119. THE EFFECT OF THE LEVEL OF CALCIUM INTAKE ON THE BIOLOGICAL VALUE OF A PROTEIN

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RANGANATHAN & RAU [1938] have reported that the addition of 2% CaCO_3 to a typical Indian diet, the "Madras diet" containing 0.124% Ca, increased the biological value of the protein, as measured on rats when fed at a 10% level of intake, from 44 to 76. In view of the importance and wide implications of this claim it was decided to submit it to independent test. If confirmed it would certainly provide a further argument in favour of increased use of milk in vegetarian dietaries based mainly on cereals.

To make differences in Ca intake more pronounced a "synthetic" diet was used in our experiments. A protein of high biological value, heat-coagulated egg white, was employed to facilitate the detection of any depressing effect of Ca deficiency.

During the course of our experiments Swaminathan [1939] published results which showed that the level of Ca intake had no effect on the biological value of the proteins of a "typical poor South Indian diet". Our results are in complete agreement with his findings.

Experimental

The biological value of the protein was determined by the method of Mitchell [Mitchell, 1924; Mitchell & Carman, 1926]. Six pairs of litter-mate female rats weighing 49–58 g. were placed in metabolic cages when 25 days old. The cages, vitamin additions, methods of collection and of analysis of the excreta and the low egg-nitrogen diet used in the standardizing period were as described in detail by Henry *et al.* [1937]. The composition and analyses of the "low" and

Table 1. *Composition and analysis of the experimental diets*

	"High" Ca	"Low" Ca
Butter-fat	15.25	15.38
Rice starch	45.76	46.14
Potato starch	8.97	9.05
Castor sugar	15.25	15.38
Ca- and P-free salts*	2.62	2.64
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	1.87	—
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	—	1.71
CaCO_3	0.72	0.12
Egg albumin†	9.56	9.56
% N	1.352	1.294
% Ca	0.8600	0.0694
% P‡	0.4306	0.3699
% Moisture	8.54	9.06

* Henry & Kon [1937].

† Commercial dried egg white was dissolved in water, coagulated by heating to 195° F. for 7½ min. and dried again.

‡ The disagreement in the P content of the diets was due to an error in the calculation of the amount of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ to be added to the "low Ca" diet.

"high" Ca diets are given in Table 1. The diets contained approximately 8.5% protein ($N \times 6.25$). The "low" Ca diet contained 0.07%, the "high" 0.86% Ca. Litter-mates were allocated by lot to the two diets and the intake was equalized within pairs. Both groups received their respective diets for two consecutive complete metabolic periods (20 days in all) to emphasize, if possible, any effects of low Ca feeding. At the end of the experiment the animals were killed with coal gas, the humeri and femora were removed and their ash contents were determined.

Results

The results of the experiment are shown in Tables 2-5. Table 2 gives the individual figures for the nitrogen metabolism data; Table 3 gives mean figures for the biological values and true digestibilities. Table 4 gives data on the Ca intakes of the animals and the bone ash contents of their bones. Table 5 shows the statistical treatment of differences. The statistical analyses were made by the paired *t*-test of "Student" [1908; 1925] as described by White [1937].

