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EFFECT OF INCORPORATION OF FRUIT PULP IN MILK FOODS ON THE GROWTH AND COMPOSITION OF BODY AND LIVER AND UTILISATION OF CALCIUM, PHOSPHORUS AND NITROGEN IN ALBINO RATS

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Cow's milk and infant foods based on cow's milk, are commonly used for feeding infants and young children in Western countries. In India and Egypt, buffalo milk is also being used along with cow's milk for this purpose.[1] For infants with poor digestive capacity or suffering from intestinal disorders, partially skimmed milk and milk foods are usually used.[2] In addition to milk, infants are usually given supplements of fruit juice and fruit pulp[2]. There is some evidence to show that the organic acids present in fruit juices may help to form a finer curd and thereby help in better digestion of the proteins.[3] Hunscher *et al.*[3] observed that the ingestion of fruit juice daily, resulted in increased retention of calcium and phosphorus in children. In an earlier paper from this laboratory, Doraiswamy *et al.*[4] reported that incorporation of dried mango or banana fruit pulp in milk foods, brought about significantly greater increases in the weight of infants as compared with milk foods containing sugar. It is likely that incorporation of mango or banana fruit pulp, resulted in better utilisation of protein, calicum and phosphorus present in the milk. In the present investigation, the effect of incorporation of mango or banana fruit pulp in milk foods on the growth and composition of liver and body and retention of nitrogen, calcium and phosphorus in albino rats has been studied.

Experimental

Materials : Half-cream milk food similar in composition to that used in an earlier study[4] was prepared by blending spray dried full-fat (26% fat) buffalo milk powder, with skim milk powder and dried mango or banana fruit pulps (at 5-6% levels). The dried fruit pulps were prepared by the method described by Siddappa *et al.*[5] The control milk food contained cane sugar in place of fruit pulp. The milk foods were fortified with vitamins and iron. The chemical composition of the foods, as determined by the methods of the Association of Official Agricultural chemists[6] is given in Table I.

Animal experiments : Three groups of freshly weaned albino rats (10 in each group and distributed according to randomized block design with respect to sex and body weight) were housed individually in cages with raised wire-screen bottoms. The animals were fed on re-constituted milk from different milk foods. The milk foods were reconstituted with 7 times their weight of warm water and fed twice daily. Records of the daily consumption of milk by the different groups of rats were maintained. The experiment lasted for eight weeks. The animals were weighed weekly.

After feeding for 8 weeks, the haemoglobin and red blood cell count of rats were determined in the blood drawn from the tail of rats according to the methods used by Korula *et al.*[7] The animals were anaesthetised with sodium amytal and were bled through the abdominal aorta to ensure a minimum and relatively uniform amount of residual blood in the livers. The whole liver was quickly removed, washed with normal saline to remove any adhering blood, wiped between filter papers and weighed.

Table I. *The chemical composition of milk foods containing dried fruit pulp*
(Values per 100g)

Constituents	Control	Half-cream milk food[1]	
		With mango pulp	With banana pulp
Moisture (g)	3.6	3.8	3.6
Protein (g)	20.3	20.5	20.6
Fat (g.)	13.0	12.8	13.1
Minerals (g)	4.7	4.6	4.8
Carbohydrates (g) (by diff)	58.4	58.3	57.9
Calcium (g)	0 90	0.94	0.95
Phosphorus (g)	0.81	0.79	0 82
Calories	432	430	431
Vitamin A value as Vitamin A (I.U.)[2]	1500	1500	1500
,, as carotene (I.U.)	—	2000	32
Vitamin D (I.U.)[2]	400	400	400
Ascorbic acid (mg)	30.0	30.8	30.4
Thiamine (mg)	0.60	0.62	0.62
Riboflavin (mg)	0.81	0 82	0.81
Niacinamide (mg)	6.2	7.0	6.6
Pyridoxine (mg)	0.30	0.31	0.32

[1] Half-cream milk food contained 5% fruit solids. The control foods contained an equivalent amount of sugar in place of fruit solids

[2] Added amounts are given

The moisture content of the liver was determined by drying to a constant weight at 90–95°C in a hot air-oven. The dried liver samples were powdered and analysed for total nitrogen and fat. Total nitrogen was determined by the micro-Kjeldahl method. Fat was determined by the method of Hawk and Elvehjem.[8]

The contents of the gastro-intestinal tract were removed by squeezing and the whole carcass was mashed in a meat mincer and then weighed. The moisture content was determined by drying at 100°C to constant weight. Addition of alcohol helped in quick drying of the material. The dry carcass was then powdered and analysed for total nitrogen, and fat by the methods used for liver. The ash content was determined by incinerating a known quantity of the material in a muffle furnace at 500-600 C. The calcium and phosphorus content of the ash was determined according to the methods used by Murthy *et al.*[9] for faeces. The initial nitrogen, calcium and phosphorus contents of the animals at the beginning of the experiment were determined separately in a group of 6 rats of similar body weight. These values were subtracted from the values obtained for the different groups at the end of the experiment, for obtaining the quantity of N, Ca and P actually retained in the body.

