

## CALCIUM BIOAVAILABILITY OF CALCIUM CARBONATE BASED DIETS FOR MALE GROWING RATS

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### ABSTRACT

Calcium (Ca) bioavailability of different levels of calcium carbonate using male Sprague-Dawley derived weanling rats was determined. Normalized value (NV), the relative bioavailability (RBV) and calcium absorption in three levels (0.14, 0.19 and 0.24 %) of three balanced diets (diets No 1, 2 and 3) of calcium carbonate ( $\text{CaCO}_3$ ) as a source of calcium were estimated. Normalized values and RBVs in femur, tibia and fibula for rats fed diet containing 0.19% calcium (No.2) were significantly ( $P < 0.05$ ) higher (81.8 and 100 %, respectively ) than those (78; 95 and 78; 95 ) for rats fed diet containing 0.14% (No.1) (78; 95 and 78; 95, respectively) and diet containing 0.24% calcium (No.3)(60.8; 74.2 and 60.2;73.6, respectively). Results also show that calcium absorption, total body calcium and serum calcium for rats fed diet No.2 were significantly ( $P < 0.05$ ) higher than those of rats fed diets No.1 and 3. It was concluded that calcium carbonate based diet containing 0.19% calcium with 1.5:1 calcium to phosphorus ratio may give high calcium bioavailability for growing rats which was considered as standard (control) diet for other diets.

### INTRODUCTION

Calcium is considered as the major nutrient among macroelements and the most abundant cation in the human body accounting between 1.5-2.2 % (Guthrie and Picciano, 1995). Calcium is very important as it plays a vital role in the human body from childhood till elderly, and it is involved in many vital functions in the body. It is an essential dietary element to maintain the integrity of skeleton and essential nutrient required in substantial amounts, but many diets are deficient in calcium making supplementation necessary or desirable (Warren Tak-Keung Lee, 1993 and Hanzlik *et al.*, 2005). There is awareness of the importance maintaining a high calcium intake throughout the life, not just for bone health, but also for the health of other systems. However, chronic low calcium intake is associated with some health disorders and the normal ratio between calcium and phosphorus is almost constant, a little more than 2:1 (Schuette and Linkswiler, 1984 and Poneros and Erdman, 1988). Bioavailability of nutrient is the proportion of dietary nutrient which is absorbed and utilized by certain organism (O'Dell, 1984). Specifically, bioavailability of calcium from foods is an important concern because calcium intakes in our diet are quite low, and most of the calcium in food is chemically bonded to other dietary constituents, and must be released in a soluble form (Allen, 1982 and 1984). This is dependent on the composition of diets in terms of levels of dietary calcium, vitamin D, phosphorus, magnesium, protein, fiber, phytate, fat, lactose and other sugars (Greger, 1988). Calcium salts

such as calcium carbonate, calcium sulfate, calcium citrate and Part of PhD Thesis of the 1<sup>st</sup> author.

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Calcium lactate are usually added to food as calcium supplements (Ranhotra *et al.*, 1997; Martini and Wood, 2002 and Hanzlik *et al.*, 2005). Calcium carbonate is higher in calcium than most other potential food grade calcium sources (Ranhotra *et al.*, 1980). Rats appear to be a good model that might be developed for predicting calcium bioavailability for human being (Mahoney and Hendricks, 1985; Weaver *et al.*, 1987 and Heaney *et al.*, 1988). The aim of this study was to evaluate the bioavailability of calcium from basal diets fortified with different levels of calcium carbonate for rats.

### MATERIALS AND METHODS

**Materials:** Ingredients were added to prepare the balanced diets as follows; Casein: Vitamin free casein, supplied by Riedel-Dehaen Co. Germany; Pure cellulose: Produced by Merchery Nagel and Co. Germany; Sucrose: Pure sucrose from BDH chemical ltd. England; Calcium carbonate (CaCO<sub>3</sub>): Supplied by Riedel-Dehaen Co. Germany; Starch; Corn starch, purchased from local market produced in Holland; Oil: Corn oil, purchased from local market produced in Turkey.

**Balanced diets preparation:** Three basal diets of calcium carbonate were prepared according to the NAS/NRC (1978) (Table 1). The diets were prepared to contain three levels of calcium 0.15, 0.20, and 0.25%. Each diet contained 12% protein, 10% fat, 10% sucrose, 5% cellulose, 2% vitamins and 1.2% minerals mixtures. All dry ingredients with corn oil were weighed and mixed, and then the weight was completed with starch to make 100 g. They were mixed again till they became homogenous and kneaded electrically in a dough mixer with addition of deionized distilled water. They were formulated; finger shaped of about 5 g. each piece, spread in large stainless steel trays and dried in a cabinet drier at 50C for 12 hours, then packed in plastic bags and kept frozen for determinations (Table 2).