Table 2. *Nitrogen metabolism; individual data for 6-day periods*

Rat no.	Level of Ca intake	Initial wt. g.	Final wt. g.	Diet intake g.	N intake mg.	Faecal N mg.	N in urine mg.	Biological value	True digestibility
Low egg-nitrogen diet									
1	—	59	65	46.23	—	88.6	80.2	—	—
2	—	56	61	44.89	—	83.6	82.8	—	—
3	—	55	62	51.00	—	101.9	82.4	—	—
4	—	57	65	51.00	—	92.9	85.0	—	—
5	—	53	56	44.47	—	90.8	83.2	—	—
6	—	49	53	42.64	—	81.7	79.8	—	—
7	—	51	56	40.91	—	86.5	73.7	—	—
8	—	50	56	43.96	—	*	78.7	—	—
9	—	52	57	39.45	—	75.8	75.4	—	—
10	—	52	54	39.50	—	77.5	79.1	—	—
11	—	53	58	48.62	—	111.9	82.4	—	—
12	—	54	61	49.00	—	95.1	77.6	—	—
1st experimental period									
1	High	76	86	44.00	594.7	102.8	140.8	92.8	98.7
2	Low	70	83	43.74	566.0	74.3	118.7	96.3	100.0
3	High	71	93	51.48	695.9	93.9	128.0	96.1	100.0
4	Low	74	95	51.48	666.2	69.3	124.5	97.4	100.0
5	High	66	74	38.83	524.9	89.2	129.9	93.3	98.4
6	Low	62	73	37.59	486.4	83.8	135.7	91.7	97.7
7	High	66	70	34.31	463.8	101.6	130.1	91.1	94.9
8	Low	63	77	40.50	522.8	75.8	134.3	92.0	100.0
9	High	64	71	38.18	516.1	129.1	217.3	73.3	89.9
10	Low	67	78	43.50	562.9	61.4	139.3	93.1	100.0
11	High	69	87	55.00	743.4	125.4	128.8	96.6	100.0
12	Low	72	90	55.00	711.7	81.6	145.6	94.1	100.0
2nd experimental period									
1	High	92	109	58.61	792.2	137.5	183.5	91.3	100.0
2	Low	91	109	59.38	768.4	112.6	176.4	91.1	100.0
3	High	102	120	61.69	833.9	129.9	194.1	90.7	100.0
4	Low	103	116	58.76	760.4	97.4	210.3	88.6	100.0
5	High	81	98	56.45	763.0	127.7	155.8	93.9	98.9
6	Low	80	97	55.85	722.7	110.6	181.2	90.9	97.4
7	High	75	85	48.62	657.2	123.4	210.6	83.7	99.1
8	Low	80	96	51.50	666.4	104.6	179.7	91.0	99.9
9	High	72	78	40.41	546.2	116.6	203.7	80.5	94.3
10	Low	84	91	42.50	550.0	56.6	199.0	84.1	100.0
11	High	95	107	51.88	701.3	131.4	168.0	93.1	98.7
12	Low	100	107	53.50	692.3	80.5	304.2	74.4	100.0

Table 2 (cont.)

Rat no.	Level of Ca intake	Initial wt. g.	Final wt. g.	Diet intake g.	N intake mg.	Faecal N mg.	N in urine mg.	Biological value	True digestibility
Low egg-nitrogen diet									
1	—	118	122	57.50	—	151.6	126.7	—	—
2	—	113	115	51.36	—	133.8	114.9	—	—
3	—	122	127	49.24	—	131.5	111.3	—	—
4	—	118	124	56.00	—	113.8	120.3	—	—
5	—	105	115	63.50	—	137.2	117.8	—	—
6	—	103	115	62.79	—	124.3	129.3	—	—
7	—	90	92	35.46	—	91.0	116.6	—	—
8	—	95	98	46.50	—	94.4	125.7	—	—
9	—	83	90	42.70	—	94.1	119.6	—	—
10	—	94	99	46.50	—	113.5	111.7	—	—
11	—	109	117	58.50	—	139.4	118.9	—	—
12	—	111	120	58.50	—	120.0	135.1	—	—

* Analysis lost. For the calculation of the basal metabolic faecal N of this rat the mean value obtained for the remaining rats was used.