The results are given in Tables II-VII.

Effect of incorporation of fruit pulp on the digestion of milk proteins in the stomach: Groups of male rats weighing about 150g. were used in this study. The animals were starved overnight of food and water. Known quantities of the reconstituted milk food were fed to the animals. The animals consumed all the milk given in about 5 minutes. Five rats, in each group were killed at the end of 15 minutes and another 5 rats at the end of 30 minutes after feeding. The stomach contents were analysed for free and total acid, total chlorides and peptic activity according to the methods used by Fawns *et al.*[10] The casein clots found in the stomach of rats were examined according to the procedure suggested by Fawns *et al.*[10] The results of analysis of stomach contents are given in Table VII.

Results

Growth rates: Table II gives the average weekly gains in body-weight of rats in a

Table II. *Average weekly increase in body weight of rats* fed different milk foods*
(Duration of experiment : 8 weeks)

Group No	Diet	Average initial body weight (g)	Average daily intake of diet (g)	Average weekly gain in body weight (g)	
A	Milk food containing cane sugar	48.1	8.6	12.70	
B	Milk food containing mango fruit powder	48.1	8.4	14.19	±0.83 (16 df)
C	Milk food containing banana fruit powder	48.2	8.7	12.76	
	Critical difference at 5% level			2.48	

* Each group contained 5 males and 5 females.

Table III. Haemoglobin and R. B. C. count in the blood of rats* fed on different milk foods

Group No	Diet	Haemoglobin (g/100cc)		Red blood cells (10^6/cu. mm.)	
A	Milk food containing cane sugar	15.28		7.69	
B	Milk food containing mango fruit powder	15.13	±0.16	7.85	±0.08
C	Milk food containing banana fruit powder	15.30	(16 df)	7.69	(16 df)
	Critical difference at 5% level	0.49		0 24	

* Each group contained 5 males and 5 females

Table IV. *Chemical composition of livers of rats fed different milk foods*
(Duration of experiment : 8 weeks : values are mean for 5 male rats in each group)

Group No	Diet	Average weight of fresh liver (g)	Composition of liver					
			Moisture (%)		Protein (Nx6.25) (%)		Fat (%)	
A	Milk food containing sugar (control diet)	6.44	71.20		19.20		3.36	
B	Milk food containing mango fruit powder	6.88	71.16	±0.38 (8 df)	19.40	±0.16 (8 df)	3.32	±0.10 (8 df)
C	Milk food containing banana fruit powder	6.41	71.06		19.38		3.18	
	Critical difference at 5% level		1.25		0.53		0.32	

Table V. *Chemical composition of the body of rats fed on different milk foods*
(Average of 5 males only)

Group No	Diet	Average body weight (g)	Composition of the body							
			Moisture (%)		Protein (Nx6.25) (%)		Fat (%)		Ash (%)	
A	Milk food containing sugar (control diet)	152.8	58.10	± 0.21 (8 df)	19.94	±0.16 (8 df)	17.32	± 0.30 (8 df)	4 26	±0 07 (8. df)
B	Milk food containing mango fruit powder	159.0	57.92		20.08		17.18		4.54	
C	Milk food containing banana fruit powder	161.0	57.98		19.96		17.28		4.50	
	Critical difference at 5 % level		0.69		053		0.97		0.21	

Table VI *Retention of nitrogen, calcium and phosphorus in rats fed on the different milk foods*
(Mean values for 5 male rats in each group in a period of 8 weeks)

Data summarized	Milk food with sugar	Milk food with mango fruit powder	Milk food with banana fruit powder
Initial body weight (g)	48.4	48.4	48.6
Final body weight (g)	152.8	159.0	161.0
Gain in weight (g)	104.4	110.6	112.4
Food intake (g)	477.6	476.4	483.7
Nitrogen intake (g)	15.5	15.6	15.9
Initial body nitrogen (g)	1.24	1.24	1.25
Final body nitrogen (g)	4.89	5.11	5.15
Nitrogen retained (g)[a]	3.65	3.87	3.90
Calcium intake (mg)	4298	4478	4595
Initial body calcium (mg)	272	272	273
Final body calcium (mg)	1824	1905	1929
Calcium retained (mg)[b]	1552	1633	1656
Phosphorus intake (mg)	3869	3763	3966
Initial body phosphorus (mg)	231	231	232
Final body phosphorus (mg)	1103	1180	1214
Phosphorus retained (mg)[c]	872	958	982

Standard error of the mean.	*Critical difference at 5 % level.*
a ±0.35 (8 d f)	1.13
b ± 125 (8 d f)	410
c ± 75 (8 d f)	246