Table (1): Diets formulation (g/100g).

Diet No.	Ca level %	CaCO <sub>3</sub>	Ingredie						
			Casein	Corn oil	Sucrose	Cellulose	Minerals mix <sup>1</sup>	Vitamins mix <sup>2</sup>	Starch
1	0.15	0.375	12.77	10	10	5	1.2	2	58.65
2	0.20	0.500	12.77	10	10	5	1.2	2	58.53
3	0.25	0.625	12.77	10	10	5	1.2	2	58.40

<sup>1</sup>The *vitamins mixture* was prepared (g/kg) as follows: alpha-tocopherol 50 g, inositol 5.0 g, menadione 1.25 g, vitamin A concentrate 4.5 g, retinyl acetate 200,000 I.U., vitamin D concentrate 0.25 g calciferol 400.000 I.U., niacin 4.5 g, riboflavin 1.0 g, pyridoxine-HCl 1.0 g, thiamine-HCl 1.0 g, ascorbic acid 45.0g, pantothenate 30.0 g, biotin 0.02 g, folic acid 0.09 g, and vitamin B<sub>12</sub> 0.00135g. The weight was completed to 1 kg by adding starch.

<sup>2</sup> The *minerals mixture* was prepared (g/kg) as follows:

KCl 296.7 g, MnSO<sub>4</sub>.H<sub>2</sub>O 12.7 g, CoCO<sub>3</sub>.6H<sub>2</sub>O 0.7 g, MgCO<sub>3</sub> 121.0 g, CuSO<sub>4</sub> 1.6 g, KI 0.8g, Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O 0.1 g, ZnSO<sub>4</sub>.7H<sub>2</sub>O 28.0 g, FeSO<sub>4</sub>.7H<sub>2</sub>O 30.0 g. The weight was completed to 1 kg by adding starch.

Table (2): Diet composition (g/100g)

Ca level %	Ingredients								Ca/P ratio	Kcal	pH
	Moisture	Protein	Fat	Fiber	Ash	CHO*	Ca	P			
0.15	12.1	11.88	9.90	4.85	1.82	59.45	0.14	0.14	1:1	376	5.7
0.20	11.1	11.82	9.80	5.10	1.84	60.34	0.19	0.13	1.5:1	376	5.9
0.25	12.2	11.86	10.0	5.00	1.80	59.14	0.24	0.12	2:1	376	5.7

\*by difference

Values are means of three determinations.

**Experimental animals:** Weanling male Sprague-Dawley rats derived rats (ministry of health, Baghdad) were housed individually in suspended mesh bottom and front stainless steel hanging cages of 25 X22 X 20 cm ( fecal collection trays underneath ) in a controlled condition, between 20 - 25°C, 12 hours light and dark cycle . After two days adaptation period, these animals were weighed and divided into 4 groups of five animals each with no statistical differences. They were fed calcium carbonate diets for 28 days. One group of five rats was sacrificed on day zero to obtain baseline animals tissues and endogenous organs for calcium determination. Animals were fed daily, their food intake was increased gradually, deionized distilled water was offered *ad libitum*. Animals were weighed weekly, feces were collected daily for each individual rat for the entire four weeks period, air dried, finely ground and stored for calcium and phosphorus determinations. At the end of feeding period (28 days) the animals were anesthetized (using ether); 2 ml of blood were withdrawn by cardiac puncture. The clotted blood was centrifuged 8 minutes to obtain serum for calcium analysis. Rats were killed and the left tibia & fibula and left femur were removed, weighed and cleaned from adhesive tissues then soaked in hexane then ether to remove fat (Poneros and Erdman, 1989) dried and saved for calcium and phosphorus determinations. Also, liver, heart, kidneys and spleen were removed, weighed, packed in plastic bags then frozen for calcium and phosphorus determination. The carcasses were placed in a heat-resistant glass jars with addition of 25 ml glacial acetic acid and 125 ml deionized distilled water. The glass jars were autoclaved for 120 minutes at pressure 1 atmosphere and 121 C. The carcasses were blended and sampled for calcium and phosphorus (total body) determination.

