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EFFECT OF PRO AND PRE –BIOTIC SUPPLEMENTATIONS IN WEANING FOODS ON SOME MINERAL ABSORPTION

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ABSTRACT

The purpose of the present study was to examine the potential synergistic effect of prebiotics and probiotics combination (synbiotics) on mineral absorption and bone strength in weaning ages. Forty two of Western strain male rats aged two months were divided into 7 treatment groups of equal mean weights 60±5 g (6 rats/ group) as follows: group 1: fed on basal diet (reference group), group 2: fed on baby cereals food (control group),group 3: fed on baby cereals food supplemented with *Bifidobacterium bifidum* (Bb) at a concentration of 8.95 log cfu/g, group 4: fed on baby cereals food supplemented with dried powder of Jerusalem artichoke tubers (Ja) at a concentration of 5%, group 5: fed on baby cereals food supplemented with Ja at a concentration of 5%+ Bb at concentration of 8.95 log cfu/ g, group 6: fed on baby cereals food supplemented with Ja at a concentration of 10%, and group 7:fed on baby cereals food supplemented with Ja at a concentration of 10% + Bb at concentration of 8.95 log cfu/g. The experimental period lasted 5 weeks. At the fifth week, a controlled metabolic study was carried out.

The obtained results indicated that bifidobacterial count was stable during storage for 6 months especially in the formula which contains 10% of Ja. Baby foods supplemented with prebiotic and / or probiotic increased the absorption and the retention of Ca and P especially at high dose of prebiotic (100 g/kg diet) and combined with bifidobacteria (group 7). Mg balance and retention showed marked improvement in all treatment groups with highest values in groups 5 and 7. Concerning mineral contents in femurs, there were significant increases in all treatment groups with higher values in group 7 for Ca and Mg and in groups 5 and 7 for P relative to the control group or reference group.

In conclusion, supplementation the baby cereal foods with bifidobacteria and Jerusalem artichoke as a source of inulin had a beneficial effect on mineral absorption and retention which have a positive impact on bone strength, particularly at 10 % of Jerusalem artichoke.

Key words : *Bifidobacterium bifidum, inulin ,mineral absorption , synbiotics.*

1. INTRODUCTION

In recent years, there has been a significant increase in research on the characterization and verification of potential health benefits associated with the use of probiotics and prebiotics. Micro-organisms most commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria (*Lactobacillus, Enterococcus*,) and to the genus *Bifidobacterium* (FAO/WHO, 2001). There is a strong evidence indicating that probiotics have preventive and therapeutic effectiveness on pathologies in pediatrics such as acute diarrhea (Dutta *et al.* 2011), and allergic pathology (Hurre *et al.* 2008), constipation (Nader and Youssef, 2007), inflammatory bowel disease (Steed *et al.,* 2010), *Helicobacter pylori* infection (Sykora *et al.,* 2004).

Prebiotic are non-active food constituents that are non-digestible by digestive enzymes in humans and shift to the colon and are then selectively fermented. The benefit to the host is mediated during selective stimulation of the growth and/or activity of one or a limited number of bacteria. The essential end components of carbohydrate metabolism are short chain fatty acids, particularly acetic acid, propionic acid and butyric acid, which are used by the host organism as an energy source (Al-Sheraji *et al. (*2013). Lactulose, galacto-oligosaccharides, fructooligosaccharides, inulin and its hydrolysates, maltooligosaccharides, and resistant starch are prebiotics normally used in the human diet.

Numerous studies have shown that some prebiotics, alone or in blends, have health benefits and reduction of several diseases such as allergies in infants, colorectal cancer, hypercholesterolemia, hyperlipidemia, diabetes and obesity (Al-Sheraji*et al.*,2013).

Some plants are considered good source of prebiotics such as inulin. Inulin occurs as an energy reserve in various plants, in particular in those of composite family such as chicory, Jerusalem artichoke tubers and dahlia (Leclercq and Hageman, 1993). Jerusalem artichoke tubers (*Helianthus tuberosus*) is considered one of the most interesting potential source of fructose. Its importance was related first to its high content of inulin (Chabbert *et al.*, 1983).

Actually, the mixtures of probiotics and prebiotics are often used in food products in order to take advantage of their synergic effects by both ensuring survival of delivered probiotics and stimulating the growth of selected indigenous bacteria. Thus, these mixtures are called synbiotics (Al-Sheraji*et al.*, 2012 a and b).