Table VII. *Analysis of gastric contents of rats fed on milk foods containing fruit pulps*
(Mean values for 5 male rats in each group)

Group	Diet	Total acidity [ml of 0.1 N HCl per 100 ml of juice]		Total Chlorides (ml. of 0.1 N HCl per 100 ml. of juice)		Peptic Index*	
		15 mins. after feeding	30 mins. after feeding	15 mins. after feeding	30 mins. after feeding	15 mins. after feeding	30 mins. after feeding
A.	Milk food containing cane sugar	26.9±3.6	29.0±2.8	19.4±1.5	30.1±2.3	79±10	76±8
B.	Milk food containing Mango fruit powder	47.5±3.6	50.7±3.9	50.6±1.9	60.6±3.6	147±12	121±11
C.	Milk food containing Banana fruit powder	36.3±2.8	41.7±3.8	39.0±2.3	41.9±2.3	123±8	129±12
Difference between groups and test of significance :	A ~ B	20.6++	21.7++	31.2†	30.5†	68++	45*
	A ~ C	9.4NS	12.7*	19.6†	11.8++	44++	53++
	B ~ C	11.2*	9.0NS	11.6++	18.7++	24NS	8NS

† Significant at 0.1% level
++ ,, ,, 1% level
* ,, ,, 5% level

* Peptic Index is experessed as the square of m.m. egg albumin digested by the gastric juice in Metts tubes.

period of 8 weeks fed on the different milk foods. No significant differences were observed in the gain in weight of rats on the three milk foods.

Composition of blood and liver: The results given in Table III and IV show that there were no significant differences in the mean haemoglobin and red blood cell count or in the mean moisture, protein and fat contents of the livers of the different groups of rats.

Composition of carcass and retention of nitrogen, calcium and phosphorus: Table V shows that the mean ash contents of the bodies of rats fed milk foods containing mango or banana fruit pulp were significantly greater ($P<0.05$) than that of rats receiving milk food, containing cane sugar. No significant differences, however, were observed in the mean moisture and protein contents in the carcass or in the mean retentions of nitrogen, calcium and phosphorus in the different groups of animals (Table VI).

Gastric digestion of milk : *Total acidity*: The values for total gastric acidity (Table VII) were considerably higher in the animals receiving milk foods containing mango or banana fruit pulp than in the control group receiving milk containing cane sugar. No trace of free HCl could be found in the stomach contents of any of the animals as evidenced by the colour of the Toepfers indicator. It is likely that the buffers and proteins of milk might have reacted with the free HCl present in the gastric juice.

Total chlorides: The total chlorides include mineral chlorides and hydrochloric acid. The values for total chlorides were considerably higher in the animals receiving milk foods containing mango or banana fruit pulp than in the control milk group.

Peptic activity: The peptic activities were also higher in rats receiving the milk foods containing fruit pulp than those of rats in the control milk group.

Protein curd: The protein curds formed in the stomachs of animals on control milk food were dense and cheesy while those formed from milk foods containing fruit pulp were soft and semi solid in nature.

Discussion

The results obtained in the present investigation have shown that the rates of growth of albino rats fed on milk foods containing mango or banana fruit pulp were not significantly higher than the control group fed on milk food not containing fruit pulp. The results of examination of gastric contents have shown that incorporation of fruit pulp in milk foods made the curd formed in the stomach less dense than the curd from the control milk food and also stimulated the gastric secretions as evidenced by increased acidity and peptic activity of the gastric contents. Investigations carried out earlier on children have, however, shown that the mean increases in weight of infants receiving milk foods containing mango or banana fruit pulp were greater than that observed with the control group fed on milk food not containing fruit pulp.[4] It is possible that in view of the efficient utilisation of nitrogen, calcium and phosphorus present in milk by the albino rat, incorporation of fruit pulps in milk food probably did not bring about any further increase in the utilisation of the nitrogen, calcium and phosphorus of milk.

Summary

1. Experiments were carried out to assess the effect of incorporation of mango and banana fruit pulp in milk foods on the growth and composition of gastric contents and of the body and liver of albino rats. There was no significant difference in the mean weekly increase in body weight of rats fed on milk foods containing banana or mango fruit pulp or cane sugar

2. The gastric contents of animals fed milk foods containing the fruit pulps showed a higher total chloride content as well as higher peptic activity than that of the gastric contents of rats fed on the milk food containing cane sugar in place of fruit pulp Microscopic examination of the casein curd formed in the stomach revealed that the curd formed from the milk foods containing fruit pulp was smaller and softer in consistency than that formed from milk food containing cane sugar.

3. The mean ash contents of the bodies of rats fed milk foods containing banana or mango fruit pulps were signficantly ($P<0.05$) greater than those of rats receiving milk food containing cane sugar No significant difference, however, was observed in the mean moisture and protein contents in the carcass in the different groups of rats

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