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EFFECT OF YOGHURT AND SOY-YOGHURT CONTAINING BIFIDOBACTERIA ON ENHANCING THE CALCIUM BIOAVAILABILITY AND BONE MINERALIZATION IN RATS

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ABSTRACT

Bioavailability of calcium from milk and other calcium sources has been an important issue of studies over recent years. The present study compared the bioavailability of calcium from basal diet containing cow milk (CM), yoghurt from cow milk (YCM), yoghurt from cow milk plus either *Bifidobacterium lactis* Bb-12 (YCMBb-12) or *Bifidobacterium longum* Bb-46 (YCMBb-46), non- fermented soymilk (NFSM), soy-yoghurt resulted from fermentation with either *B.lactis* Bb-12 (YSMBb-12) or *B.longum* Bb-46 (YSMBb-46) and basal diet alone (control). The obtained results indicated that there were no significant differences in food intake, body weight gain and food efficiency among the different groups of rats fed experimental diets in comparison to control. During the experimental period, feeding diets containing probiotics significantly increased serum calcium concentration and significantly reduced the faecal calcium (mg / day) compared to the control diet. The apparent absorption % of calcium was significantly increased too. The caecum acidity (pH), cecal carboxylic acids and bifidobacterial count were also increased in the groups of rats fed probiotic. These cecal parameters have a positive correlation with calcium bioavailability. The levels of femoral and tibial strength were also significantly increased by the probiotic diet. It is worthy to note that diet containing YCMBb-12 was the most effective and in general probiotic cow milk yoghurts were much better than those of soymilk yoghurt in enhancing the bioavailability of calcium. Thus the results in the present study proved the beneficial effects of cow milk yoghurts and soymilk yoghurts containing probiotics in enhancing calcium bioavailability and subsequently lowering loss of body calcium.

Keywords: Yoghurt, soy-yoghurt, calcium bioavailability, femur and tibia bone.

INTRODUCTION

Calcium is the most abundant mineral in the human body. About 99 % of total body calcium is stored in bones and teeth, while the remaining 1 % is found throughout the body in blood, muscle, and the fluid between cells. It is needed for muscle contraction, blood vessel contraction and expansion, the secretion of hormones and enzymes, and nerve conduction (Shills, 1999).

There is a growing awareness of the importance of maintaining a high calcium intake throughout life, not just for bone health (Food and Nutrition Board, Institute of Medicine, 1997), but also for the health of other body systems (Thys-Jacobs et al., 1998; Baron et al., 1999; Appel et al., 1997; Bucher et al., 1996).

The recommended calcium intake is 1000 mg / day, varying between countries and age groups (Weavers, 2000). Milk and milk products account for about 70 % of calcium sources in western countries (Osler et al., 1997).

Milk and milk products are considered good sources of calcium due to their high calcium content and bioavailability (Erba et al*.,* 2001).

Popular nutrition literature suggests that calcium may be more readily absorbed from products subjected to partial digestion by bacteria during manufacture (Recker et al*.,* 1988).

Recently, Scholz-Ahrens et al*.* (2007) reviewed the effect of prebiotics, probiotics and synbiotics on mineral absorption, bone mineral content and bone structure. Some researchers reported that probiotic could have a potential effect on bone accretion independent of that of prebiotics (Crittenden et al*.,* 2003; Hill, 1997; Hancock, Viola, 2001 and Igarashi et al*.,* 1994). It was also reported that probiotic yoghurts containing strains of *Lactobacillus casei, Lactobacillus reuteri, and Lactobacillus gasseri* increased apparent calcium absorption and bone mineral content in growing rats (Ghanem, Badawy, Abdel-Samam, 2004).

Another, popular source of calcium especially for vegans and for those with milk aversions is soymilk (Heaney et al*.,* 2000). Interest in soybeans and soy-beans products has grown significantly in the last two decades due to their reported nutritional and health promoting benefits (Setchell and casidy, 1999).This is in part because the isoflavones in soymilk can have many benefits such as bone-sparing effects over the long term (Setchell and Lydeking-Olsen, 2003) not only by attenuating bone loss (Chen et al*.,* 2003; Atkinson et al*.*, 2004) but also by enhancing calcium absorption (Zafar et al*.,* 2004). Another potential way to improve the nutritional content and therapeutic effect of soymilk is through fermentation with probiotics (Abd El-Gawad et al*.,* 2004 and 2005; Stanton et al*.,* 2005).

Previously, the anti-tumors and the hypocholestraemic effects of yoghurt and soy-yoghurt containing *Bifidobacterium lactis* Bb-12 or *Bifidobacterium longum* Bb-46 were investigated (Abd El-Gawad et al*.,* 2004 and 2005). As a further investigation, the objective of the present study was to examine the effect of yoghurt and soy-yoghurt containing *Bifidobacterium lactis* Bb-12 or *Bifidobacterium longum* Bb-46 on enhancing bioavailability of calcium and bone mineralization in rats.

MATERIALS AND METHODS

Yoghurt was made according to the method of (Tamime and Robinson, 1985). Cows' milk was inoculated with 3 % (v / v) of *Lactobacillus delbreuckii* sub sp *bulgaricus* and *Streptococcus salivarius* sub sp *thermophilus* (Chr. Hansen Laboratories, Copenhagen, Denmark), and divided into three portions. One portion (without added bifidobacteria) served as control yoghurt and was denoted as YCM; a second portion was inoculated at a level of 0.07 % (w / v) with a freeze-dried *Bifidobacterium lactis* Bb-12 and served as experimented yoghurt and was denoted as YCMBb-12. The third portion was inoculated at a level of 0.07 % (w / v) with a freeze-dried *Bifidobacterium longum* Bb-46 and served as experimented yoghurt and was denoted as YCMBb-46. The two strains of bifidobacteria were obtained from Chr. Hansen Laboratories (Copenhagen, Denmark). After the incubation period, the yoghurts were stored at 4± 1°C.

Fresh and non-beany flavoured soymilk was prepared from soybeans according to the method of Kamaly (1997), and divided into three portions. One portion served as a non-fermented soymilk and was denoted as NFSM; the other two portions were used for preparation of soy-yoghurts Bb-12 and Bb-46 according to the method described by El-Sayed et al. (1998), using 0.07 % (w / v) *B.lactis* Bb-12 or *B.longum* Bb-46. After inoculation at 37°C for 4-5 h, both yoghurts were stored at 4± 1°C.