Breastfed infants are generally healthier than formula-fed infants, especially with respect to their ability to fend off infections. Some of the health benefits of human milk have been attributed partly to factors that modulate the development of a normal gut microbiota.These factors called human milk oligosaccharides (HMOS) (Newburg, 2005).

Hence, the development of improved infant formulas has focused on emulating the beneficial effects of breast milk by, among other approaches, supplementing formulas with specific probiotics or oligosaccharides and inulin (prebiotics) that selectively stimulate the growth or metabolic activity of potentially beneficial indigenous bacteria such as bifidobacteria (Chouraqui *et al.*, 2008).

Several studies suggested that food supplements containing probiotic bacteria and /or prebiotics have beneficial effects on mineral absorption, Ca and Mg (Demigné *et al.*, 2008) as well as Fe (Tako *et al.*, 2008).

So, the present study was conducted in order to evaluate if baby food cereals fortified with bifidobacteria alone or with Jerusalem artichoke as a source of inulin (prebiotic) have the positive effect on Ca, Mg and P absorption and their bioavailability in bones.

2. MATERIALS AND METHODS

2.1. Preparing the formulas of baby cereals

- All materials used for processing of formulas of baby cereals were taken from the Arab company for medicinal food factory. Ismailia, Egypt.
- The ingredients of baby cereal formulas are shown in Table (1).
- *Bifidobacterium bifidum* (Bb-12) were obtained from Chr.Hansn-Denemark. (Freeze- dried Bifido Bactria were prepared according to the method of Saarela *et al.* (2006).
- Jerusalem artichoke tubers (*Helianthus tuberosus*) were obtained from Horticultural Researh Institute, Agricultural Research Center, Dokki,Giza.
- All formulas were prepared and dried using Drum drier according to the method of (Perez–Conesa *et al*., 2002). After drying the formulas, thebifidobacteria were added under a septic condition. The end products were packaged in five layer bags with modified atmosphere under nitrogen gas.

2.2.Chemical analysis of baby cereal formulas

- The chemical compositions of different formulas of baby cereals were determined according to AOAC (2005).
- The inulin content was extracted and

Table (1): The composition of baby cereal formulas.

Ingredients %	F1	F 2	F3	F4	F5	F6
Rice flour	**70**	**67**	**65**	**62**	**60**	**57**
Skimmed milk	**8**	**8**	**8**	**8**	**8**	**8**
Soy isolate	**5**	**5**	**5**	**5**	**5**	**5**
Lecithin	**1**	**1**	**1**	**1**	**1**	**1**
Sun flower oil	**3**	**3**	**3**	**3**	**3**	**3**
Jerusalem artichoke	**-**	**-**	**5**	**5**	**10**	**10**
Freeze dried Bifido bacteria*	**-**	**3**	**-**	**3**	**-**	**3**
Tri calcium phosphate	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**
Sugar	**8**	**8**	**8**	**8**	**8**	**8**
Vitamins and Minerals mixture	**4.7**	**4.7**	**4.7**	**4.7**	**4.7**	**4.7**
Total	**100**	**100**	**100**	**100**	**100**	**100**

***3 g of** *Bifidobacterium bifidum* **contain 8.95log cfu/g.**

determined by HPLC according to the method of Molnar-Perl (2000).

2.3. *Bifidobacterium bifidum* count

Bifidobacterium bifidum (Bb-12) counts were determined according to Dave and Shah (1996), using modified MRS agar supplemented with 0.05% L-cysteine and 0.3% lithium chloride. The plates were anaerobically incubated at 37°Cfor 48 hrs

2.4. Biological assay

Forty two of the Western strain male rats aged two months with an initial body weight of approximately 60±5 g were housed in an animal house of Ophthalmology Research Institute at 12-h light/dark cycle in a temperature and humidity-controlled room. The animals were allowed free access to feed on basal diet (BD) based on AIN -93G formulation with some modification (Reeves *et al.,*1993) and water *ad libitum* in the acclimatization period (one week). After this period, the animals were divided equally into 7 treatment groups of equal mean weights (6 rats/group) as follows :

- Group 1 fed on BD[1] (reference group).
- Group 2 fed on baby cereal formula (formula 1) as shown in Table (1), control group
- Group 3 fed on baby cereal formula supplemented with *Bifidobacteriumbifidum* (Bb) at concentration 8.95 log cfu/g (formula 2).
- Group 4 fed on baby cereal formula supplemented with dried powder of Jerusalem artichoke tubers at concentration 5% (formula3).
- Group 5 fed on baby cereal formula supplemented with dried powder of Jerusalem artichoke tubers at concentration 5%+ Bb at concentration 8.95 log cfu/g (formula 4).
- Group 6 fed on baby cereal formula supplemented with dried powder of Jerusalem artichoke tubers at concentration 10% (formula 5).
- Group 7 fed on baby cereal formula supplemented with dried powder of Jerusalem artichoke tubers at concentration 10% + Bb at concentration 8.95 log cfu/g (formula 6).