**Chemical analysis:** Moisture and ash were determined according to the methods of AOAC (1980). Protein (Macrokjeldahl method), fat (Soxhlet method) and fiber were determined according to the methods mentioned by Pearson (1976). pH was determined according to the methods mentioned by Pearson (1976) and Ling (1963) using pH meter ( Philips , England ).

Calcium determination: Calcium was determined according to the method mentioned by Pearson (1976) using flame photometer with the following modifications:

(A): Standard calcium stock solution: Transfer 2.497 g of CaCO<sub>3</sub> to 1 liter volumetric flask using 100 ml of deionized distilled water and slowly adds 60 ml of M hydrochloric acid. When the CaCO<sub>3</sub> has dissolved it was diluted to the mark with water (1 ml = 1 mg Ca)

(B): Standard working solutions: Many dilutions were prepared from solution (A) to contain 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppm of calcium. Calcium concentrations were conducted using flame photometer apparatus, type Corning 400, England. The mean of three readings was plotted for each dilution against calcium concentration.

(C): Calcium determination in the samples: A suitable quantity of sample was ashed in muffle furnace (AOAC, 1980), moisten by deionized distilled water in a porcelain crucible and carefully 10 ml of 6M hydrochloric acid were added. Samples were evaporated to dryness on a water bath and continue the heating for a further half hour. The samples were cooled and 20 ml of water and 10 ml of the hydrochloric acid were added then boiled and filtered using 42 whatman filter paper into 100 ml volumetric flask and the volume was made up to 100 ml. Solutions were kept in Stoppard polyethylene 50 ml bottles till determination.

Phosphorus determination: Phosphorus was determined colorimetrically by Vanado-Molybdate colorimetric method after ashing samples by muffle furnace at 550C (Pearson, 1976 and AOAC, 1980) using spectrophotometer type CECIL (England) at wave length 470 nm.

**Biochemical determinations:** Serum calcium: Animals were anesthetized by ether in a jar. Two ml of blood were withdrawn by cardiac puncture using a medical syringe. The clotted blood was centrifuged at medium speed for 8 minutes in Karl Kolb centrifuge. Serum was withdrawn using a minisyringe then diluted 1: 100 with 1% LaCl<sub>3</sub>.7H<sub>2</sub>O. Standard curve of CaCO<sub>3</sub> was plotted using 1% LaCl<sub>3</sub>.7H<sub>2</sub>O by the same modified procedure as in standard curve of calcium determination. Readings were taken by flame photometer.

**Hemoglobin determination (HB):** Hemoglobin concentration was measured (g / dl) according to Sahli method which was described in Jain (1986) by withdrawal of 20 microliter of blood in a capillary tube.

**Calculations:** Apparent calcium absorption was calculated as follows:

$$\text{Apparent calcium absorption \%} = \frac{\text{Consumed Ca (g)} - \text{fecal calcium (g)}}{\text{Consumed Ca (g)}} \times 100$$

$$\text{Relative apparent calcium absorption \%} = \frac{\text{Apparent calcium absorption (diet)}}{\text{Apparent calcium absorption (control)}} \times 100$$

Normalized (NV) and Relative bioavailability values (RBV) were calculated as

follows:

$$NV = \frac{\text{Total calcium in bone (mg)}}{\text{Calcium consumed (g) NV (diet)}}$$

$$RBV \% = \frac{\text{NV (control diet)}}{\text{NV (control diet)}} \times 100$$

Dry matter absorption (DMA) was calculated as follows:

$$DMA \% = \frac{\text{Diet consumed (g) - feces (g)}}{\text{Diet consumed (g)}} \times 100$$

On dry weight basis.

Protein efficiency ratio (PER) was calculated as follows:

$$PER = \frac{\text{Body weight gain, g}}{\text{Consumed protein, g}}$$

Energy consumed (Kcal) was determined according to diet digestion factors in the body according to Atwater factors (Al-Zuhayri, 2000)

**Statistical analysis :** The data were analyzed statistically by complete randomized design using Duncan's multiple range test for means comparison on probability of  $P < 0.05$  ( Steel and Torrie , 1980 ) and using SAS Program (1989).