Forty -eight male albino rats of average weight ranged between (113-148g) were housed in cages with screen bottom in a temperature and humidity controlled room. All animals kept under normal healthy conditions and fed a basal diet for one week**.** After this adaptation period, the rats were divided randomly into 8 experimental groups, each of 6 animals. One group received a basal diet throughout the experimental period of 45 days and served as control group. The other seven groups were fed on a basal diet plus the feeding diets treatments. The feeding diets used in the trial are: milk, yoghurt, probiotic yoghurt Bb-12, probiotic yoghurt Bb-46, soymilk, probiotic soy-yoghurt Bb-12, and probiotic soy-yoghurt Bb-46. The experimental groups of rats and diets used in the trial are described in Table (1). The basal diet and the other diets were supplemented with a constant amount of the studying minerals (Ca, Mg, P, Fe, and Zn). The rats were allowed free access to experimental diet and water. The daily food consumption of each rat and weekly body weights was monitored throughout the test period. At the end of 45 days experimental period, Blood samples were collected from the eye vein under diethyl ether anesthesia; the samples were taken into tubes and then centrifuged at 3000rpm for 10min to obtain the serum, which stored at -20ºC until analysis. Feces were collected every three weeks and removed hair and food attached were removed and feces were kept frozen until analysis. Each rat was anesthetized and killed by drawing blood.

Table 1. Experimental groups of rats and diets used in the trial [x, y].

Diet treatment	Diet code	Diet formulae
Basal diet	Control diet	100g basal diet + 50 ml water
Basal diet+ Cow milk	CM	100 gm basal diet + 50 gm CM
Basal diet+ yoghurt from Cow milk (YCM)	YCM	100 gm basal diet + 50 gm YCM
Basal diet+YCM with added *B.lactis* Bb-12	YCMBb-12	100gm basal diet+ 50 gm YCMBb-12
Basal diet+YCM with added *B.longum* Bb-46	YCMBb-46	100 gm basal diet + 50 gm YCMBb-46
Basal diet+ non-fermented soy milk	NFSM	100 gm basal diet + 50 gm NFSM
Basal diet + yoghurt from soy milk fermented with *B.lactis* Bb-12	YSMBb-12	100 gm basal diet + 50 gm YSMBb-12
Basal diet + yoghurt from soy milk fermented with *B.longum* Bb-46	YSMBb-46	100 gm basal diet + 50 gm YSMBb-46

[x] The basal diet consisted of: 20 % (w / w) casein, 53.2 % (w / w) corn starch, 7 % (w / w) corn oil, 3.5 % (w / w) mineral mixture, 1 % (w / w) vitamin mixture, 5 % (w / w) cellulose, 0.3 % (w / w) L.cysteine and 10 % (w / w) sucrose.

[y] The mineral mixture consisted of [mg(except as noted) / kg diet]:Ca HPO_4, 15 g; K_2HPO_4, 2.5 g;KCl, 5 g; NaCl, 5 g; $MgCl_2$, 2.5 g; Fe_2O_3, 2.5 g; $MnSO_4$, 125; $CuSO_4$. 5 H_2O, 25; CoSO4. 7H_2O, 0.2; $ZnSO_4$. H_2O, 100; and KI, 0.4.

Then the organs (Liver, Kidney, Spleen and Heart) were excised immediately and weighed, the whole caecum was taken and weighed and kept frozen until analysis, after rats were killed, the femur and tibia from each rat were removed, cleaned and weighed, then were kept until analysis.

Protein and fat content were determined by microkjeldahl procedure and rose-gottlieb method according to AOAC (1984), and AOAC (1990) respectively.Wherease sugars were determined by HPLC according to the method of Black and Bagley (1978). Mineral content (Ca, Mg, Fe, and Zn) in the diet was determined by dry ashing by atomic absorption according to the method of Ohta et al. (1997). Whereas mineral content (Ca, Mg, Fe, and Zn) in the products was determined by wet ashing by atomic absorption according to the method of Parker et al. (1967). Furthermore, Phosphorus in the diet and products was determined by spectrophotometer according to the method of Astm (1975). Table 2 shows the chemical composition (protein, fat, sugar, and mineral content) of the different products used in this study.

The count of bifidobacteria in single culture (soy-yoghurt) was enumerated by poured plate method on Lactobacilli MRS-Agar medium according to the method described by Samona and Robison (1991). Whereas the count of bifidobacteria in mixed cultures (probiotic yoghurt) was enumerated according to Dinakar & Mistry (1994).

Yoghurt culture (*Lactobacillus delbreuckii* sub sp *bulgaricus* and *Streptococcus salivarius* sub sp *thermophilus*) was enumerated according to the method of Lee et al. (1973). Table 3 shows the viable count of yoghurt culture and bifidobacteria in the experimental products.

Calcium in serum was determined using calorimetric O-cresolphtalein complexone (CPC) method according to the method of Kaplan *et al.* (1996).

The minerals (Ca, Mg, P, Fe, and Zn) in feces were determined by wet ashing method (Oxidation procedure) according to the method of parker *et al.* (1967).

Table 2. Chemical composition of the products used in the study [x].

Product	Chemical composition							
	Protein (%)	Fat (%)	Sugar (%)	Minerals (mg/ kg)				
				Ca	Mg	P	Fe	Zn
CM	3.07	3.10	4.45	1147.66	107.31	881.50	0.43	3.30
YCM	3.20	3.30	2.65	1196.63	110.25	895.11	0. 64	4.01
YCMBb-12	3.38	3.32	2.10	1189.38	109.55	887.23	0.60	4.53
YCMBb-46	3.31	3.29	2.21	1190.34	110.85	898.57	0.57	3.90
NFSM	4.47	2.32	0.95	246.63	44.20	530.11	0.09	4.30
YSMBb-12	4.73	2.39	0.45	265.09	48.27	548.50	0.10	5.60
YSMBb-46	4.86	2.37	0.55	253.26	46.63	539.71	0.16	4.80

[x] **see Table 1 and text for details of diet treatments.**

For measuring the pH of caqecum, 0.5 gm of sample (caecum content) was blended in 10 ml distilled water for 2 min. then filtered through glass wool. The pH was determined by pH meter (Jenway) as following the method of Kotula *et al.* (1976).

The count of Bifidobacteria in rat's caecum was determined by plate method on Lactobacilli MRS-Agar medium according to the method described by Chen *et al.* (1999).

Carboxylic acids (Acetic + Lactic + Propionic + Pyruvic) in caecum were determined by HPLC according to the method of Wodecki *et al.* (1991).

Table 3. The viable count of yoghurt culture and Bifidobacteria in experimental products [x, y, z, w,]

product	Yoghurt culture count	Bifidobacterial count
	(cfu ×10^8 / ml) [a]	
YCM	75.8	ND [b]
YCMBb-12	68.9	3.1
YCMBb-46	47.7	3.8
YSMBb-12	ND	4.6
YSMBb-46	ND	5.3

[x] values are means (n = 3).
[y] see Table 1 and text for details of diet treatments.
[z] yoghurt culture: *Lactobacillus delbreuckii* sub sp *bulgaricus* and *Streptococcus salivarius* sub sp *thermophilus*.
[w] Bifidobacteria : *B.lactis* Bb-12 or *B.longum* Bb-46.
[a] cfu: colony forming unit.
[b] ND: Not determined.

