EFFECT OF CALCIUM ON THE UTILIZATION OF PROTEIN CONCENTRATE

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A simple method for the preparation of calcium salts of various proteins has been evolved by which the quantity of calcium increases from 1 to 3%. This method will prove to be more economical than the corresponding known method.[8] The growth tables show the utility of these calcium salts in the development of the body. Protein efficiency ratio (PER) and digestibilities have been determined and their values indicate the acceptance of these salts both nutritionally and organolepticallly.

Introduction

Calcium is an essential constituent of our teeth and bones. It is absorbed in the stomach in the presence of vitamin D, ascorbic acid, protein and moderate amounts of fat. The absence or lack of calcium in the diet leads to esteoporosis of bones and is responsible for adult rickets and the 'rheumatic pains' of the old age. It has also been observed recently that the potential hazards from ingestion of foods contaminated with radiostrontium (Sr^{89}, Sr^{90}) are somewhat diminished in proportion to the calcium and phosphate concentration existing in the food naturally or by enrichment.

Our normal diets do not provide proper amount of calcium[1] as, meat is of little value as a source of calcium while fruits and vegetable are useless for this purpose. Brown bread has unavailable calcium while white bread has quite a small amount of it. It has been suggested in the literature that whole meal flour should be fortified with 200 mg calcium per 100 g flour. It has been proposed to supplement our diets with calcium or increase the use of milk per capita in order to improve the nutritional standard.

But there is considerable concern over the limited milk supplies in the country. The climate and the degree of technological development are not yet suitable for the large-scale distribution of milk. The work carried out in India [3,4,5] points to the beneficial effect of calcium supplementation 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. Therefore, it was considered worthwhile to prepare the calcium salts of proteins and thus determine the beneficial effects of calcium on the utilisation of protein.

Experimental

Casein free from fat and vitamins (BDH) was added to $Ca(OH)_2$ (1 g CaO/50 ml water) in small portions with continuous stirring till the pH of the reaction mixture decreased from 11 to 7. The product was washed thoroughly and then dried in the sun.

Raho fish procured from the local market was dried in an oven at 105°C. It was then crushed and defatted in the soxhlet apparatus. This product was pulverized to a fine powder and treated with $Ca(OH)_2$ as described earlier.

The four diets were prepared and named as (a) casein diet, (b) Ca caseinate, (c) fish powder diet and (d) the diet prepared from the calcium salt of the fish powder to be referred as "Ca proteinate" hereinafter.

The nitrogen content of the protein concentrates was determined by Kjeldhal method followed by micro-distillation according to the method of Markham[6] and converted into protein by multiplying with 6.25. The protein concentrates were incorporated in a formula diet by replacement of maize starch in such a proportion that the protein content was about 11%. The protein concentrates used as such after treatment with $Ca(OH)_2$ are detailed as below:

General formulation for 1 kg diet.

Potato starch	100 g
Glucose	150 g
Fat (Vanaspati)	150 g
Vitamin mixture*	50 g
Mineral mixture*	50 g
Test diet	*x*
Maize starch	500—*x* g

*Formulae adopted by the Human Nutrition Research Unit, Mill Hill, London.

The quantity (x) of test diets and maize starch used are given in Table 1.

TABLE 1.

Diet	Protein %	Calcium %	Quantity used x g	Maize starch g
Casein ..	82.0	0.27	137	363
Ca caseinate	80.0	1.36	142	358
Fish powder	84.0	2.9	135	365
Ca proteinate	81.3	5.7	139	361

Chemical Analysis of the Diets.—The A.O.A.C. methods[7] were followed for the determination of moisture, fat, Ca and protein. The results are given in Table 2.

TABLE 2.

Diet	Fat %	Moisture %	Protein %	Calcium %
Casein ..	15.6	5.0	11.3	0.54
Ca caseinate	15.3	5.5	11.3	0.74
Fish powder	15.8	5.0	11.3	1.05
Ca proteinate	15.2	6.0	11.3	1.40

Grouping, Feeding and Weight Gain Measurement of Rats.—Sixteen albino rats weighing between 40–50 g were divided into 4 groups of 4 rats each and arranged in such a way that the average weight of the four groups was identical.