## RESULTS AND DISCUSSION

The nutritional status of rats fed calcium carbonate based diets is shown in Table (3). The results show that there were significant differences ( $P < 0.05$ ) in the body weight gain (86.2 g) of the rats in respect to calcium carbonate diet containing 0.19 % calcium (No. 2). The average increase in the weight of rats fed diet containing 0.14 % calcium (No.1) was 79.8 g, while for rats fed diet containing 0.24 % calcium (No.3); it was 80.0 g. Ranhotra *et al.* (1980) mentioned that growth of rats can be an indicator of absorbing and retention of calcium. Protein efficiency ratio (PER) can be an indicator of growth which combined with body weight gain and the protein intake. PER in rats fed diet No.2 was very close to that in rats fed carbonate diet containing 0.24 % calcium at 2.19 and 2.86, respectively. This result was higher than that mentioned by Al-Qubati (2003) which was 2.16 but it was closer to our finding in the first group which fed carbonate diet containing 0.14 %, it was 2.27. This result may be due to low calcium content in the diet for the first group (No.1) compared to other two groups fed calcium carbonate (diet No.2 and 3). Previously, Boelter and Greenberg (1941) mentioned that calcium content in rat's diets as low as 0.01 % caused growth retardation due to the lack of diet consumption. Insignificant statistical differences ( $P < 0.05$ ) were found (Table 3) among the weights of endogenous organs (liver, heart, kidneys and spleen) of rats fed diets fortified with calcium carbonate as source of calcium. The average weights of livers for rats fed diets No. 1, 2 and 3 were 4.42, 3.97 and

3.84g respectively; which were lower than those values found in our laboratory under similar conditions (Al- Rawashdeh, 1997). Heart weight for rats fed the three diets were all similar (0.45 g) but higher than that value (0.39 g) mentioned by Al- Rawashdeh ( 1997 ) while kidneys weight in our study were lower than that value (1.03 g) found by Al-Rawashdeh (1997). On the other hand, spleen weight in all three groups of rats were higher (0.35 g) than that found by Al-Rawashdeh (1997). Almost, similar results for rats were found by Sharaf (1998) under the same conditions. Hemoglobin values in rats fed calcium carbonate diets were close to each other and within normal value; they were 13.60, 13.02 and 13.07 g / dl

Table (3): Nutritional status of growing rats fed calcium carbonate based diets.

Diet No.	Ca Level %	Diet Consumed g	Energy Consumed Kcal	Body Weight g		PER*	Hb g/dl	Endogenous Organs g			
				Initial Weigh	Weight Gain			Liver	Heart	Kidneys	Spleen
1	0.14	297	1116	43.0 a	79.8±2.77 b	2.27±0.09 b	13.60±0.15 a	4.42±0.65 a	0.69±0.05 a	0.69±0.05 a	0.45±0.05 a
2	0.19	250	941	43.2 a	86.2±3.56 a	2.90±0.12 a	13.02±0.10 b	3.97±0.60 a	0.45±0.06 a	0.68±0.07 a	0.43±0.08 a
3	0.24	236	888	41.6 a	80.0±3.67 b	2.86±0.18 a	13.07±0.35 b	3.84±0.52 a	0.45±0.05 a	0.63±0.03 a	0.37±0.02 a

\* PER = Protein Efficiency Ratio. Hb = Hemoglobin. Values are means of five growing rats.

Means sharing the same superscript letter within a column are not significantly different (P<0.05).

Table (4): Calcium bioavailability of calcium carbonate based diets for growing rats.

Diet No.	Ca %	Body Wt Gain g	Ca Consumed g	Tibia & Fibula bones				Femur bone				Tibia, Fibula & Femur		
				Weight g	Ca mg	NV <sup>1</sup>	RBV <sup>2</sup> %	Weight g	Ca mg	NV	RBV %	Weight g	Ca mg	RBV %
1	0.14	79.8±2.77 b	0.41±0.01 c	0.22±0.01 a	32.5±1.6 b	78.0±4.3 a	95.0 b	0.22±0.01 a	32.5±2.0 b	78.0±4.8 a	95.0 a	0.44±0.02 a	65.0±3.2 b	95.0 a
2	0.19	86.2±3.56 a	0.47±0.01 b	0.23±0.01 a	38.9±2.1 a	81.8±3.7 a	100 a	0.24±0.02 a	38.4±3.2 a	81.6±4.1 a	100 a	0.47±0.04 a	77.8±4.9 a	100 a
3	0.24	80.0±3.67 b	0.56±0.01 a	0.22±0.01 a	34.4±1.4 b	60.8±2.5 b	74.2 c	0.22±0.01 a	34.2±1.2 b	60.2±1.9 b	73.6 b	0.44±0.01 a	68.6±2.5 b	73.8 b
BL*	--	--	--	0.08±0.01 b	9.4±0.74 c	--	--	0.08±0.01 b	9.8±0.2 c	--	--	0.16±0.01 b	19.2±0.8 c	--

<sup>1</sup> NV = Normalized Value <sup>2</sup> RBV = Relative Bioavailability \* BL = Baseline animals.