The length and thickness of bone (femur and tibia) was measured with a vernier caliper according to the method of Arjmandi *et al.* (1998).

The femur and tibia bone was cut at the mid-diaphysis and the marrow washed out. Bone volume and density were measured by Archimedes principle according to the method of Kalu *et al.* (1991).

The breaking force of femur and tibia bones was determined with digital force gauge model FGN-50 Chisel head NIDEC-SHIMPO Corporation, Kyoto, Japan following the procedure described by Gomez-Aldapa *et al.* (1999).

The dry weight of femur and tibia was determined according to the method of Ohta *et al.* (2002). Bones were brought to constant weight by placing them at 80ºC for 18 h and weighing.

The ash weight of femur and tibia was determined according to the method of Ohta et al. (2002). The dried bones were ashed at 550ºC for 24 h.

The amount of calcium in the ashed samples of femur and tibia was determined according to the method of Ohta et al. (2002), the ashed samples of femur and tibia bones were dissolved in 4 mL of 2 mol/ HCl, and then diluted appropriately with distilled water for atomization.

Data are presented as means and standard deviation. The significance differences between groups / treatments were evaluated using the general linear model procedure of Statistical Analysis System (SAS) (1990) (SAS Institute, Inc, Cary, NC, USA) to analysis the biological examination data by least significant difference (LSD) at $P<0.05$.

RESULTS AND DISCUSSION

Data presented in Table (4) indicated that there were no significant differences in food intake, body weight, body weight gain and food efficiency (Food efficiency (%) =Body weight gain / Food intake ×100) among the eight experimental rat groups throughout the study period (45 days). This is in agreement with the results obtained by Kruger *et al.* (2009) who found similar results when growing male and ovariectomised female rats were fed high

calcium milk powder plus or minus probiotics (L. rhamnosus HN001) . A similar trend was recorded by Tsuchita *et al.* (1992), Narva *et al.* (2007).

Table (4): Body weight gain and food intake of rats fed yoghurt or soy-yoghurt containing bifidobacteria for 45 days [x, y].

Treatment	Final Body weight (g)	Body weight gain / day [z]	Food intake (g/d)	Food efficiency (%)
Control	235.67 [a] ± 21.83	2.51 [a] ± 0.31	15.95 [a] ± 0.57	13.66 [a] ± 2.76
CM	235.00 [a] ± 18.68	2.39 [a] ± 0.60	15.94 [a] ± 1.12	14.89 [a] ± 3.27
YCM	231.00 [a] ± 15.10	2.295 [a] ± 0.02	16.03 [a] ± 1.14	14.96 [a] ± 1.93
YCMBb-12	229.33 [a] ± 15.01	2.32 [a] ± 0.17	16.66 [a]± 1.52	14.03 [a] ± 1.10
YCMBb-46	235.33 [a] ± 26.50	2.41 [a] ± 0.92	16.38 [a] ± 0.23	14.66 [a] ± 5.45
NFSM	235.33 [a] ± 21.36	2.41 [a] ± 0.08	16.22 [a] ± 0.69	14.84 [a] ± 0.19
YSMBb-12	235.67 [a] ± 20.03	2.36 [a] ± 0.23	16.52 [a] ± 1.32	14.11 [a] ± 0.10
YSMBb-46	234.33 [a] ± 6.03	2.41 [a] ± 0.36	16.30 [a] ± 0.94	14.86 [a] ± 2.93

[x] values are means ± Standard deviation (n = 3). [y] See table 1 and text for details of diet treatments. [z] Body weight gain / day = (Final body weight – Initial Body weight) / study period.

Table (5):Organ weights of rats fed on experimental diets for 45 days [x, y].

Treatment	Organs weight (g)			
	Liver	Kidney	Spleen	Heart
Control	6.40 [b] ± 0.70	1.10 [b] ± 0.10	0.93 [a] ± 0.25	0.63 [ab] ± 0.12
CM	6.50 [b] ± 0.80	1.23 [ab] ± 0.06	1.03 [a] ± 0.12	0.63 [ab] ± 0.06
YCM	7.03 [ab] ± 0.90	1.47 [a] ± 0.32	1.10 [a] ± 0.20	0.73 [ab] ± 0.06
YCMBb-12	6.50 [b] ± 0.40	1.43 [a] ± 0.21	1.23 [a] ± 0.21	0.77 [ab] ± 0.31
YCMBb-46	7.53 [a] ± 0.29	1.37 [ab] ± 0.06	1.23 [a] ± 0.16	0.83 [a] ± 0.06
NFSM	6.63 [ab] ± 0.49	1.20 [ab] ± 0.10	1.00 [a] ± 0.17	0.57 [b] ± 0.06
YSMBb-12	6.77 [ab] ± 0.51	1.23 [ab] ± 0.06	0.97 [a] ± 0.16	0.63 [ab] ± 0.06
YSMBb-46	6.43 [b] ± 0.50	1.17 [ab] ± 0.06	0.97 [a] ± 0.12	0.77 [ab] ± 0.12

[x] values are means ± Standard deviation (n = 3).
[y] See Table 1 and text for details of diet treatments.

Although the food intake was slightly lower in rats fed basal diet (control), their body weight gain per day was slightly higher than that of rats fed experimental diets (Table 4). This might be due to the reported data which indicated firstly that more calcium was utilized when rats were fed milk or soy milk products subjected to partial digestion by bacteria especially probiotics during manufacture (Smith *et al.,* 1985 and Tang *et al.,* 2007) and secondly that the high the calcium intake, the lower was the body weight and weight gain (Zemel *et al.,* 2001).

The results presented in Table (5) exhibited that there were no significance in liver, kidney, spleen and heart weights between rats fed soy milk, soy probiotic yoghurt and basal diet (control). Regarding the effect of feeding cow milk (CM) and cow milk yoghurt (YCM), cow milk yoghurt containing bifidobacteria *B. lactis* Bb-12 (YCM Bb-12) and cow milk yoghurt containing *B. longum* Bb-46 (YCMBb-46) on the weight of the above mentioned organs, it is clear that feeding YCMBb-12 led to a significant

increase in weight of kidney, while feeding YCMBb-46 resulted in an significant increase in liver weight as compared with that of the control groups.