The experimental period continued for 5 weeks. During the first four weeks, water was allowed *ad-libitum*, the body weight was recorded weekly and the food intake was recorded each two days. At the fifth week, a controlled metabolic study was carried out. Through this period, daily food intake was recorded. At the same time, daily excretions of stool and urine were collected. The total urine volume was recorded at the end of the study. The stool and urine were frozen immediately after the daily collection.

At termination of the experiment, the diet of each group was removed 6 h before anethesing the rats. Blood samples were collected from the orbit ocular vein.The serum was separated by centrifugation at 4°C (3000 rpm for 10 min) and stored frozen at -20°C for subsequent chemical analyses. The caecum was separated immediately and weighed, its contents collected after cutting off the caecal wall and weighed. Weight of total caecum and its contents were calculated relative to body weight. The caecal contents were diluted with four times of deionized water and homogenized by homogenizer. The pH of homogenate was measured by pH meter. Right and left Femurs were dissected, cleaned, freed from the surrounding tissues and filled in physiological saline and stored at−20 °C.

2.4.1.Determination of serum and urine chemistry

Serum and urine of the total calcium and phosphorus were estimated calorimetrically by the method given by Weatherburn *et al.* (1982) as cited in Linear Chemicals. Serum and urine magnesium were estimated according to the method of Bohuon *et al.* (1962) as cited in Quimica Clinica Aplicada.

2.4.2. Determination of minerals in bones, diet and feces

Dry femurs were ashed in muffle furnace at 600. The ash was dissolved in HCl (3.0 ml) then evaporated to about 1.0 ml on a hot plate. After cooling, it was made up to 25 ml with deionized water. Portions of the dry sample (0.35 of each diet or feces) were ashed at 600°C for 10 h then dissolved in concentrated nitric acid (3.0 ml) and hydrogen peroxide (1.0 ml) at 130°C until discoloration. Final volume (25 ml) was made by 3 % nitric solution. The concentrations of each element in prepared samples of bone, feces and diets were measured by flame atomic absorption spectrophotometer AAS (Model3300,

[1] BD ingredients (g/100 g diet) consist of : Casein 20 (13 % protein) ,corn oil 9,cellulose 5,mineral mix 3.5,vitamin mix 1,sucrose 10 ,L-cysteine 0.18,choline bitartrate 0.25 corn starch 51 (based on AIN -93G formulation).

Perkin-Elmer, Beaconsfield, UK) according to the method of Robberecht *et al.(*1994).

Mineral apparent absorption, balance, retention and dietary mineral efficiency were calculated according to Scholz-Ahrens *et al.* (2002) using the following equations:

$$\textbf{Mineral apperent absorption\%} = \frac{(\textbf{Daily mineral intake} - \textbf{daily fecal})\textbf{x100}}{\textbf{Daily mineral intake}}$$

$$\textbf{Mineral balance} = \textbf{daily mineral intake} - (\textbf{fecal mineral} + \textbf{urinary mineral})$$

$$\text{Mineral retention \%} = \frac{\textbf{mineral balance x 100}}{\textbf{daily mineral intake}}$$

$$\text{Dietary mineral efficiency\%} = \frac{\textbf{mineral content x 100}}{\textbf{total mineral intake}}$$

2.5. Statistical Analysis

Data were presented as mean ±S.E. and analyzed by one-way analysis of variance (ANOVA) and Duncan's test ($P \leq 0.05$) were used to establish the significance of differences according to the method of Snedecor and Cochran (1989). The result was performed using the SPSS software version 19 windows program.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of baby cereals

The chemical compositions of baby cereal prepared from different formulas were analyzed and the data showed in Table (2). From the data in the table, inulin content was 3.2 % in F3 and F4, while 6.3 % in F5 and F6. This result indicates that the concentration of inulin in Jerusalem artichoke was high (about 64 % on dry matter) compared to other plants such as onion, leek, garlic and artichoke (Deleenheer, 1996). The other chemical composition carbohydrates, protein, fat, fiber, ash and moisture were slightly non- significant affected by adding Jerusalem artichoke in all formulas. The chemical composition of the formulas was agreed with the Egyptian infant food based from cereals standard 3284/2005.