Values are means of five animals.

Means sharing the same superscript letter within a column are not significantly different (P<0.05)

for rats fed carbonate diets no. 1, 2, and 3 respectively. Sharaf (1998) mentioned that hemoglobin for rats were between 12.32 to 12.34 g/dl. Al-Qubati (2003) also, found hemoglobin values in rats between 12.70 to 13.50g/dl under similar conditions. However, these results were lower than that (14.80 g/dl) for the rats published by Ralston Purina Company (1984).

Results in Table (4) show calcium bioavailability for growing rats fed diets containing different levels of calcium carbonate. Data show that calcium consumption by the animals was increased with increasing the level of calcium carbonate in the diets. Insignificant differences ( $P<0.05$ ) were found among weight of tibia for the three groups of rats fed different levels of calcium carbonate diets. Weights of tibia were ranged between 0.22 to 0.23 g but total calcium of tibia and femur for rats fed diet No.2 was higher than those fed diet No.1 and 3. Calcium percent for these treatments were 16.9 and 16.0 %, respectively. Ranhotra *et al.* (1997) found that femur calcium content of growing rats was 17.1 % which fed bread diet fortified with  $\text{CaCO}_3$ . Tibia and femur calcium contents for baseline animals were 11.7 and 12.2 %, respectively which were higher than that (10.7 % femur calcium content) mentioned by Ranhotra *et al.* (1997). Normalized values were significantly ( $P<0.05$ ) low in rats fed diet No.3 comparing to those fed diets No.1 and 2. The result may be due to the amounts of calcium consumed which lead to reduce absorption efficiency. This result was also in agreement with those conducted by Behling and Greger (1990). In human, Heaney *et al.* (1990) found that absorption of calcium was 64 % by women subjects fed small amounts of calcium but it was 28.6 % when the amount of ingested calcium increased. Bone calcium content and calcium absorption data can be a good indicator to assess relative bioavailability of calcium in rats (Ranhotra *et al.*, 1980 and 1997). Tibia calcium content was utilized as an indicator of calcium bioavailability since it reflects calcium retention by rats (Heaney, 1986). Rats fed diet No.2 containing 0.19 % calcium can be a control (standard) group according to normalized value which was higher than other groups (NV was 81.8 mg calcium per g of tibia). So, bioavailability arbitrarily set at 100 % for rats fed  $\text{CaCO}_3$  diet containing 0.19 % calcium. RBV for rats fed diets No. 1 and 3 were 95.0 % and 74.2 %, respectively. Table (4) also revealed femur weights for the three rat groups fed  $\text{CaCO}_3$  diets, were between 0.22 to 0.24 g with no significant differences. Femur calcium in rats fed calcium carbonate diet containing 0.19 % calcium was significantly ( $P<0.05$ ) higher comparing with other groups, therefore, there is higher NV for this treatment which was 81.6 mg / g. This result was very close to NV in tibia for the same group, while NV in tibia and fibula for animals fed diets No.1 and No.3 were 78.0 and 60.8, respectively. These results were very close to NV in femur for the same groups (78.0 and 60.2, respectively). It was concluded that rat group fed diet No.2 considered as control group according to calcium content of tibia and femur, in which, RBV in this group was higher (100%) than others (95% and 73.8% for rats fed diet No.1 and 3, respectively). The result may be due to predominance of active transport of calcium in intestinal



tract more than diffusion transport under low levels of dietary calcium comparing with the results of Poneros and Erdman (1989).