Serum calcium concentration (mg/dl) in rats fed yoghurt or soy-yoghurt containing bifidobacteria during 45 days is indicated in (Table 6) .It was obvious that serum calcium concentration upon feeding the different experimental diets as compared with that of control (Table. 6). Furthermore, the calcium concentration in serum of all rats was increased throughout the study period. At 22 days of study, the increment in serum calcium concentration was significant in rats group fed YCMBb-12 when compared with that of NFSM, YCM, CM and Control group. At the end of study (45 days) there were significant differences between values of serum calcium concentration in rats of all groups and that of control. On the other hand, no significant differences were existed between serum calcium concentrations in rats of these groups. It is worthy to note that feeding diets containing probiotic bacteria led to significant increase in serum calcium concentration as compared with that of the control and cow milk probiotic yoghurt had greater effect than that of its correspondence of soy probiotic yoghurt (Table 6). This might be due to the partial digestion occurred during manufacturing of probiotic cow milk or soy milk products which increase the soluble calcium and consequently enhance its bioavailability and absorption.

Table (6): Serum calcium concentration (mg/dl) in rats fed on experimental diets during 45 days [x, y].

Treatment	Serum calcium concentration (mg/dl) [z]	
	22 day	45 day
Control	7.14 ^{fg} ±1.17	7.19 ^{fg} ± 2.03
CM	9.31 ^{ef}± 0.40	11.98 ^{abcd} ±1.79
YCM	9.39 ^{ef} ±1.40	12.44 ^{abc}±1.95
YCMBb-12	12.53 ^{abc}±1.91	14.31 ^{a}± 2.38
YCMBb-46	12.17 ^{abcd} ±1.92	13.48 ^{ab}± 3.30
NFSM	9.73 ^{de}± 0.93	12.19 ^{abcd} ±1.37
YSMBb-12	10.58 ^{cde} ±1.81	13.72 ^{ab} ± 2.31
YSMBb-46	11.40 ^{bcde}± 2.02	13.19 ^{ab} ±1.48

[x] values are means ± Standard deviation (n = 3).
[y] See Table 1 and text for details of diet treatments.
[z] Serum Ca concentration in zero time = 6.48.

Calcium intake, excretion and apparent absorption (%) as affected by feeding cow milk yoghurt and soy-yoghurt containing bifidobacteria during 45

Are presented in (Table 7). Figures in Table (7) illustrated that there were no significant differences in calcium intake (mg / day) between rats fed all experimental groups. The fecal calcium (mg / day) was significantly higher in case of rats fed basal diet (control) than that of other experimental group. The incorporation of YCMBb-12, YCMBb-46, YSMBb- 12 and YSMBb – 46 in the diet significantly lowered the calcium excretion in rat feces (Table 7) and subsequently in proved the apparent absorption percentage of calcium. The

obtained results are in accordance with the results observed by **Perez-**Conesa *et al.* (2006) and Ghanem *et al.* (2004).

Table (7). Apparent absorption of calcium in rats fed on experimental diets during 45 days [x, y].

Treatment	Ca intake (mg/d)	Fecal Ca excretion (mg/d)	Ca apparent absorption(%) [z]
Control	70.35 [a] ±2.54	23.87[a] ±2.25	66.17[d]± 4.33
CM	70.45 [a] ±5.11	16.65 [bc] ± 0.51	76.28 [c]±1.90
YCM	70.68 [a] ±4.97	15.75 [bc] ± 0.49	77.63 [bc]±1.70
YCMBb-12	73.48 [a] ±6.70	12.79 [cd] ± 1.91	82.56[a]± 2.33
YCMBb-46	72.26 [a] ±1.04	12.91 [cd] ± 0.77	82.13[ab]±1.39
NFSM	71.57 [a] ±3.04	19.26 [b] ± 3.09	73.19 [c] ±3.26
YSMBb-12	72.86 [a] ± 5.81	11.99 [d]± 1.33	83.55 [a] ±1.19
YSMBb-46	71.92 [a] ± 4.16	11.79 [d]± 2.26	83.43[ab]±3.92

[x] values are means ± Standard deviation (n = 3).
[y] See Table 1 and text for details of diet treatments.
[z] Ca apparent absorption % = Ca intake – Ca excretion / Ca intake x100.

Caecum weight (g),pH and bifidobacterial count in rats fed yoghurt and soy - yoghurt containing bifidobacteria for 45 days are indicated in (Table 8).

The oral application of the experimental diets clearly showed no significant differences in the total weight of caecum of different groups rats either fed probiotic containing or probiotic free diets (Table 8) as compared with that of the control. On the other hand, results in the same table indicated that feeding on each of the experimental diets led to significant decrease in the rat's caecum pH as compared with control (basal diet). Moreover, the incorporation of probiotics in rat's diet resulted in significant reduction in pH of rat's caecum than probiotic-free diets. It is worthy to note that *B.lactis* Bb-12 was more effective on pH reduction than *B.longum* Bb-46 either when present in yoghurt from cow or soy milk (Table. 8).The obtained results are in general agreement with those of Campbell et al*.* (1977) and perez-Conesa *et al.* (2007) who illustrated that the addition of bifidobacteria and / or prebiotic to the diets changed the pH of the cecal content.

Regarding the bifidobacterial count, it was found that the addition of *B.longum* Bb-46 significantly increased the count of bifidobacteria as compared with that of *B.lactis* Bb-12, yoghurt culture and control treatment respectively. This is in line with the data obtained by Ghanem *et al.* (2004) and that reviewed by Scholz-Ahrens *et al.* (2007).

Carboxylic acids up to six carbon atoms in length are the major end products of microbial fermentation in the ruminant digestive tract (Find-health articles.com). They also formed in lower part of the intestinal tract of non-ruminant animals and they may be called organic acids or short chain fatty acids. It is obvious from Table (8) that presence of probiotic B.lactis Bb-12, B.longum Bb-46 or yoghurt culture in the rate diet increased significantly the amount of acetic acid as compared with control and microorganisms-free cow milk or soymilk. In general similar trend was seen when other carboxylic

acids were determined in the cecal content. From the results in Table (8) that registered in Table (7) and the above mentioned Table (7) it is clear that presence of probiotic bacteria in the diet led to decrease the pH, increase the amount of carboxylic acids and apparent absorption % of calcium. These findings are in accordance with the previously reported data by van den Heuvel *et al.* (1999) who concluded that probiotic bacteria produce fermentation by-products including short chain fatty acids (SCFA, essentially acetate, propionate and butyrate) and other organic acids (e.g. lactate) which by contributing to a reduced luminal pH and increased amount of soluble calcium, may increase calcium absorption. In addition, Cashman et al. (2003) illustrated that, it is also possible that bacterially produced SCFA directly influence calcium absorption by other means. Unal *et al.* (2005)

Table (8). Caecum weight, pH and Bifidobacterial count in rats fed experimental diets for 45 days [x, y].