Table 3. Mean biological values and true digestibilities of egg-white in low or high Ca diets

	High Ca intake		Low Ca intake	
	Biological value	True digestibility	Biological value	True digestibility
Period I	90.52	96.98	94.10	99.62
Period II	88.88	98.50	86.68	99.55
Mean	89.70	97.74	90.39	99.58

Table 4. Gains in weight, Ca intakes and ash content of dry, defatted femora and humeri of rats receiving the high and low Ca diets

Rat no.	Level of Ca intake	1st experimental period			2nd experimental period			% bone ash*
		Gain in wt. g.	Ca intake mg.	Ca, mg. intake/g. gain in wt.	Gain in wt. g.	Ca intake mg.	Ca, mg. intake/g. gain in wt.	
1	High	10	378.4	37.84	17	504.0	29.65	59.9
2	Low	13	30.4	2.34	18	41.2	2.29	55.8
3	High	22	442.7	20.12	18	530.5	29.47	60.0
4	Low	21	35.7	1.70	13	40.8	3.14	56.4
5	High	8	333.9	41.74	17	485.5	28.56	58.6
6	Low	11	26.1	2.37	17	38.8	2.28	55.3
7	High	4	295.1	73.78	10	418.1	41.81	57.4
8	Low	14	28.1	2.01	16	35.7	2.23	56.6
9	High	7	328.3	46.90	6	347.5	57.92	57.5
10	Low	11	30.2	2.75	7	29.5	4.21	57.5
11	High	18	473.0	26.28	12	446.2	37.18	59.1
12	Low	18	38.2	2.12	7	37.1	5.30	57.6
Mean High Ca intake		11.5	375.2	41.11	13.3	455.3	37.43	58.75
Mean Low Ca intake		14.7	31.5	2.22	13.0	37.2	3.24	56.53

* Each figure is the mean of duplicate determinations on one femur and one humerus.

It can be seen from Table 3 that the biological values of the proteins of egg-white were very similar at both levels of Ca intake. In the first experimental period a higher value was given by the "low" Ca group while in the second period the "high" Ca group was slightly superior. Table 5 shows that the differences lack any statistical significance either for the separate or the pooled periods. True digestibilities were in both periods higher in the "low" Ca group but the differences between the two groups were again statistically not significant.

Table 5. *Statistical treatment of the results*

		Difference high-low Ca	Standard error of mean	P*
Biological value	Period I	-3.58	± 3.37	1:1
	Period II	+2.20	± 3.67	1:1
	Periods I and II	-0.69	± 2.52	1:1
True digestibility	Period I	-2.64	± 1.72	1:5
	Period II	-1.05	± 1.06	1:3
	Periods I and II	-1.84	± 0.98	1:12
% bone ash		+2.22	± 0.68	1:44a

* P=probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population. a =over 1:20.

The percentages of bone ash in both groups of animals were 58.7 and 56.5 for the "high" and "low" Ca diets respectively. At first sight the figure of 56.5% for animals receiving only 2-3 mg. Ca/g. gain in weight would seem high; it must be remembered, however, that the experimental periods (totalling 20 days) were sandwiched between two periods (10 days each) in which the supply of Ca was adequate. Table 5 shows that the % bone ash of the "high" Ca rats was significantly greater than that of the "low" Ca animals.

According to Sherman & Macleod [1925] female rats growing from 60 to 110 g. (the range covered in this experiment) normally deposit about 10 mg. Ca/g. gain in weight. It can be seen from Table 4 that the intake for the "high" Ca group was considerably above this figure (41 and 37 mg./g. gain respectively for the 1st and 2nd periods) while the figure of 2.2-3.2 mg. for the "low" Ca group was much below the normal. The difference in Ca intakes was probably sufficiently marked for any fall in the biological value of protein caused by low Ca intake to become evident. As no differences were found it must be concluded that the level of Ca intake had, in these experiments, no effect on the biological value or digestibility of the protein. This is in complete agreement with the findings of Swaminathan [1939] but in opposition to the report of Ranganathan & Rau [1938].

SUMMARY

1. The biological values of the nitrogen of two diets, both containing egg white as a source of protein but one having a Ca content of 0.86% and the other of 0.07%, were measured on rats by the balance sheet method of Mitchell.

2. No differences were found in the biological values and true digestibilities of the protein of the two diets. The availability of dietary nitrogen is apparently not decreased when the diet is deficient in Ca.

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