EFFECT OF DIFFERENT LEVELS OF PROTEIN AND CALCIUM ON GROWTH, EFFICIENCY OF FOOD AND PROTEIN UTILIZATION AND BODY COMPOSITION OF RATS

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The effect of adding varying levels of calcium carbonate to six semi synthetic diets containing high and low level of protein has been investigated. It was found that at low level of protein intake the addition of calcium does not improve growth and body performance of rats. At a higher protein intake there is a significant improvement in the growth and body performance by increasing the level of calcium in the diet. Protein content of the rats on different dietary regimens remains the same while body water and fat content varies. The significance of these findings in relation to food enrichment programme in Pakistan is discussed.

Introduction

The diets of the people of Indo-Pakistan subcontinent, particularly of those living in rice eating areas have been found to be low in calcium. Suggestions have, therefore, been made to augment the supplies of calcium from other sources to improve the nutritional standard of the people inhabiting these areas. [1,2] Work carried out in India points to the beneficial effect of calcium supplementation [3-6] but in these studies comparatively less attention has been paid to the protein content of the diet, which is known to play an important role in the utilisation of calcium. It has been shown that protein deficient diets greatly reduce calcium absorption and mineral deposition.[7-9] Ali and Miller[10] carried out Net Protein Utilization (N.P.U.) studies on rice and wheat-based diets resembling those eaten in East and West Pakistan and found that supplementation with 5 percent Glaxo Salt Mixture, which contained 12 percent calcium, did not improve the NPU of the mixture and had actually lowered the protein value of the diet. In the light of these observations it was considered of interest to investigate the effect of different levels of protein and calcium on the growth and body performance of rats.

Experimental

Semi-synthetic diets containing high (21 percent) and low (5.6 percent) levels of protein were selected for this study. Calcium carbonate was added to them so as to provide 0.69, 0.24 and 0.045 percent calcium which is equivalent to approximately 1720, 620 and 120 mg. calcium per 1000 calories, respectively. The composition of the six different diets is shown in Table 1.

Animals.—Male albino weanling rats, 28-30 days old and weighing 50-60 g. were divided into six groups of 12 animals each in such a manner that each group had almost the same average weight. The rats were housed in wire-mesh cages in an air-conditioned room maintained at 80°F. Food and distilled water were given ad libitum. One drop of vitaminised oil [Vitamin A palmitate (1 million units/g.) 5 ml ; calciferol 0.2 g.,α-tocopherol 50 g., menadione 20 g., sesame oil 1 litre] was given orally to each rat per week.

Weight Gain Measurements.—The rats in each group were weighed twice a week for 56 days. Average weight curves are shown in Fig. 1. Initial and final body weights are given in Table 2. Food intake, Feed Efficiency (F.E) and Protein Efficiency Ratio (P.E.R.) measured according to the method described earlier,[11] are also shown in this table.

Carcass Analysis.—At the end of the experimental period the rats were killed with chloroform. Incisions were made into the skull, thoracic and body cavaties. Carcasses of each group were dried separately to constant weight at 105°C. (48 hours). The loss in weight was reported as body-water and expressed as percentage (Table 4).

The carcasses of each group were then pulverised and fat was determined in the sample as petroleum ether extract according to the method of A.O.A.C.[12] Nitrogen was determined according to the usual Kjeldahl method[12] and converted to protein by multiplying with 6.25.

The results of the above determinations were reported on wet weight basis.

Results and Discussion

Body Weight.—From an examination of average weight curves of rats (Fig. 1) it will be observed

TABLE 1.—COMPOSITION OF THE EXPERIMENTAL DIETS.

Diets	Casein g.	Hydrogenated oil (Star Vanaspati) g.	Maize starch g.	Glucose g.	Potato starch g.	Vitamin* mixture g.	Mineral† mixture (-Ca salts) g.	Calcium carbonate (BDH) g.	Calcium %	Protein %
Low-Protein High-Calcium (LPHC)	50	150	450	150	100	50	50	15	0.69	5.60
Low-Protein Medium-Calcium (LPMC)	50	150	450	150	100	50	50	5	0.24	5.60
Low-Protein Low-Calcium (LPLC)	50	150	450	150	100	50	50	1	0.045	5.66
High-Protein High Calcium (HPHC)	200	150	300	150	100	50	50	15	0.68	20.60
High-Protein Medium-Calcium (HPMC)	200	150	300	150	100	50	50	5	0.24	21.00
High-Protein Low-Calcium (HPLC)	200	150	300	150	100	50	50	1	0.045	21.10

*Aneurine hydrochloride, 0.120 g.; Riboflavin, 0.400 g.; Pyridoxine-hydrochloride, 0.080 g.; Nicotinamide, 8 g.; Inositil 8, g.; P-Amino-benzoic Acid, 24 g.; D. biotin, 0.08 g.; Folic acid, 0.08 g.; Cyanocobolamin, 0.002 g.; Choline chloride 24, g.; Ca-pentothenate 2.4 g.; Maize Starch—tomake 2 kg.