Treatment	Caecum		
	Weight (g)	pH	Bifidobacterial count (CFU/ 10^7)
control	1.80 ^{a} ± 0.09	7.04 ^{a} ± 0.14	3.38 ^{c} ± 0.20
CM	1.80 ^{a} ± 0.24	6.69 ^{b} ± 0.06	3.98 ^{bc} ± 0.42
YCM	1.90 ^{a} ± 0.24	6.48 ^{bc} ± 0.18	4.20 ^{b} ± 0.05
YCMBb-12	2.07 ^{a} ± 0.04	5.94 ^{d} ± 0.04	4.44 ^{b} ± 0.22
YCMBb-46	2.00 ^{a} ± 0.29	6.02 ^{d} ± 0.11	5.18 ^{a} ± 0.77
NFSM	1.86 ^{a} ± 0.11	6.57 ^{bc} ± 0.34	3.90 ^{bc} ± 0.09
YSMBb-12	2.01 ^{a} ± 0.25	5.95 ^{d} ± 0.06	4.28 ^{b} ± 0.22
YSMBb-46	2.10 ^{a} ± 0.39	6.34 ^{c} ± 0.15	4.18 ^{b} ± 0.18

[x] values are means ± Standard deviation (n = 3).
[y] See table 1 and text for details of diet treatments.

Concluded that rats fed yoghurt showed considerable increase in calcium bioavailability. Tang *et al.* (2007) indicated that fermentation of calcium fortified soymilk with selected probiotics potentially enhanced calcium bioavailability due to increased calcium solubility and bioactive isoflavone aglycone enrichment.

Femur calcium, bone density and breaking force in rats fed yoghurt and soy-yoghurt containing bifidobacteria for 45 days are shown in Table (9). Results presented in Table (9) showed increase in values of femur calcium, bone density and breaking force for the groups of rats fed experimental diets other than basal diets (control). Among the experimental diets, feeding the YCMBb-12 containing diet significantly increased the values of the three measured parameters. While bone density and breaking force values were significantly increased than that of control when YSMBb-12 was fed.

Regarding the breaking force, it was noticed from the same table that incorporation of YCMBb-12 and YSMBB-12 significantly increased this property in comparison to that of control treatment.

From the above mentioned results and the results excised in Table (10) especially that of femur ash weight (higher calcium content in the femoral bones of rats), it is clearly seen that inclusion of cow milk yoghurt containing probiotics may be of help for those people with low calcium absorption as

reported by Heaney *et al.* (1994) when examined diet containing caseinphospgopeptide. These results are in line with those of Choi *et al.* (2005), perez-Conesa *et al.* (2007). It is worthy to note that neither thickness nor length of all experimented rat femur were significantly affected on feeding probiotics diets. These findings are in agreement with the reported data in the literature.

Table (9). Femur Calcium content, bone density and breaking force in rats fed yoghurt and soy-yoghurt containing bifidobacteria for 45 days [x, y].

Treatment	Femur calcium (g/kg)	Bone density (g/cm^3) [z]	Breaking force (Neioten [w])
Control	450.22 [b] ± 80.90	1.27 [c] ± 0.13	85.97 [b]± 2.24
CM	569.03 [a] ± 25.94	1.33 [bc] ± 0.12	92.97 [ab]± 4.23
YCM	570.00 [a] ± 75.91	1.38 [bc] ± 0.07	93.73 [ab] ± 5.14
YCMBb-12	578.20 [a] ± 48.68	1.53 [a] ± 0.30	103.78 [a] ± 8.93
YCMBb-46	582.96 [a] ± 17.14	1.41 [ab] ± 0.18	97.47 [ab] ± 2.23
NFSM	527.68 [ab]± 19.87	1.31 [c]± 0.24	87.43 [b] ± 1.71
YSMBb-12	537.42 [ab] ± 80.15	1.47 [ab] ±0.19	100.08 [a] ± 4.79
YSMBb-46	533.73 [ab] ± 56.87	1.39 [abc] ±0.02	94.95 [ab] ± 2.01

[x] values are means ± Standard deviation (n = 3).
[y] See table 1 and text for details of diet treatments.
[z] Bone density (g/cm^3) = was measured by Archimedes principle. [w] Neioten = kg × 9.18.

Table (10). Femur ash weight, thickness and length in rats fed the experimental diets for 45 days [x, y].

Treatment	Ash weight (g)	Thickness (mm)	Length (mm)
Control	0.11 [b] ± 0.007	0.31 [c] ± 0.005	26.32 [a] ± 0.61
CM	0.11 [b] ± 0.01	0.33 [abc] ± 0.01	27.41 [a] ± 0.57
YCM	0.13 [ab] ± 0.01	0.35 [ab] ± 0.02	28.91 [a] ± 0.63
YCMBb-12	0.14 [a] ± 0.02	0.32 [abc] ± 0.01	28.71 [a] ± 1.54
YCMBb-46	0.13 [a] ± 0.02	0.31 [bc] ± 0.03	28.87 [a] ± 1.09
NFSM	0.12 [ab] ± 0.004	0.34 [abc] ± 0.03	26.96 [a] ± 1.5
YSMBb-12	0.13 [ab] ± 0.006	0.33 [abc] ± 0.003	28.47 [a] ± 1.69
YSMBb-46	0.13 [ab] ± 0.005	0.33 [abc] ± 0.01	28.47 [a] ± 1.52

[x] values are means ± Standard deviation (n = 3).
[y] See table 1 and text for details of diet treatments.

Tibia calcium content, bone density and breaking force in rats fed yoghurt and soy-yoghurt containing bifidobacteria for 45 days are presented in Table (11) .The results in Table (11) show the effect of feeding cow and soy milks, cow milk yoghurt, cow milk yoghurt containing *B.lactis* Bb-12 or *B.longum* Bb-46 and soymilk fermented only with *B.lactis* Bb-12 or B.longum Bb-46 to get soy-yoghurts on the tibia calcium, its bone density and breaking force.

Table (11). Tibia Ca content, bone density and breaking force in rats fed yoghurt and soy-yoghurt containing bifidobacteria for 45 days.

