CCXLI. THE EFFECT OF FEEDING CACAO SHELL TO COWS ON THE VITAMIN D CONTENT OF BUTTER (MILK).

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It has recently been reported by Knapp and Coward [1934] that cacao shell, an important by-product of chocolate manufacture, is rich in vitamin D. The sample examined by them contained 28 International Units (I.U.) per g. and was therefore about one-quarter as potent as an average cod-liver oil. For a vegetable product the potency is quite exceptional.

As cacao shell is mainly used in cattle food, feeding tests with milch cows have been carried out at this Institute in collaboration with Mr A. W. Knapp and Mr A. Churchman of Messrs Cadbury Bros., Ltd., of Bournville and it is proposed to present here the results of vitamin D assays carried out on butters from cows which received cacao shell in their rations and on butters from control animals.

EXPERIMENTAL.

Estimation of the vitamin D potency of the cacao shell used in feeding experiments.

As a preliminary to the experiment proper the vitamin D content of the sample of cacao shell used in the cow feeding experiments was estimated in curative and protective experiments on rats. The cacao shell was part of a half-ton sample of the commercial product sent to us by Messrs Cadbury Bros., Ltd. This was a carefully taken sample representative of a bulk of 3467 cwt. of shell. For the rat tests the shell was ground to a fine powder.

(a) *Curative test.* Young rats were weaned when weighing about 60 g. and were placed on the 2965 diet of Steenbock for from 3 to 3½ weeks until definitely rachitic. They were then given in one single dose at the beginning of the 10-day test either cacao shell or the international standard for vitamin D (v.D. 9). Of the latter, two levels, 2 and 5 units, were given. The corresponding levels of cacao shell were calculated on the basis of the value given by Knapp and Coward [1934], namely 28 I.U. per g. For feeding the finely ground cacao shell was mixed with a little castor sugar and water and offered in palette dishes in quantities of 71 mg. and 179 mg. (respectively equivalent on the above basis to 2 and 5 I.U. of vitamin D). The consumption was complete in all cases. After 10 days the rats were killed by coal gas and the wrist bones were placed in 4% formaldehyde for several days. They were then split longitudinally, placed in palette dishes containing a 1.5% solution of silver nitrate and exposed to light. The degree of healing was then estimated by comparing the deposition of calcium salts in the bones with the graded scale in use in this Institute for assessment of extent of healing. The results are given in Table I.

In the course of the last few years numerous estimations of the healing effect of graded doses of the international standard for vitamin D have been

Table I. *The vitamin D content of cacao shell.*

Curative experiments.									
Dose	Rat	Litter	Score		Dose	Rat	Litter	Score	
			Indi- vidual	Av.				Indi- vidual	Av.
2 units inter- national standard	4336	1	2.0		5 units inter- national standard	4340	1	2.0	
	4350	2	1.5			4346	2	0.5	
	4354	3	0.5			4358	3	1.5	
	4372	4	0.5	1.1		4368	4	5.5	
						4377	5	1.0	2.1
71 mg. cacao shell	4337	1	2.25		179 mg. cacao shell	4341	1	2.25	
	4351	2	1.00			4347	2	2.0	
	4355	3	0.50			4359	3	3.5	
	4373	4	2.00	1.4		4369	4	3.5	2.8

carried out in this laboratory. From the results the following equation relating the degree of healing (score) expressed in arbitrary units to the dose has been constructed [Kon]:

$$\text{Score (arbitrary units)} = 4.22 + 3.79 \log \text{dose (I.U.)}.$$

By calculating from this equation it is found that the ratio of scores $\frac{1.4}{1.1}$ obtained for the lower level of cacao shell and 2 units of international standard for vitamin D respectively corresponds to a ratio of dosages $\frac{0.18}{0.15}$. Therefore 71 mg. of cacao shell contain $\frac{0.18}{0.15} \times 2 = 2.4$ I.U. and 1 g. of cacao shell $\frac{2.4 \times 1000}{71} = 34$ I.U. of vitamin D.

A similar calculation for the higher levels of shell and international standard shows that the ratio of scores $\frac{2.8}{2.1}$ corresponds to a ratio of dosages $\frac{0.42}{0.28}$ and that 179 mg. of cacao shell contain 7.5 I.U. Hence 1 g. of cacao shell contains $\frac{7.5 \times 1000}{179} = 42$ I.U. of vitamin D.

Taking an average of the two values the curative test gives a content of 38 I.U. of vitamin D per g. of cacao shell.