3.2. Storage stability of Bifidobacteria on baby cereals formulas

The storage actual stability of the different formulas of baby cereals with bifidobacterium was studied for 6 months. The bifidobacterial count was 8.95(log cfu/g) at zero time and after 6 months, it ranged from 7.81-8.46 (log cfu/g). The results indicated that the bifidobacterial count was stable during storage for 6 months especially in the formula No. 6 which contained 10% of Jerusalem artichoke. These results are in agreement with Gibson and Wang (1994) who stated that in pure cultures; most species of bifidobacteria are adept at the use of inulin-type fructans**.**

3.3. Body weight gain and feed efficiency

As shown in Table (3), supplementation of baby foods with bifidogenic Jerusalem artichoke (inulin) maintained the normal growth rate and feed efficiency as the control group or the reference group. The obtained results agree with the previous results who reported that no significant differences were found neither in the daily feed intake nor in the body weight gain between the control group and those fed on diets contained inulin or resistant starch as reported by Younes *et al.* (2001).

3.4. Fecal bulk and caecal weight and pH

As shown in Table (4), the fecal excretion was significantly increased in all the treated groups except group 3 supplemented with bifidobacteria only in comparison to the control group. The highest value (9.26 g) was recorded for group 7 that consumed formula containing

Table (2): Chemical composition of different formulas of baby cereals per 100g on dry weight basis.

Material (%)	F1	F2	F3	F4	F5	F6
Moisture	5	4.8	5	4.8	4	4.8
Dry matter	95	95.2	95	95.2	96	95.2
Protein	13.0	12.92	12.90	12.91	12.80	12.82
Fats	9	9.0	8.98	8.97	8.98	8.96
Ash	4.5	4.5	4.7	4.7	5.0	5.0
Crude fiber	1.5	1.48	1.65	1.66	1.8	1.79
Total carbohydrates*	72	72.1	71.77	71.76	71.42	71.43
Inulin	-	-	3.2	3.2	6.3	6.3

F1: control baby food formula **F2:control baby food formula +Bb**
F3:control baby food formula +5%Ja. **F4:control baby food formula+Bb+5%Ja.**
F5:control baby food formula+ 10% Ja . **F6:control baby food formula +Bb+ 10% Ja.**
***Total carbohydrates calculated by difference according to the following equation:**
Total carbohydrates % =100 - (protein% +fat %+ ash %+ crude fiber %) on dry weight matter.

Table (3): The effect of different baby cereal formulas on body weight gain, feed intake and feed efficiency.

Treated groups	B.W gain (g)	Daily B.W gain (g/d)	Daily feed Intake (g/d)	Feed Efficiency (g/g)
G1	41.33 c ± 0.67	1.47 b ±0.02	11.83 a ±0.03	0.12 bc ±.003
G2	43.00 bc ± 1.73	1.53 ab ±0.06	12.83 a ±0.01	0.12 abc ±0.005
G3	40.00 c ±1.15	1.42 b ±0.04	12.85 a ±0.03	0.11 c ±0.003
G4	47.33 ab ±1.20	1.68 a ±0.04	12.83 a ±0.01	0.13 ab ±0.005
G5	47.33 ab±1.76	1.68 a ±0.06	12.83 a ±0.01	0.13 ab ±0.006
G6	48.66 a ±0.88	1.73 a ±0.03	12.84 a ±0.01	0.13 a ±0.003
G7	45.33 abc ±3.17	1.61 ab ±0.11	12.84 a ±0.01	0.12 abc ±0.008

Different letters in the same column denote statistical significance (P<0.05).

Table(4): The effect of different baby cereal formulas on fecal bulk and weight% and pH of cecum.

Treated groups	Fresh Fecal Weight (g)	Fecal Moisture (%)	Total caecal Weight %	Caecal pH
G1	8.53 ab ±0.03	26.00 cd ±1.00	0.35 e ±0.01	7.13 a ±0.12
G2	4.56 d ±0.49	23.83 cd ±1.64	0.62 d ±0.01	6.93 ab ±0.08
G3	3.66 d ±0.27	20.40 d ±2.13	0.78 d ± 0.08	6.80 bc ±0.17
G4	6.13 c ±0.12	28.83 bc ±2.80	0.98 b ±0.03	6.56 cd ±0.03
G5	7.73 b ±0.66	28.00 bc ±3.60	0.95 b ±0.08	6.30 de ±0.05
G6	9.10 a ±0.20	35.00 b ±2.51	1.0 a ±0.08	6.10 ef ±0.05
G7	9.26 a ±0.26	47.66 a ± 1.33	1.36 a ±0.01	5.83 f ±0.03

Different letters in the same column denote statistical significance (P<0.05).

bifidobacteria + 10% Ja. Fecal moisture percent was significantly increased in groups 6 and7 as compared with the control group. The present results are in a good agreement with earlier reports which confirmed that non-digestible carbohydrates increased the fecal bulking (wet weight and moisture percentage of feces) due to increase in the biomass (Pascoal *et al.,* 2005).