Treatment	Wet weight (g)	Dry weight (g)	Ash weight (g)	Non-mineral weight (g) [z]	Thickness (mm)	Length (mm)
Control	0.33 [a] ±0.03	0.24 [a] ± 0.028	0.12 [c] ±0.01	0.126 [a] ±0.027	0.288 [a] ±0.016	34.34 [a] ±0.055
CM	0.33 [a] ±0.01	0.24 [a] ± 0.01	0.14 [abc] ±0.02	0.1000 [a] ±0.028	0.288 [a] ±0.023	34.57 [a] ± 0.025
YCM	0.36 [a] ±0.02	0.26 [a] ± 0.02	0.13 [bc] ±0.03	0.134 [a] ±0.034	0.345 [a] ±0.051	36.49 [a] ±0.044
YCMBb-12	0.34 ±0.05	0.25 [a] ± 0.03	0.17 [a] ± 0.01	0.079 [a] ±0.018	0.318 [a] ±0.010	35.60 [a] ±0.111
YCMBb-46	0.33 [a] 0.01	0.24 [a] ± 0.01	0.16 [ab] ± 0.01	0.081[a] ±0.009	0. 319 [a] ±0.035	3596 [a] ±0.077
NFSM	0.33 [a] 0.04	0.24 [a] ±0.005	0.13 [bc] ± 0.003	0.111[a] ±0.003	0.300 [a] ±0.035	35.47 [a] ±0.077
YSMBb-12	0.34 [a] ±0.06	0.24 [a] ±0.04	0.14 [abc] ±0.02	0.106[a] ±0.036	0.323 [a] ±0.031	35.35 [a] ±0.241
YSMBb-46	0.32 [a] ±0.03	0.24 [a] ±0.02	0.13 [bc] ±0.014	0.110 [a] ±0.039	0.298 [a] ±0.021	35.13 [a] ±0.070

[x] values are means ± Standard deviation (n = 3).
[y] See table 1 and text for details of diet treatments.
[z] Bone density (g/cm^3) = was measured by Archimedes principle.

Table (12). Tibia wet weight, ash weight, thickness and length in rats fed on experimental diets for 45 days [x, y].

Treatment	Tibia calcium (g/kg)	Bone density (g/cm^3) [z]	Breaking force (Neioten [w])
control	408.66 [b] ± 58.10	0.66 [b]± 0.04	62.90 [d]± 2.97
CM	471.46 [ab]± 52.34	0.68 [b] ± 0.05	65.03 [cd]± 8.82
YCM	478.58 [ab] ± 36.60	0.83 [b] ± 0.07	71.23 [bcd]± 4.56
YCMBb-12	502.90 [a] ± 46.23	1.35 [a] ± 0.33	93.57 [a]± 3.86
YCMBb-46	501.53 [a] ±14.31	1.31 [a]± 0.026	91.57 [a]± 5.39
NFSM	453.90 [ab] ± 40.24	0.71 [b] ± 0.02	67.70 [bcd] ± 16.97
YSMBb-12	502.44 [a] ± 23.07	0.92 [b] ± 0.05	79.90 [ab]± 8.64
YSMBb-46	493.97 [ab] ± 7.66	0.89 [b] ± 0.10	79.66 [abc]± 5.33

[x] values are means ± Standard deviation (n = 3).
[y] See table 1 and text for details of diet treatments.
[z] Non-mineral weight (g) = dry weight – ash weight

The obtained results clearly indicated that incorporation of YCMBb-12 or YCMBb-46 significantly increased the tibia calcium content, its bone density and breaking force.

Similar trend was observed when soy-yoghurts were incorporated but the values of only bone density and breaking force were lower than that of its correspondence from cow milk yoghurt, where the tibia calcium content was almost similar to that of its cow based correspondence.

The results in Table (12) illustrated the tibia weight and dry wet weight, ash weight, non- mineral weight, thickness and length. It could be seen that ash weight of tibia excised from rats fed YCMBb-12 or YCMBb-46 was higher than that of its correspondence (i.e. those fed YSMBb-12 or YSMBb-46).

Conclusions:

The reported results of the present work enabled us to conclude that yoghurts containing probiotics either made from cow milk or soymilk have significantly increased the calcium bioavailability and subsequently increased the femoral and tibial calcium content as well as strengthen both of them, ince the breaking force required to break each of them was increased.

REFERENCES

Abd El-Gawad, I. A.; El-Sayed, E. M.; Hafez, S. A.; El-Zeini, H. M. and Saleh, F. A. (2004). Inhibitory effect of yoghurt and soy-yoghurt containing bifidobacteria on the proliferation of Ehrlich ascites tumour cells In vitro and In vivo in a mouse tumour model. British journal of nutrition. Vol. 92, 81-86.

Abd El-Gawad, I. A.; El-Sayed, E. M.; Hafez, S. A.; El-Zeini, H. M. and Saleh, F. A. (2005).The hypocholesrolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. International dairy journal. 15, 37-44.

AOAC (1990). Official methods of analysis, 15th ed. Association of official analytical Chemists, Inc, USA.

AOAC, (1984). Official methods of analysis, 13th ed. Association of official analytical Chemists, Washington, USA.

Appel, L.J.; Moore, T.J.; Obarzanek, E.; et al. (1997). A clinical trial of the effects of dietary patterns on blood pressure. N. Engl. J. Med. 336: 1117–1124.

Arjmandi, B. H.; Alekel, L.; Getlinger, M.J.; Goyal, V.V.; Hasler, C.M.; Juma, S.; Drum, M.L.; Hollis, B.W. and Kukrreja, S. C. (1998). Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. Am. J. Clin. Nutr. 68(Suppl): 1358S-1363S.

Astm (1975). Annual book of American Society of Testing Materials Standard Water. Part 31Dsis-72-Philadelphia, PA

Atkinson, C.; Compston, J.E.; Day, N.E.; Dowsett, M.; Bingham, S.A. (2004). The effects of phytoestrogen isoflavones on bone density in women: a double blind, randomized, placebo-controlled trial. Am. J. Clin. Nutr. 79:326–33.

Baron, J.A.; Beach, M.; Mandel, J.S.; et al. (1999). Calcium supplements for the prevention of colorectal adenomas. N. Engl. J. Med. 340:101–107.

Black, L. T. and Bagley, E. B. (1978). Determination of oligosaccharides in a soybeans by High pressure liquid chromatography using an internal standard. J. of the American oil chemistry society. Vol. 55(2): 228.

Bucher, H.C.; Guyatt, G.H.; Cook, R.J.; et al. (1996). Effect of calcium supplementation on pregnancy-induced hypertension and preeclampsia. JAMA. 275:1113–1117.

Campbell JM, Fahey GC and Wolf BW, (1997). Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *J Nutr* 127:130–136.

Cashman, K.D. (2003) Prebiotics and calcium bioavailability. Curr. Issues Intest. Microbiol. *4*, 21–32. Coudray, C., Bellanger, J., Castiglia-Delavaud, C.,Rémésy, C., Vermorel, M., and Rayssignuier, Y. (1997) Effect of soluble or partly soluble dietary fibers supplementation on absorption and balance of calcium, magnesium, iron, and zinc in healthy young men. Eur. J. Clin. Nutr. *151*, 375–380.