†NaCl 154 g.; KCl 249.4 g.; MgSO4 (Anhydrous) 76.6 g.; K_2HPO_4 437.4 g.; $MgCO_3$. Mg $(OH)_2$ $3H_2O$ 70.2 g.; Trace mineral mixtureS 33.4 g.; Maize Starch—to make 2 kg.

$ Fe. NH_4, Citrate, 91.41 g.; $CuSO_4$.$5H_2O$ 5. 98 g,; NaF 0.76 g.; $MnSO_4.2H_2O$ 1.07 g.; K. Al $(SO_4)_2$ 0.54 g.; KI 0.24 g.; Total: 100 g.

that at low protein levels increase of calcium in the diet has no beneficial effect. The groups fed Low-Protein High Calcium (LPHC), Low-Protein Medium Calcium LPMC, Low-Protein Low-Calcium (LPLC) diets lost weight initially and then maintained it throughout the experimental period. The final body-weights appear to have increased slightly-with increasing level of calcium in the diet, but statistical analysis showed that difference between the means was insignificant (Table 3). However, when the level of protein was increased in the diet, there was good growth in the High-Protein High Calcium (HPHC), High-Protein Medium Calcium (HPMC), High-Protein Low-Calcium (HPLC) groups which paralleled the increase of calcium in the diet. Better growth in HPHC group might be due to higher food intake than that of HPMC and HPLC groups but in HPMC group it was observed to be due to the higher level of calcium in the diet as compared with HPLC group. Although HPLC group ate slightly more food than HPMC group, but still they grew less than HPMC group which indicated that at higher levels of protein, the requirements of calcium are also correspondingly increased for optimal growth in animals.

It is evident from Fig. 1 that a low calcium intake of 0.045 percent resulted in better growth in HPLC group when compared with low-protein groups, which indicated that the deficiency of proteins in the diet was the major limiting factor and should be given due attention in the national food-planning.

Feed Efficiency and Protein Efficiency Ratio (*Table* 2).—In the case of high-protein groups there was a significant increase ($P < 0.01$) in the efficiency of food and protein utilization as the level of calcium was increased which indicates that by augmenting the level of protein in the diet, there was need for corresponding increase of calcium for maximum utilization of protein and calories.

Body Composition (*Table* 3).—(i) BODY WATER: It will be observed from Table 3 that the rats fed on low protein diets contained more water as

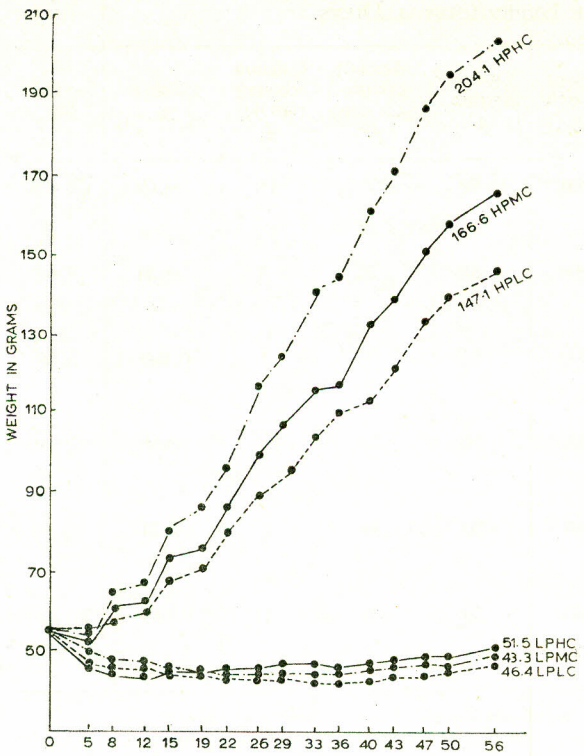

Fig. 1.—Showing effect on growth of rats with different levels of proteins and calcium in diets.