The results of the total caecal weight %, (relative to body weight) and caecal pH are shown in Table (4). It was clearly showed that the consumption of the formula were containing Ja 5% or 10% was associated with a significant increase in the total caecal weight. Moreover, this increase was obvious when probiotic bacteria were combined with 10% Ja in group 7. In contrast, the caecal pH tended to decrease significantly when the weaning formula was supplemented with Ja and bifidobacteria. Group 7 recorded the lowest value in caecal pH. This result suggests that the increase in caecal weight was due to wall thickening and that the colonization of the caecal with bifidobacteria was successful in response to long term feeding of bifidogenic inulin. Our results agree with the previous studies which demonstrated that non-milk oligosaccharides from inulin are able to mimic the prebiotic effects of breast milk by significantly increasing *Bifidobacterium* spp., by increasing the production of short chain fatty acids (SCFA) and lowering caecal pH (Kim *et al.,* 2007).

3.5. Absorption, balance and retention of calcium

As shown from the obtained data in Table (5), in the case of serum Ca, feeding on baby formulas supplemented with 10% Ja without or with probiotic recorded the highest mean values (groups 6 and 7) and were significantly higher than the control group. Fecal Ca excretion was decreased significantly in rats fed on formulas supplemented with bifidobacteria and / or Jerusalem artichoke except group 4 with the lowest value recorded in group 7 relative to control formula. Also, group 7 showed a significant increment in apparent Ca absorption percentage. Concerning the urinary Ca excretion, no statistical differences were found in the groups 5 and 6, but groups 3, 4 and 7 showed significant differences when compared with the control group (G2). There was a marked increment of Ca balance and retention % in the treatments of groups 5, 6 and 7 which recorded the highest mean value. It is suggested that the diet supplemented with prebiotic and / or probiotic may be increased the absorption and retention of Ca especially at high dose of prebiotic (100 g/kg diet) and combined with Bifidobacteria. The trend observed was consistent to the results of Gopal *et al.,* (2003) Table (5): The effect of baby cereal formulas on absorption, balance and retention of calcium.

Treated groups	Serum Ca (mg/dl)	Ca Intake (mg/d)	Fecal Ca (mg/d)	Apparent Ca Absorption (mg/d)	Apparent Ca Absorption (%)	Urinary Ca (mg/d)	Ca balance (mg/d)	Ca retention %
G1	7.66 b ±0.37	205.00 c ±2.88	110.00 b ±2.86	95.00 cd ±2.88	46.34 b ±3.46	1.40 cd ±0.34	93.60 cd ±3.75	45.66 b ±3.17
G2	7.61 b ±0.25	214.00 abc ±3.46	125.00 a ±1.73	89.00 d ±3.46	41.59 b ±4.04	2.20 a ±0.23	86.80 d ±2.60	40.56 b ±2.88
G 3	8.37 ab ±0.22	206.66 bc ±1.66	105.00 b ±2.88	101.00 bc ±3.46	49.03 b ±1.85	0.92 d ±0.078	100.74 bc ±2.88	48..75 b ±3.45
G 4	8.25 ab ±0.25	208.33 abc ±2.88	120.00 a ±2.88	88.33 d ±2.18	42.40 b ±2.88	1.27 d ±0.15	86.73 d ±3.46	41.70 b ±2.88
G 5	8.16 ab ±0.25	212.00 abc ±2.88	106.33 b ±3.48	105.67 b ±4.48	49.84 b ±3.46	1.50 bcd ±0.28	104.17 b ±3.46	49.14 b ±2.88
G 6	8.56 a ±0.20	215.00 ab ±2.88	105.00 b ±2.85	110.00 b ±2.88	51.16 ab ±3.48	2.10 ab ±0.28	107.90 b ±2.88	50.19 b ±2.74
G 7	8.77 a ±0.15	216.00 a ±2.30	85.00 c ±2.88	131.00 a ±2.88	60.65 a ±2.88	0.95 d ±0.05	130.05 a ±2.88	60.21 a ±2.88