Chen, R.M.; Wu, J.J.; Lee, S. C. (1999). Increase of intestinal Bifidobacterium and suppression of coliform bacteria eith short-term yoghurt. J.of Dairy Science, 82: 2308-2314.

Chen, Y.M.; Ho, S.C.; Lam, S.S.H.; Ho, S.S.S.; Woo, J.L.F. (2003). Soy Isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. J. Clin. Endocrinol. Metabol. 88(10):4740–7.

Choi, I; Changhwa, J.; Choi, H.; Kim, C. and Ha, H. (2005). Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones. Food Chemistry 93 (2005) 577–583.

Crittenden, R.G.; Martinez, N.R.; Playne, M.J. (2003). Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria. Int. J. Food Microbiol. 80:217–222.

Dinakar, P. and Mistry, V. V. (1994). Growth and viability of Bifidobacterium bifidum in chedder cheese. Journal of Dairy Science. 77: 2854-2864.

El-Sayed, E.M.; Hefny, A.A.; Saleh, F.A. and Abd El Gawad, I.A. (1998). Bifidobacteria as a starter for the manufacture of soy-yoghurt products. 7th Egyptian Conf. Dairy Sci. & Tech., 269-294.

Erba, D.; Ciappellano, S.; Testolin, G. (2001). Effect of caseinphosphopeptides on inhibition of calcium intestinal absorption due to phosphate Nutrition Research. 21: 649–656.

Find-health articles.com.

Food and Nutrition Board, Institute of Medicine. (1997). Dietary reference intakes for calcium, magnesium, phosphorus, vitamin D, and fluoride.Washington, DC: National Academy Press.

Ghanem, K.Z.; Badawy, I.H. and Abdel-Samam, A,M. (2004). Influence of yoghurt and probiotic yoghurt on the absorption of calcium, magnesium, iron and bone mineralization in rats. Milchwissenschaft. 59:472–5.

Gomez-aldapa, C.; Martinez-Bustos, F.; Figueroa, C.J. and Ondorica, F.C. (1999). A composition of the quality whole corn tortillas made from instant corn flours by traditional or extraction. Int. J. Food Sci. Tech., 34:391-399.

Hancock, R, D. ; Viola, R. (2001). The use of micro-organisms for L-ascorbic acid production: current status and future perspectives. Appl. Microbiol. Biotechnol.56:567–76.

Heaney, R. P., Saito, Y., & Orimo, H. (1994). Effect of casein phosphopeptide on absorbability of co-ingested calcium in normal postmenopausal women. Journal of Bone Mineral Metabolism, 12, 77–81.

Heaney, R. P.; Dowell, M. S.; Rafferty, K. and Bierman, J. (2000). Bioavailability of the calcium in fortified soy imitation milk, with some observations on method. Am. J. Clin. Nutr. 71:1166–9.

Hill, M.J. (1997). Intestinal flora and endogenous vitamin synthesis. Eur.J. Cancer Prev. 6: Suppl 1:S43–5. human health. J Nutr 129 (Suppl):758s–67s.

Igarashi M, Liyama Y, Kato R, Tomita M, Asami N, Ezawa I. Effect of Bifidobacterium longum and lactulose on the strength of bone in ovariectomised osteoporosis model rats. Bifidus. 1994;7:139–47.

Kalu, D.N.; Liu, C.C; Salerno, E.; Hollis, B.; Echon, R.; Ray, M. (1991). Skeletal response of ovarectomized rats to low and high doses of 17 B-estradiol. Bone Miner. 14: 175-187.

Kamaly, K.M. (1997). Bifidobacteria fermentation of soybean milk. Food Research International 30, 675–682.

Kaplan, L.A.; Pesce, A. J (1996). Clinical Chemistry, MosbyEd.

Kotula, A. W.; Twigg, G. G. and Young, E. P. (1976). Evaluation of beef batties containing protein during 12-month frozen storage. J. food Sci. 41:1142-1147.

Kruger, M. C.; Fear, A.; Chua, W. H.; Pilmer, G.G.; Scollum, L. M. (2009). The effect of lactobacillus rhamnosus HN001 on mineral absorption and bone health in growing male and ovarictomised female rats. Dairy Science Technology. Article puplished by EDP science.

Lee, S.Y.; Vedemuthu, E.R. Washam, C.J. and Reinbold, B.W. (1973). An agar medium for the differential enumeration of yoghurt starter J. Milk Food Tech., 37: 272.

Narva, M.; Rissanen, J.; Halleen, J.; Vapaatalo,m H.; Vaananen, K.; Korpela, R. (2007). Effects of Bioactive Peptide, Valyl-Prolyl-Proline (VPP), and *Lactobacillus helveticus* Fermented Milk Containing VPP on Bone Loss in Ovariectomized Rats. Ann Nutr Metab. 51:65–74.

Ohta, A.; Baba, S.; Ohtsuki, M.; Takizawa, T.; Adashi, T.; & Hara, H. (1997). In vivo absorption of calcium carbonate and magnesium oxide from the large intestine in rats. J. Nutr. Sci. vitaminol. , 43: 35-46.

Ohta, A.; Uehara, M.; Sakai, K.; Takasaki. M.; Adlercreutz, H.; Morohashi, T. and Ishimi, Y. (2002). A compination of dietary fructooligosaccharides and isoflavone conjugates increase femoral bone mineral density and equol production in ovariectomized mice. J. Nutr. 132: 2048-2054.

Osler, M.; Heitmann, B.L.; Schroll, M. (1997). Ten year trends in the dietary habits of Danish men and women. Cohort and cross-sectional data. Eur. J. Clin. Nutr. 51:535-541

Parker, M.M.; Humoller, F.L. and Mahler, D.J. (1967). Determination of copper, zinc and other trace elements in biological materials. Clin. Chem., 13: 40.

P´erez-Conesa D, Lopez G, Abell´an P and Ros G, (2006). Bioavailability of calcium, magnesium and phosphorus in rats fed probiotic, prebiotic and synbiotic powder follow-up infant formulas and their effect on physiological and nutritional parameters. *J Sci Food Agric* **86**:2327–2336.

Perez-Conesa, D.; Lopez, G. and Ros, G. (2007). Effects of probiotic, prebiotic and synbiotic follow-up infant formulas on large intestine morphology and bone mineralisation in rats. J Sci Food Agric 87:1059–1068

Recker, R. P.; Bammi, A.; Barger-Lux, J. and Heaney, R. P. (1988). Calcium absorbability from milk products, an imitation milk, and calcium carbaonate. Am. J. Clin. Nutr. 47: 93-95.

