copper	10ppm
Phosphorous	0.08%	Zinc	37ppm
Sulphur	0.45%	Selenium	1ppm
Iron	0.05%	Molybdenum	39ppm
Boron	16.5ppm	Iodine	20ppm
Fluorine	200ppm	Manganese	125ppm
Sodium	310ppm	Nickel	30ppm

Nutritional Functionality

AquaCal is not just a calcium source. It contains 34% calcium, 3.2% magnesium and other minerals and trace elements essential for bone health.

Magnesium is involved in Calcium metabolism and in the conversion of vitamin D into its active form as well as maintaining bone integrity. Magnesium plays an important role in Calcium transportation. Boron plays a role in converting Vitamin D into it's active form and thus participating in the Calcium absorption process with Copper and Zinc playing a role in bone metabolism, cell growth and division.

The AquaCal product range is formulated to allow incorporated into different food systems e.g. oil vs. aqueous systems examples include Dairy & Soya systems, dairy spreads, cereals, confectionery & dietary supplements.

Inclusion of the fortifying ingredient into the food systems is only part of the equation as subsequently the "active" ingredients must be available for absorption and its efficacy in terms of benefits provided in this case, bone density increases must be verified. Therefore the main purpose of the following trial is to check the bioavailability of AquaCal and the minerals therein versus other calcium sources such as Limestone. AquaCal has a far greater surface area than Limestone and also its availability in acid has been shown to be approximately twice than that of limestone. Both of these factors would indicate that the bioavailability of AquaCal should be greater than lime. Thus the objectives of this study were to carry out a competitive bioavailability study in two groups of rats, in a single-dose parallel study, of Calcium from two sources (AquaCal & ground Limestone). In addition, two groups of rats were monitored in a continuous feeding study whereby one group is supplemented with 50mg/kg/day Calcium from AquaCal and the other one supplemented with the same dose of Calcium from ground limestone.

MATERIALS AND METHODS

The study was conducted by the "Independent Pharmaceutical Research Unit, Department of Biochemistry, Trinity College, Dublin, Republic of Ireland with Marigot supplying the test material.

It was necessary to evaluate the levels of Calcium in both calcified seaweed product and limestone in order to ensure that each group of rats were fed the same levels of Calcium. Both AquaCal and limestone were radiolabelled with Ca^{47} prior to feeding. Atomic Absorption Spectroscopy was employed as a method of analysis.

Comparative Bioavailability of Calcium in Aquamin versus Ground Limestone

Sixteen 7 week old male rats, Wistar strain, were randomised into two groups (n = 8), and fed ad libitum a purified diet (AIN-76) (Table 2) and given distilled water ad libitum for 2 weeks. Rats were maintained at a temperature of 21 +/- 2°C with a 12 hr dark – light cycle with faeces and urine collected separately. On the fifteenth day, after fasting for 10 hours, the animals were fed overnight a meal of AIN-76 diet, containing 5g of Calcium per kg (as either Seaweed or Limestone as the sole Calcium source in replacement for calcium carbonate), which was extrinsically labelled with ^{47}Ca as $^{47}CaCl_2$. On verification of meal consumption the rats were replaced on the AIN-76 diet. ^{47}Ca was determined in quantitative daily faecal collections over the subsequent 7 days. Urine collections were made for each group for 3 days after feeding the labelled meal and cumulative urinary loss over the first three days expressed as a percentage of the dose. Animals were killed after 7 days and ^{47}Ca in the femur was determined.

Table 2. Composition of the modified AIN-76 diet

Ingredients	Content (g/kg)
Casein	200.0
DL Methionine	3.0
Cornstarch	150.0
Sucrose	487.5
Fibre	50.0
Corn Oil	50.0
AIN Mineral Mix	35.0
AIN Vitamin Mix	10.0
Calcium Carbonate	12.5
Choline Bitartrate	2.0
	1000.0

Ca absorption was determined by the single tracer faecal ^{47}Ca recovery method as follows: -

In the faecal ^{47}Ca recovery method quantitative collections of faeces were carried out daily for 7 days after administration of the isotopically labelled meal. ^{47}Ca in the administered doses of food and in the faecal collections was determined in a well gamma counter. Faecal ^{47}Ca loss was expressed as a percentage of the administered dose for each day (Days 1 – 7) for each rat. ^{47}Ca absorption (%) was calculated as the difference between the total dose (100%) and the cumulative faecal ^{47}Ca loss (%) for each day, for each rat.

Continuous Feeding Comparative Study for Six weeks

Two groups of three rats were fed ad lib on Standard Laboratory Chow (Table) for a period of six weeks. Each group was given a daily gavage dose of either Seaweed or Limestone based on a human dose defind by the sponsor as 3g/day. The daily dose of Seaweed in a 200g rat is 10mg or 0.25

mg/g/week. In order to standardise the daily doses of calcium, 10mg of seaweed contains 3.18 mg calcium. The equivalent amount of calcium in limestone is 8.3mg Limestone. Thus the daily gavage of calcium was kept constant for the six week period by increasing the concentration of the test material in the gavage according to the increased weight of the rats.