Table (5) shows calcium absorption in growing rats fed calcium carbonate diets. Fecal calcium were 0.20 , 0.17 and 0.22 g for rats fed diets No.1 , 2 and 3 which were containing 0.14 , 0.19 and 0.24 % , respectively .It was known that upon higher calcium absorption there was lower fecal calcium (Ranhotra *et al.* 1997). Fecal calcium was high (0.22 g) in rats consumed higher amount of calcium (0.56 g) in rats fed diet No. 3. It was concluded that with increasing calcium consumption more calcium was loss in the feces therefore, decreasing calcium absorption (Behling and Greger, 1990 and Heaney *et al.* 1990). The results show that apparent absorption in group No.2 was 62.6 % while it was 51.0 and 60.6 % in group fed diets No.1 and 3, respectively which means higher absorption efficiency of calcium in rats fed diet containing 0.19 % calcium compared to other groups. Ranhotra *et al.* (1997) referred that apparent absorption of calcium in rats was higher when consumed calcium amount at marginal level. Relative apparent absorption for rats fed diet No.1 and No.3 were 81.2 and 96.8 % , respectively comparing to control group (No.2). Ranhotra *et al.* (1997) found that the relative apparent absorption ranged between 94.6 to 98.0 % in growing rats fed bread diet fortified with calcium carbonate, sulfate, citrate and lactate. Many factors increase fecal calcium such as high content of fat in diet and or the presence of some substances e. g. oxalate, phytate or fibers (Allen, 1982; Turnlund, 1987; Weaver *et al.*1987 and Guthrie and Picciano, 1995). No significant differences ( $P<0.05$ ) were found among dry matter absorption ( DMA % ) for rats fed diets No.1 , 2 and 3 , they were 91.8 , 92.0 and 91.6 % , respectively .This result may be due to the similarity of ingredients of the diets. Similar result of DMA for rats was found by Behling and Greger (1990) in their study on mature female rats fed similar levels of dietary salts.

Table (6) shows body calcium profile and endogenous organs of the rats .Calcium contents of liver, heart, kidneys and spleen for rats fed diet No.2 were 8.3, 13.8, 15.5 and 20.8 mg calcium per gram of each organ, respectively. Calcium content of these organs was higher than those for rats fed diets No.1 and 3. The calcium content of endogenous organs for rats fed diet No.1 was as follows: 8.0, 13.0, 14.2 and 19.8 mg/g of liver, heart, kidneys and spleen, respectively. In rats fed diet No.3 calcium content in these organs were 7.9, 13.0, 14.8 and 19.5 mg/g at the same order. This data confirmed what had mentioned earlier about calcium content in tibia and femur and emphasize that rats fed diet No.2 was control group. Total body calcium was 1.90, 1.94 and 1.89 % for rats fed diets No.1, 2 and 3, respectively. Group fed diet No.2 showed the highest (1.94 %) of calcium may be due to balanced ratio between calcium and phosphorus in the diet (1.5: 1) (Guthrie and Picciano, 1995 and Al-Zuhayri, 2000). Insignificant differences ( $P<0.05$ ) in the average initial body weight of baseline animals (43.0 g) comparing with the body weight of the rats in tested groups fed  $\text{CaCO}_3$  diets. Weight of liver, heart, kidneys and spleen for baseline animals were 2.52, 0. 27, 0.52 and 0.08 g, respectively. Calcium content of these organs was significantly ( $P<0.05$ ) lower comparing with those for animals grown up on  $\text{CaCO}_3$  diets for 28 days (Table 6). The amounts of calcium in liver,

heart, kidneys and spleen of this group found to be 5.7, 7.1, 7.4 and 10.9 mg 1 g, respectively, while total calcium in the whole body was 1.61 %, this result was significantly ( $P < 0.05$ ) lower than those for other animals which fed carbonate diets.

Table (7) shows calcium and phosphorus interaction and their absorption in growing rats. Results show that rats fed diet No.1 consumed 0.41 g of each calcium and phosphorus (ratio 1: 1 ) , the other group fed diet No .2 , consumed 0.47 g of calcium and 0.32 g of

Table (5); Calcium absorption of calcium carbonate based diets for growing rats.

Diet No.	Ca level %	Body weight g		Diet Consumed g	Ca consumed g	Feces Ca g	Ca absorption %	Relative Ca absorption	Dry matter absorption %
		Initial	Gain						
1	0.14	43.0	79.8±2.77 b	297	0.41±0.01c	0.20 b	51.0±3.93 b	81.2±6.41 b	91.8±0.54 a
2	0.19	43.2	86.2±3.56 a	250	0.41±0.01b	0.17 c	62.6±1.14 a	100.0 a	92.0±0.44 a
3	0.24	41.6	80.0±3.67 b	236	0.56±0.01a	0.22 a	60.6±2.19 a	96.8±3.49 a	91.6±1.00 a

Values are means of five animals. Means sharing the same superscript letter within a column are not significantly different (P<0.05).

Table (6): Calcium profile of body tissues of growing rats fed calcium carbonate based diets.