Different letters in the same column denote statistical significance ($P<0.05$).

who suggested that synbiotic stimulated the fecal bacteria counts of *Lactobacilli* and *Bifidobacteria* in human subject, but little effect was seen when only the probiotic (*Bifidobacteriumlactis* HN019) or the prebiotic (galacto-oligosacchrides) was given. The explanation contributed to the enhanced mineral absorption is the trophic effect of prebiotics on the gut (cell growth and functional enhancement of the absorptive area) (Raschka and Daniel, 2005).

3.6. Absorption, balance and retention of magnesium

As shown in Table (6), no significant differences were observed in serum levels of Mg between the treated groups and the control group. Supplementation of baby food with bifidobacteria and/or Jerusalem artichoke as a source of inulin did not affect the daily Mg intake, while fecal Mg showed a significant decreasing compared to the control (G2) or the reference group(G1). This was accompanied with significant increasing in apparent Mg absorption values (absolute or percent) of the treated groups. Although the treated groups (4, 5, 6 and7) recorded higher values of urinary Mg excretion relative to the control, but Mg balance and retention showed marked improvement in all treated groups with the highest values in rats supplemented with prebiotics and probiotics (groups 5 and 7). The present data were confirmed by Demigné *et al.* (2008) who reported that, in rat model, both native inulin and reformulated inulin exerted similar effects as to caecal fermentation by the production of short-chain fatty acids (SCFA), especially butyric acid and stimulation of Ca and Mg digestive absorption and affect the bone mineral density.

3.7.Absorption, balance and retention of phosphorus

The data presented in Table (7) illustrated that group 7 only showed the pronounced increment in serum P. Supplementation the baby formula with prebiotic and/or probiotic did not affect significantly the fecal P excretion values and apparent P absorption percentage. In contrary, urinary excretion of P decreased significantly in all treatment groups except group 4 as compared to rats fed on the control formula. Feeding on baby formulas with *bifidobacteria* and/or inulin caused increment in P balance and retention with higher value in group 7. These data are in line with those given by Rideout and Fan (2004) who reported that in growing pigs, inulin had no effect on P absorption but reduced urinary P excretion. Another study on rats revealed significantly higher P retention at the medium dose of oligofructose (50 g/kg) after 8 weeks and at the high dose oligofructose (100 g/kg) after 4 weeks. This effect was mediated by a reduction of urinary P excretion (Scholz-Ahrens and Schrezenmeir, 2007).

3.8. Mineral contents in femurs

As shown in Table (8), calcium and phosphorus contents in femurs showed significant increases in the treated groups especially group 7 for Ca and groups 5 and 7 for P relative to the control group or the reference

Table (6): The effect of different weaning cereal foods on absorption, balance and retention of magnesium.

Treated groups	Serum Mg mg/dl	Mg Intake (mg/d)	Fecal Mg (mg/d)	Apparent Mg Absorption (mg/d)	Apparent Mg Absorption (%)	Urinary Mg (mg/d)	Mg balance (mg/d)	Mg retention %
G1	2.69 a ±0.22	10.26 a ±0.80	5.93 b ±0.41	4.33 b ±0.69	42.20 d ±1.16	0.15 c ±0.015	4.18 bc ±0.61	40.74 e ±1.43
G2	2.70 a ±0.04	10.60 a ±0.37	6.98 a ±0.75	3.62 b ±0.37	34.15 e ±1.73	0.41 bc ±0.07	3.21 c ±0.15	30.28 f ±1.45
G3	2.76 a ±0.04	11.20 a ±0.60	4.15 c ±0.51	7.05 a ±0.86	62.95 c ±4.05	0.19 c ±0.01	6.86 a ±0.92	61.25 b ±1.45
G4	2.55 ab ±0.02	11.66 a ±1.06	4.12 c ±0.23	7.54 a ±0. 83	64.67 c ±1.27	1.43 a ±0.14	6.11ab ±0.34	52.40 d ±2.00
G5	2.58 a ±0.19	12.33 a ±0.88	3.50 d ±0.43	8.83 a ±1.17	71.61 ab ±1.27	1.10 a ±0.28	7.73 a ±0.82	62.69 b ±0.90
G6	2.53 ab ±0.05	12.46 a ±1.29	4.22 c ±0.23	8.24 a ±1.01	66.13 bc ±1.07	1.18 a ±0.13	7.06 a ±1.06	56.66 c ±1.32
G7	2.91 a ±0.06	11.90 a ±0.49	3.13 d ±0.45	8.77 a ±0.69	73.70 a ±1.45	0.65 b ±0.11	8.12 a ±0.18	68.24 a ±0.96

Different letters in the same column denote statistical significance ($P<0.05$).