compared with rats fed on high protein diets, irrespective of the level of calcium in the diet. Statistical analysis of the results showed that there was no significant difference between the percent body water of rat carcasses in the same group (i.e. low protein or high protein groups) except in LPMC group which had a significantly higher percentage of body water than that of LPHC group at 5 percent level but insignificant at 1 percent level of probability. (ii) FAT: As contrasted with body water the rats fed on low protein diets had significantly less percentage of fat ($P<0.01$) as compared with the rats fed on higher protein diets. Like body water there was no statistically significant difference between the mean fat percentages in the same group except in HPHC group which had significantly less percentage of fat (significant at 5 percent level but just significant at 1 percent level of probability). This may be due to slightly higher protein content of carcass in this group. (iii) PROTEIN: The rats fed on high protein diets contained slightly higher percentage of protein in the carcass than those fed on low protein diets, but the differences between the mean values were not statistically significant except in the case of HPHC group which has significantly higher ($P<0.01$) percentage of protein in the body as compared with those of the low protein groups. The difference between the mean values in the high protein groups was not significant. Thus the protein content of rat carcasses (expressed as percentage) was found to be almost constant, and fat was the only variable which replaced water in the rats fed on high protein diets. This is also in

TABLE 2.—FOOD EFFICIENCY, (FE) PROTEIN EFFICIENCY RATIO (PER) OF THE EXPERIMENTAL DIETS.

Diet/group	Food intake* g.	Protein† intake	Initial weight	Final weight g.	Weight gain or loss	F.E.**	P.E.R.††
LPHC ..	178.7	10.0	54.5	51.5	—3.0	—	—
LPMC ..	163.0	9.1	54.6	49.3	—5.3	—	—
LPLC ..	179.3	19.3	54.7	46.4	—8.3	—	—
HPHC ..	455.7	93.9	54.4	204.1	+149.7	3.04	1.59
HPMC ..	389.7	81.9	54.6	166.6	+112.0	3.48	1.33
HPLC ..	394.0	83.1	54.7	147.1	+92.4	4.27	1.11
				Difference highly significant ($P<0.01$) in the high protein groups, but insignificant in the low-protein groups.		Differences highly significant ($P<0.01$)	Differences highly significant ($P<0.01$)

(Experimental period 8 weeks; results expressed as mean values per group of 12 rats)
*Dry weight basis; †Refer to Table 1 for % protein in the diets; **Food required to gain one gram of body weight; ††Weight gain per gram of protein intake.

TABLE 3.—BODY COMPOSITION OF RATS FED ON EXPERIMENTAL DIETS.

Diet/group		Body water %	Fat %	Protein %
LPHC	..	66.1	8.5	18.6
LPMC	..	68.4	8.5	18.7
LPLC	..	66.3	9.0	19.0
HPHC	..	63.5	11.1	21.1
HPMC	..	63.4	12.0	19.9
HPLC	..	63.5	12.6	19.8
		Differences significant between high and low protein groups but insignificant in the same group.		Differences insignificant except between HPHC and low protein groups.

agreement with the observations of Moulton[13] and McCance and Widdowson.[14]

Discussion

The results of the above experiments have demonstrated that utilization of calcium depends upon protein intake and if a diet is initially deficient in protein, the body is unable to make use of excess calcium and there is a growth failure. The weight curves at different levels of calcium show that at low level of protein intake the requirement of calcium is also correspondingly reduced and no benefit can accrue by increasing protein in the diet. However, the e should be minimum requirement of calcium in the diet in the absence of which the body cannot make efficient use of available calories and protein in the diet. This aspect of the problem needs re-investigation under the conditions prevailing in tropical countries.

From these investigations it can also be inferred that in any food enrichment programme due attention should be paid to other dietary factors and particularly to proteins which fortunately, are generally associated with many important nutrients. Due to an apparent role in the formation of skeleton, sometimes too much emphasis is given to calcium nutrition at the expense of other equally important constituents of the diet. Although much of the world's population consumes diet which is very low in calcium, as compared to the current standards, yet there is no convincing evidence of any disease due to calcium deficiency alone. Syndromes associated with other nutrients such as iron and iodine are reasonably well characterised. The minimum requirement of calcium has not yet been established but the general consensus of opinion is that there is no firm evidence of harm at intakes of even slightly less than 300 mg. per day in a normal individual.[15] This, in any case, is provided in an average Pakistani diet except that mainly consisting of milled rice which is definately harmful due to lack of many other nutrients besides calcium.

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