Diet No.	Ca level %	Body weight gain g	Ca consumed g	Endogenous organs								Total body calcium %
				Liver		Heart		Kidneys		Spleen		
				Weight g	Ca mg/g	Weight g	Ca mg/g	Weight g	Ca mg/g	Weight g	Ca mg/g	
1	0.14	79.8±2.77 b	0.41±0.01 c	4.42±0.65 a	8.0±0.63 a	0.45±0.06 a	13.0±0.44 b	0.69±0.05 a	14.2±0.52 c	0.45±0.05 a	19.8±0.25 b	1.90±0.01
2	0.19	86.2±3.56 a	0.41±0.01 b	3.97±0.60 a	8.3±0.29 a	0.45±0.06 a	13.8±0.33 a	0.68±0.07 a	15.5±0.31 a	0.43±0.08 a	20.8±0.74 a	1.94±0.01 a
3	0.24	80.0±3.67 b	0.56±0.01 a	3.84±0.52 a	7.9±0.20 a	0.45±0.05 a	13.0±0.23 b	0.63±0.03 a	14.8±0.32 b	0.37±0.02 a	19.5±0.21 b	1.89±0.02 b
BL*	--	--	--	2.52±0.38 b	5.7±0.04 b	0.27±0.04 b	7.1±0.42 c	0.52±0.05 b	7.4±0.15 d	0.08±0.01 b	10.9±0.50 c	1.61±0.01 c

\* Baseline animals. Values are means of five animals. Means sharing the same superscript letter within a column are not significantly different (P<0.05).

Table (7): Calcium and phosphorus interaction and their absorption in growing rats fed calcium carbonate based diets.

Diet No.	Body weight gain g	Ca consumed g	P consumed g	Ca/P	Tibia & Femur		Serum Ca mg/dl	Feces Ca g	Ca absorption %	Fecal P g	P absorption %	% DMA	Total Body	
					Ca mg	P mg							Ca %	P %
1	123±4.2 b	0.41±0.01 c	0.41±0.01 a	1:1	65.0±3.2 b	33.9±1.5 a	8.6±0.29 b	0.20±0.01 b	51.0±3.9 b	0.15±0.01 a	63.0±0.79 a	92±0.5 a	1.9±0.01 b	0.88±0.01 a
2	129±5.3 a	0.47±0.01 b	0.32±0.01 b	1:1.5	77.8±4.9 a	34.5±4.6 a	10.1±0.30 a	0.17±0.01 c	62.6±1.1 a	0.11±0.01 b	65.1±0.96 a	92±0.4 a	1.9±0.01 a	0.88±0.01 a
3	122±4.0 b	0.56±0.01 a	0.28±0.01 c	1:2	68.6±2.5 b	28.4±2.9 b	8.2±0.38 b	0.22±0.01 a	60.6±2.2 a	0.11±0.01 b	59.4±1.40 b	92±1.0 a	1.89±0.02 b	0.81±0.08 b

DMA= Dry Matter Absorption. Values are means of five animals.

Means sharing the same superscript letter within a column are not significantly different ( $P < 0.05$ ).

phosphorus ( ratio 1.5 : 1 ) , while the rats fed diet No. 3, consumed 0.56 and 0.28 g of each of calcium and phosphorus respectively( ratio 2 : 1 ). Calcium phosphate plays an important role in bone formation in which 90% of phosphorus found in the body in the form of calcium phosphate (Hydroxyapatite) in bones and teeth and bone calcification is affected when the ratio between calcium and phosphorus in blood is unbalanced (Williams, 1985). Dietary recommended amounts of phosphorus represented 2: 3 of calcium.