Table (7): the effect of different baby cereal formulas on absorption, balance and retention of phosphorus.

Treated groups	serum P (mg/dl)	P Intake (mg/d)	Fecal P (mg/d)	Apparent P Absorption (mg/d)	Apparent P Absorption (%)	Urinary P (mg/d)	P balance (mg/d)	P retention %
G1	8.69 d ±0.17	91.66 c ±4.41	40.00 a ±2.88	51.66 d ±2.02	56.36 b ±0.88	0.70 d ±0.17	51.96 e ±3.78	55.60 d ±0.88
G2	9.81 bc ±0.46	98.33 bc ±1.66	28.66 bc ±0.66	69.67 d ±1.20	71.85 a ±3.78	6.86 b ±0.84	62.81d ±1.76	63.88 c ±2.31
G3	9.20 c ±0.05	110.33 ab ±2.88	25.00 c ±1.15	85.33 ab ±2.90	77.34 a ±1.45	4.36 c ±0.16	80.97ab ±1.20	73.39 ab ±2.40
G4	9.11 c ±0.26	112.00 a ±2.31	31.33 b ±0.66	80.67abc ±1.20	72.03 a ±1.15	8.00 a ±0.42	72.67 bc ±1.20	64.88 c ±1.15
G5	9.61 bc ±0.22	105.66 ab ±6.88	31.00 b ±3.05	74.66 cd ±2.88	70.66 a ±1.15	3.60 c ±0.30	71.06 cd ±4.33	67.25 bc ±1.52
G6	10.08 b ±0.13	110.00 ab ±2.88	31.33 b ±1.33	78.67 bc ±2.90	71.52 a ±4.16	4.33 c ±0.88	74.34 bc ±3.17	67.58 bc ±2.31
G7	11.47 a ±0.78	112.00 a ±2.88	23.33 c ±1.66	88.67 a ±3.75	79.17 a ±2.88	2.66 c ±0.33	86.01 a ±2.88	76.79 a ±2.88

Different letters in the same column denote statistical significance ($P<0.05$).

Table (8): The effect of baby formulas on, Ca, Mg and P content in femurs and dietary mineral efficiency percent.

Treated groups	Ca (mg/f)	Mg (mg/f)	P (mg/f)	Dietary Ca efficiency %	Dietary Mg efficiency %	Dietary P efficiency %
G1	184.33 c ±1.85	3.57 e ±0.01	37.66 cb ±0.33	3.21 c ±0.01	1.24 e ±0.02	1.47 c ± 0.01
G2	181.33 c ±1.66	4.04 d ±0.15	35.33 c ±2.33	3.03 d ±0.01	1.36 cd ±0.003	1.28 e ± 0.003
G3	200.00 b ±3.05	4.09 d ±0.098	40.33 b ±0.88	3.46b ±0.01	1.30 d ±0.003	1.31d ± 0.005
G4	204.66 ab ±6.96	4.62 c ±0.30	41.33 b ±1.45	3.51 b ±0.01	1.42 b ±0.005	1.32 d ± 0.01
G5	205.33 ab ±0.88	4.95 ab ±0.16	49.33 a ±1.33	3.46 b ±0.01	1.43b ±0.005	1.67a ± 0.003
G6	207.66 ab ±3.66	4.94 b ±0.34	41.66 b ±1.20	3.45 b ±0.02	1.42b ±0.005	1.35 d ± 0.01
G7	215.66 a ±6.09	5.42 a ±0.26	50.00 a ±0.57	3.57 a ±0.03	1.63 a ±0.005	1.60 ab ± 0.003

Different letters in the same column denote statistical significance ($P<0.05$).