Table 3. Standard Laboratory Chow

Ingredient	Content (%)
Crude Protein	17.0
Crude Oil	3.5
Crude Fibre	7.0
Crude Ash	8.0
Moisture	14.0
Digestible energy	11.8 (Mj/kg)
Calcium	1.3
Phosphorous	0.8
Salt	0.7
Vitamin A	9,000 (i.u./kg)
Vitamin D_3	2,000 (i.u./kg)
Lycine	0.85
Methionine	0.32
Threomine	0.64

After 7 days the animals were sacrificed and the Ca^{47} content of the femurs was determined. The femurs were removed, cleaned of muscle tissue, weighed and digested in concentrated citric acid. Following digestion, the Calcium and Magnesium levels were measured by Atomic Absorption Spectroscopy.

Statistical analysis involved the comparison of group means by the Student's T-test.

RESULTS AND DISCUSSION

Calcium levels in the test products were determined to be 47.9% & 39.8% for Limestone and Aquamin respectively.

Comparative Bioavailability of Calcium in Aquamin versus Ground Limestone

Fractional absorption of ^{47}Ca was estimated by extrapolating the linear portion (Days 3 – 7) of the plot of log [100-cumulative faecal ^{47}Ca (% administered ^{47}Ca)] vs. time back to the time of isotope administration. There was no significant difference in bioavailability based on calcium absorption

Table 4. Calcium absorption from AquaCal and Limestone containing meals in 9 week old male rats.

Method		Ca Absorption (%) Faecal ^{47}Ca Recovery
	N	(Mean +/- SEM)
AquaCal	8	49.5 +/- 4.2
Limestone	8	53.9 +/- 2.5

between the two products. In addition cumulative urinary calcium loss from the dose was similar in both groups.

Fractional Ca absorption in 9 week old rats was similar from AquaCal and Limestone containing meals.

Continuous Feeding Comparative Study for Six weeks

Whereas the percentage of Calcium administered was slightly lower in the femurs from the AquaCal group compared to the Limestone group, the percentage of calcium absorbed in the femurs from the AquaCal group was slightly higher (Table 5). Neither group reached statistical significant.

Appendix 1 demonstrated an increased weight gain in the AquaCal –fed group compared to the Limestone fed group.

In addition, the weights of the femurs were significantly greater in the AquaCal group. The calcium content of the femurs in the AquaCal group was significantly lower, whereas the magnesium and silica content was similar.

It should be noted that the calcium content was kept constant for both studies. This was done in order to compare the effect of feeding both sources of Calcium in each study. In view of the fact that there was a greater increase in weight gain in the AquaCal-fed group and the femurs showed a statistically significant increase in weight, the apparent loss of calcium could be attributed to some other nutrients being absorbed or perhaps increased metabolism with the bones. Elemental analysis of other nutrients demonstrated changes between the two groups but these are only a few of the possible

Table 5. ^{47}Ca retention in the femurs of 9 week old rats post administration of ^{47}Ca-labelled AquaCal or limestone containing meals.

	% ^{47}Ca administered/femur	% ^{47}Ca absorbed/femur
	(Mean +/- SEM)	
AquaCal	1.93 +/- 0.11	3.90 +/- 0.23
Limestone	2.03 +/- 0.09	3.77 +/- 0.17

Table 6 Analysis of femurs after six week study

	Mean weight of Rats (g)	Mean Weight of Femurs (g)	Calcium Content (mg/g bone)	Magnesium Content (mg/g bone)	Silicon ontent (mg/g bone)
			(Mean +/- SEM)		
AquaCal	412.6	1.43 +/- 0.037	78.15 +/- 5.34	2.72 +/- 0.19	0.015 +/- 0.003
Limestone	396.0	1.27 +/- 0.016	91.56 +/- 3.16	2.72 +/- 0.07	0.011 +/- 0.003
Significance		p<0.005	p<0.05	N.S.	N.S.

Table 7 Elemental Analysis of Composites of rat femur extracts

Element	AquaCal (mg/g bone)	Limestone (mg/g bone)
P	52.74	56.14
K	1.60	1.57
Na	3.64	3.70
Cu	0.007	< 0.001
Fe	0.075	0.05
Mn	<0.01	<0.01
Zn	0.154	0.143
B	0.006	0.003
F	15.94	17.90
Co	< 0.001	< 0.001
Se	<0.07	<0.07

nutrients, which might be affected.

The trace minerals, such as Zinc, in the product can possibly explain the impact of AquaCal on the growth of the rats. Zinc plays a critical role in growth, when cells are rapidly dividing, growing or synthesising proteins, collagen etc. Furthermore additional minerals, such as Boron, could also have contributed to proper bone growth. Boron is necessary for the conversion of Vitamin D into its active form, this is one of the reasons why Boron deficiency has been shown to affect calcium metabolism and bone formation. The aim in the future is to extend these studies into clinical trials on the bioavailability of AquaCal.

In conclusion AquaCal presents a great potential over other calcium sources because of its structure and its composition of additional minerals to Calcium including Magnesium and boron. As a natural and organic mineral, AquaCal has many different fields of application.

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Appendix 1 Weight Gain of Rats During Study Period