recommended amounts, this ratio is similar to the ratio of these elements in human milk and similar to their ratio in bones (Williams, 1985 and Guthrie and Picciano, 1995). Total calcium content in tibia and femur was 77.8 mg in rats fed diet No.2. This result was significantly ( $P<0.05$ ) high comparing to rats fed  $\text{CaCO}_3$  diets No. 1 and 3 which contain 65.0 and 68.6 mg, respectively. Concerning phosphorus bioavailability, tibia and femur of rats fed  $\text{CaCO}_3$  diet No. 2 retained 34.5 mg of phosphorus, while in rats fed diet No.1 and 3 phosphorus amounts were 33.9 and 28.4 mg, respectively . Calcium absorption depends on the ratio between calcium and phosphorus in diet, if the ratio less than 2: 1 in adults or less than 1: 1 in some categories e.g. children, or nutrition during pregnancy or nutrition of growing animals. It has been known that the amount of absorbed calcium decreases due to interference in sites of absorption. The ratio of calcium to phosphorus in human body is 2: 1, whereas the optimum ratio of calcium to phosphorus in diet is (1.5:1) for more calcium absorption (Williams, 1985 and Al-Zuhayri, 2000). The results in Table (7) show that low amount of fecal calcium was indicated and high calcium absorption for rats fed diet No.2 which consumed calcium to phosphorus ratio of 1.5: 1. Fecal calcium in this group was 0.17 g which significantly ( $P<0.05$ ) differed as compared to rats fed  $\text{CaCO}_3$  diets No.1 and 3 in which the calcium lost in feces were 0.20 and 0.22, respectively. Serum calcium in rats fed carbonate diets No.1, 2 and 3 were 8.6, 10.1 and 8.2 mg / dl, respectively, all in the normal limit. Ranhotra *et al.* (1997) found that serum calcium in growing rats was 6 - 12 mg / dl. Ralston Purina Company (1984) referred that serum calcium level in rats was 12.4 mg / dl. Serum can be constant parameter to predict the response toward minerals e. g. calcium (Wood, 2000). Weaver *et al.* (2002) determined calcium in Asian women by appearance of  $\text{Ca}^{45}$  in serum. Apparent absorption of calcium was 51.0, 62.6 and 60.6 % for rats fed diets No.1, 2 and 3, respectively. Fecal phosphorus amounts were 0.15, 0.11 and 0.11 mg for rats fed diets No. 1, 2 and 3 ,respectively, as a result the apparent absorption of phosphorus were 63.0, 65.1 and 59.4 % , respectively. The higher apparent absorption of phosphorus was in rats fed diet No.2 with calcium to phosphorus ratio of 1.5: 1. It has been known that this ration is preferable (Williams, 1985 and Al-Zuhayri, 2000). Dry matter absorption (DMA %) for rats fed diets No.1, 2 and 3 were 91.8, 92.0 and 91.6 %, respectively. The results show that there were no significant differences ( $P<0.05$ ) between the values, however, higher DMA% was found in rats fed diet No. 2. Whole body calcium in rats fed diet No.2 was 1.94% , it was significantly ( $P<0.05$ ) higher than that of other groups , while whole body

phosphorus was similar in rats fed diets No.1 and 2 (0.88% ) but significantly ( $P<0.05$ ) decreased in rats fed diet No .3 ( 0.81% ).

## التوافر الحيوي للكالسيوم من أغذية كاربونات الكالسيوم لذكور الجرذان النامية

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### الخلاصة

تم تقدير التوافر الحيوي للكالسيوم في مستويات مختلفة من كاربونات الكالسيوم باستخدام ذكور الجرذان النامية من نوع سبراكو داولي . قدرت قيمة الموازنة (المعايرة) والتوافر الحيوي النسبي وامتصاص الكالسيوم في ثلاث أغذية موزونة بمستويات ١٤ ، ١٩ ، ٢٥ ، ٣٠ % من الكالسيوم مصدره كاربونات الكالسيوم. بينت النتائج أن قيمة الموازنة والتوافر الحيوي النسبي في كل من عظم الفخذ والساق لمجموعة الحيوانات المغذاة الغذاء رقم ٢ كانت عالية معنوياً (  $P < 0.05$  ) ( ٨١ و ١٠٠ % على التوالي) مقارنة بتلك لمجموعة الحيوانات المغذاة بالأغذية رقم ١ ( ٧٨ و ٩٥؛ ٧٨ و ٩٥ % ، على التوالي) ورقم ٢ ( ٣٠ ، ٧٤ و ٦٠ ، ٧٣ % ، على التوالي). كذلك أشارت النتائج إلى أن امتصاص الكالسيوم والكالسيوم الجسم والكالسيوم السليم للحيوانات المغذاة الغذاء رقم ٢ كانت أعلى معنوياً (  $P < 0.05$  ) من مثيلاتها في مجموعة الحيوانات المغذاة بالأغذية رقم ١ و ٣. استنتج من ذلك أن غذاء كاربونات الكالسيوم والتي احتوت على ١٠ % كالسيوم وبنسبة ١ : ١ كالسيوم : فسفور أعطى توافراً حيوياً للكالسيوم عالياً في الجرذان النامية واعتبرت غذاءاً قياسياً مقارنة للأغذية الأخرى.

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