The four groups of rats were housed separately in wire mesh cages and each group was given the respective diet and water *ad lib.* daily.

The rats in groups were weighed on alternate days for four weeks and the growth of the rats recorded (Table 3).

Food Intake and Digestibility.—The diets were placed in pots especially designed to minimise spillage. Spilled diet was collected on filter paper placed in the trays below the cages. The food was separated from the faeces by sifting, and both were dried in an oven at 105°C to constant weights. The total food intake for four weeks for the four groups of rats was as follows :

(*a*) Casein .. .. .. 240 g
(*b*) Ca caseinate .. .. 519 g
(*c*) Fish powder .. .. 633 g
(*d*) Ca proteinate .. .. 672 g

The coefficient of apparent digestibility (D) of the diets was calculated by using the following formula:

$$D = \frac{I-F}{I} \times 100$$

where I = Total food intake in g, and F = weight of total faeces in g.

Feed Efficiency and Protein Efficiency Ratio.—Feed Efficiency (F.E.), *i.e.* food in g required to gain 1 g body weight, and protein efficiency ratio (P.E.R.), *i.e.* gain in body weight per g of protein intake, are given in Table 4.

Discussion

It is obvious from the experimental details that calcium has marked effect upon the development of the body. The rats fed on casein showed variations in their body weight whereas the rats fed on Ca caseinate showed a uniform development. Similarly the rats fed on Ca proteinate showed better growth than the rats fed on fish powder. This brings out the utility of calcium proteinate in the development of the body.

It has been reported[8] that Ca proteinate can be prepared with the use of calcium hydroxide in sucrose solution, for the solubility of calcium hydroxide in 26% sugar solution is 50 times more

TABLE 3.—GROWTH OF THE RATS.

Days	Casein g	Ca caseinate g	Fish powder g	Ca proteinate g
0	185	185	185	185
2	191	193	207	225
4	195	202	227	245
6	204	215	250	265
8	213	228	261	291
10	218	237	271	309
12	230	242	290	320
14	225	260	310	345
16	237	270	318	360
18	245	290	343	380
20	258	295	370	390
22	255	312	387	407
24	270	321	405	419
26	267	330	415	432
28	272	330	419	445

TABLE 4.—BIOLOGICAL EVALUATION OF TEST DIETS.

Diet	Total food intake (g)	Total faeces (g)	Gain in body wt (g)	*D*	F.E.	P.E.R.
Casein ..	240	38	87	88.8	3·91	2·27
Ca caseinate ..	519	48	145	90·7	3·58	2·47
Fish powder ..	633	60	234	90·5	3·58	2·47
C proteinate .	672	94	260	86.0	2·59	3·43

The figures are for each group of 4 rats during the 4-week period.

than could be in water. But this method is expensive and results in the loss of sugar. Since proteins contain a number of free carboxyl groups and since the calcium can combine with the Zwitter[9] ion $COO^-RN^+H_3$ as well as with the protein ion NH_2RCOO^-, the treatment of protein with CaO in water, increased the calcium content of casein by 1.09% and of fish powder by 2.8%.

People live satisfactorily on 300 mg to 1000 mg of calcium per day. In East Pakistan most of the people consume diets which are inadequate in calcium. In order to improve the diets of the general population, particularly under emergencies such as war, calcium salts, *e.g.* calcium carbonate, di- and tricalcium phosphates, calcium sulphate calcium lactate and calcium gluconate, have been used in the enrichment of foodstuff. But Miller and Ali[10] observed that 5% Glaxo salt mixture containing 12% calcium did not improve NPU of the food. Ali and his coworkers[11] observed that calcium carbonate has adverse effect on the P.E.R. of casein, and hence the protein utility is lowered. So it was considered of high importance to prepare certain calcium compounds which not only supply the requisite amount of calcium but also enhance the utility of protein concentrates.

It has been observed that the daily loss of calcium from the body in the urine is about 300 mg. To make up this loss safely 600 mg calcium must be taken and whenever whole cereals, *e.g.* whole-meal bread, shredded wheat, oatmeal and unpolished rice, are taken regularly, the food should contain extra calcium. An average Pakistani diet consisting of milled rice and like things is insufficient even to meet the minimum requirement of calcium.

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