Samona, A.; and Robison, R.K. (1991). Enumeration of Bifidobacteria in dairy products. J.of the society of dairy Tech. 44(3), August.

Scholz-Ahrens, K. E.; Berit Marten, P. A.; Weber, P.; Timm, W.; Asil, Y.; Gluer, C. C; and Schrezenmeir, j. (2007). Prebiotics, Probiotics, and Synbiotics Affect Mineral Absorption, Bone Mineral Content, and Bone Structure. J. Nutr. 137: 838S-846S.

Setchell, K.D.R.; Cassidy, A. (1999). Dietary isoflavones: biological effects and relevance to

Setchell, K.D.R.; Lydeking-Olsen, E. (2003). Dietary phytoestrogen and their effect on bone: evidence from in vitro and in vivo observational and dietary intervention studies. Am. J. Clin.Nutr.78 (Suppl):593S–609S.

Shils, M.E., (1999). Modern Nutrition in Health and Disease, 9th edR Williams and Wilkins Inc., Baltimore.

Smith, T. M.; Kolars, J. C. and Savaiano, D. A. (1985). Absorption of calcium from milk and yoghurt. Am. J. Cli. Nutr.

Stanton, C.; Ross, R.P.; Fitzgerald, G.; Van Sinderen, D. (2005). Fermented functional foods based on probiotics and their biogenic metabolites. Curr. Opin Biotechnol. 16:198–203.

Tang, A.L.; Shah, N.P.; Wilcox, G.; Walker, K.Z. And Stojanovska, L. (2007).Fermentation of Calcium-Fortified Soymilk with *Lactobacillus*: Effects on Calcium Solubility, Isoflavone Conversion, and Production of Organic Acids. Journal of Food Science. Vol. 72, Nr. 9.

Tamime, A. Y. and Robinson, R. K.(1985). Yoghurt, science and technology Paramo press, Oxford, England.

Thys-Jacobs, S.; Starkey, P.; Bernstein, D.; Tian, J. (1998). Calcium carbonate and the premenstrual syndrome: effects on premenstrual and menstrual symptomatology. Am. J. Obstet. Gynecol. 179: 444–452.

Tsuchita, H.; Kuwata, T.; Sakamaki, C.; Kuwano, K., Shinoda, S. and Yoshida, T. (1992). Bioavailabilities of calcium, phosphorus and magnesium from whey mineral complex in growing male rats. Z ErnAhrungswiss 31:258-268.

Unal, G.; El, S. N. and Kilic, S.(2005). In vitro determination of calcium bioavailability of milk, dairy products and infant formulas International Journal of Food Sciences and Nutrition, 56(1):13-22.

Van den Heuvel, E.G.H.M, Muijs, T., Van Dokkum, W., and Schaafsma, G. (1999a) Lactulose stimulates calcium absorption in postmenopausal women. J. Bone Miner. Res.14,1211-1216.

Weaver, C.M. (2000). The growing years and prevention of osteoporosis in later life. Proc. Nutr. Soc. 59:303-306.

Wodecki, Z.J.; Torlop, B. and Slebioda, M. (1991). Chromatographic separation of citric acid for monitoring the mould process.J. Chromatogr. 558: 302-305.

Zafar, T.A.; Weaver, C.M.; Jones, K.; Moore, D.R.; Barnes, S. (2004). Inulin effects on bioavailability of soy isoflavones and their calcium absorption enhancing ability. J. Agric. Food Chem. 52:2827–31.

Zemel MB. (2001). Calcium modulation of hypertension and obesity: mechanisms and implications. J Am Coll Nutr. 20:428S–35S.

تـأثير الزبـادى وزبـادى الصـويا المحتويـه عل بكتريـا البيفيدو علـى الاتاحـه الحيويـه للكالسيوم وتكلس العظام فى الفئران

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الاتاحه الحيويه للكالسيوم من اللبن ومصـادر الكالسيوم المختلفه اصبح موضـوع هام فى السنوات الاخيره. واجريت هذه الدراسه لمقارنة الاتاحه الحيويه الكالسيوم من وجبة الاساس المحتويه علـى اللبن البقرى (CM) ,الزبـادى المصنع من اللبن البقرى(YCM) , الزبـــادى المصـــنع مـــن اللـــبن البقـــرى والمحتـــوى علـــى بكتريـــا *B.lactis* Bb-12 or *B.longum* Bb-46 (YCMBb-12 or YCMBb-46), او المحتويـه علـى لبن الصـويا غير المتخمر(NFSM) او زبـادى الصـويا المتخمر بواسطة *B.lactis* Bb-12 or *B.longum* Bb-46 (YSMBb-12 or YSMBb-46) او وجبة الاساس (Control). النتائج المتحصل عليها تشير الـى انـه لاتوجد اختلافات معنويـه فـى الغـذاء المـأكول , زيـادة وزن الجسـم وكفـاءة التغذيـه بـين المجـاميع المختلفـه للفئـران بالمقارنه بالكونترول. اثناء فترة التجربه , التغذيه على الوجبات المحتويـه علـى البروبيوتيك تزيد معنويا تركيز الكالسيوم فـى السيرم و تقلل معنويـا الكالسيوم المفرز فـى البول مقارنـة بـالكونترول. كمـا ازدادت نسـبة الامتصـاص الظـاهرى للكالسيوم ايضـا. ايضـا لـوحظ زيـادة تركيز الاحماض الكربوكسيليه وزيادة اعداد بكتريا البيفيدو فـى محتـوى ال caecum فـى مجاميع الفئران المغذاه على البروبيوتيك. كما ينخفض caecum pH فى هذه المجـاميع. ولهذه القياسـات علاقـه ايجابيـه بالاتاحـه الحيويـه للكالسيوم.ايضـا يـزداد محتـوى عظـام الفخذ والسـاق مـن الكالسـيوم وهكـذا تـزداد كثافـة وقـوة كسـر هـذه العظـام وذلـك بالتغذيـه علـى البروبيوتيـك. ومـن امفيـد ملاحظـة ان الوجبـات المحتويـه علـى *B.lactis* Bb-12 (YCMBb-12) كانـت هـى الاكثـر تـأثيرا , و بصـفة عامـه فـان الزبـادى البروبيوتيـك المصنع من اللبن البقرى كان افضل من زبادى الصويا فى تحسين الاتاحه احيويه للكالسيوم. وهكذا فان نتائج هذه الدراسه تثبت االتأثيرات المفيده للزبادى المصنع من اللبن البقرى او من لبن الصويا والمحتويه على البروبيوتيك فى تحسين الاتاحه الحيويه للكالسيوم وبالتـالى تقليل فقد الكالسيوم من الجسم.