group. Concerning magnesium content in femurs, there were marked increasing in groups 5, 6 and 7 with higher value observed in group 7. These results are confirmed by the results of dietary Ca, P and Mg efficiency percentage, where the treated groups showed significant increases, particularly group 7 for Ca and Mg and groups 5 and 7 for P as compared to control group (Table 8). The present study is in agreement with the previous studies which revealed that although there were no significant differences in ash content, but femur Ca and Mg content, dietary Ca and Mg efficiency increased significantly among rats consuming diets containing inulin or fructooligosaccharides (Mabrok, 2006). Hirama *et al*. (2003) reported that high phosphorus content in the surface of cortical bone at the femoral neck was observed in rats after feeding diet containing indigestible carbohydrates. Because butyrate is the major fermentation product of inulin, whereas mostly acetate and lactate are produced on short-chain inulin-type fructans and butyrate is a potent stimulator of calbindin D9K expression and thus of the active calcium absorption pathway mediated *via* the increase in 1, 25 di-hydroxycholicalciferol receptor binding activity (Abrams *et al.,* 2005).These findings can explain the advantage of inulin over oligofructose with respect to bone mineralization *via* this mechanism (Rossi *et al.,* 2005).

In conclusion, supplementation the baby cereal foods with bifidobacteria and Jerusalem artichoke as a source of inulin had a beneficial effect on Ca, Mg and P absorption and retention, particularly at 10 % of Jerusalem artichoke. Consequently, a positive impact on mineral deposition in bone was observed in baby ages. Further studies are needed with other sources of indigestible carbohydrated and other types of probiotics.

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تأثيرامداد أغذية الفطام بالمدعمات الحيوية ومنشطاتها على امتصاص بعض المعادن

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ملخص

كان الهدف من هذه الدراسة اختبار التأثير المتعاون بين المدعمات الحيوية (البروبيوتك) ومنشطاتها (البريبيوتك) على امتصاص بعض المعادن وتمثيلها فى العظام في سن الفطام. تمت التجربة علي 42 فاراً من الذكور يتراوح اعمارها حدود شهرين وتم تقسيمها الي 7 مجموعات وكل مجموعة تحتوي علي 6 فئران وتم تغذيتها كالاتي:

المجموعة الاولي: غذيت علي الوجبة الأساسية (مجموعة مرجعية).

المجموعة الثانية: غذيت علي اغذية الأطفال المصنعة من الحبوب (المجموعة الضابطة).

المجموعة الثالثة: تم تغذيتها علي اغذية الاطفال المدعمة ببكتيريا البفيدوبكتيريم .

المجموعة الرابعة: تم تغذيتها علي اغذية الاطفال المدعمة بدقيق الطرطوفة بتركيز 5%.

المجموعة الخامسة: تم تغذيتها علي اغذية الاطفال المدعمة بدقيق الطرطوفة 5% بالاضافة الي بكتيريا البفيدوبكتيريم.

المجموعة السادسة: تم تغذيتها علي اغذية الاطفال المدعمة بدقيق الطرطوفة 10% .

المجموعة السابعة: تم تغذيتها علي اغذية الاطفال المدعمة بدقيق الطرطوفة 10% بالاضافة الي بكتيريا البفيدوبكتيريم.

استمرت التجربة لمدة 5 اسابيع و في الاسبوع الخامس اجريت دراسة التحكم في عملية التمثيل الغذائي .

اشارت النتائج الي ان عدد بكتيريا البفيدوبكتيريم انخفض قليلا اثناء التخزين لمدة 6 اشهر وهذا الانخفاض كان غير معنوى وكانت تركيبة الاغذية المدعمة بنسبة 10% من دقيق الطرطوفة الأكثر ثباتاً.

كما اسفرت النتائج علي ان تدعيم اغذية الاطفال بالبروبيوتك والبريبيوتك زود من معدل امتصاص عنصر الكالسيوم والفوسفور والاحتفاظ بهما وخاصة عند تركيز 10% من البريبيوتك + البفيدوبكتيريم (مجموعة7) وكان اتزان والاحتفاظ بعنصر الماغنسيوم كان واضحا بكل المجاميع المعالجة و أعطي اعلي قيم في مجاميع5 ,7.

وبالنسبة لمحتوي المعادن في عظمة الفخذ إتضحت بعض زيادات معنوية في كل المجاميع المعالجة وأعطي أعلي قيمة لعنصراً الماغنسيوم والكالسيوم في مجموعة 7 اما عنصر الفوسفور فقد اعطي أعلي قيمة في المجاميع 5 و7 بالمقارنة بالمجموعة القياسية.

نستنتج من خلال هذه الدراسة ان تدعيم اغذية الاطفال المصنعة من الحبوب ببكتيريا البفيدوبكتيريم ودقيق الطرطوفة كمصدر جيد للانيولين يعتبران ذى تاثير نافع في امتصاص المعادن التي لها تاثير ايجابي علي بناء العظام في مرحلة الفطام.