(b) *Protective test.* In this test the vitamin D contents of cacao shell and of cacao shell fat were measured in terms of the international standard for vitamin D. Young rats were weaned when weighing about 60 g. and were placed on the 2965 diet of Steenbock. The various addenda were given from the start daily for 5 weeks with the exception of Sundays. Finely ground cacao shell was diluted 10 times with icing sugar and given at 2 levels of intake, namely 6.7 mg. and 13.4 mg. daily (equivalent to 0.187 and 0.375 I.U. respectively on the assumption that 1 g. of cacao shell contains 28 units).

A sample of cacao shell fat extracted in the laboratory of Messrs Cadbury was also tested. Two levels, 0.62 mg. and 1.24 mg. were fed dissolved in olive oil. The cacao shell contained, according to an analysis carried out by Messrs Cadbury, 4.6% fat. On this basis and assuming that the whole of the antirachitic potency of cacao shell is present in the fat the above levels were equivalent to 0.375 and 0.75 I.U. of vitamin D. The international standard for vitamin D (V.D. 9) was fed at a single level of 0.41 unit. In addition one group of rats was given no supplement to the rachitogenic diet and served as negative control.

At the end of 5 weeks the rats were killed by coal gas, their femora and humeri after cleaning were defatted by extraction with several changes of alcohol for

72 hours in a Soxhlet extractor, dried and ashed at 700° in an electric furnace. For each rat a duplicate determination on one femur and humerus was carried out (Table II).

Table II. *The vitamin D content of cacao shell and of cacao shell fat.*

Protective experiments.									
Dose	Rat	Litter	Ash %		Dose	Rat	Litter	Ash %	
			Indi- vidual	Av.				Indi- vidual	Av.
Cacao shell 6.7 mg.	4419	8	43.3		Cacao shell	4443	7	51.5	
	4450	4	43.3		13.4 mg.	4430	5	46.7	
	4459	3	41.9			4455	3	46.0	
	4470	1	42.0	42.6		4465	2	49.0	48.3
Cacao shell fat 0.62 mg.	4416	8	42.9		Cacao shell fat	4444	7	45.0	
	4449	4	34.8		1.24 mg.	4432	6	49.8	
	4460	3	39.3			4456	3	48.2	
	4474	1	45.1	40.5		4466	2	50.3	48.3
Standard 0.41 I.U.	4420	8	41.9		Negative con- trols	4440	9	29.1	
	4431	6	46.5			4435	6	26.7	
	4462	2	50.5			4469	2	26.7	
	4473	1	51.7	47.6		4477	1	26.2	27.2

The gains in percentage of ash over the negative controls were as follows:

Cacao shell	6.7 mg. level	+15.4
Cacao shell	13.4 „	+21.1
Cacao shell fat	0.62 „	+13.3
Cacao shell fat	1.24 „	+21.1
International standard 0.41 unit level +20.4		

In the same way as for curative tests the following equation relating the degree of protection to the dose has been worked out for protective experiments:

$$\text{Gain in ash \%} = 26.33 + 16.6 \log \text{dose (I.U.) [Kon].}$$

Calculations similar to those described above in the case of curative tests show that the ratio of gains in ash percentage $\frac{15.4}{20.4}$ given by the 6.7 mg. level of cacao shell and the standard is equivalent to a dose ratio of $\frac{0.22}{0.44}$ and that 6.7 mg. of cacao shell contain 0.205 I.U. and 1 g. of cacao shell = 31 I.U. of vitamin D.

For the higher level of cacao shell the ratio of doses works out at $\frac{0.48}{0.44}$ and 13.4 mg. of cacao shell contain 0.45 I.U. From this 1 g. of cacao shell contains 34 I.U.

Taking an average of the two figures, a value of 32.5 I.U. of vitamin D per g. of cacao shell is obtained from protective tests and as the average of the curative tests worked out at 38 units, a value of 35 I.U. of vitamin D per g. of cacao shell may be accepted as the result of all the tests carried out in this Institute. We have been informed by Messrs Cadbury that a sample of the same consignment of cacao shell as sent to this Institute was submitted to Dr K. H. Coward at the laboratories of the Pharmaceutical Society of Great Britain and that she reported for it a value of 28 I.U. of vitamin D per g., identical with that previously published by Knapp and Coward [1934]. Our value is slightly higher than that obtained by Dr Coward but, in view of the small number of animals used by us, the agreement is very satisfactory.

The calculations for the cacao shell fat give the following results:

Lower level of cacao shell fat. Ratio of doses $\frac{0.164}{0.44}$. Hence 0.62 mg. of fat contains 0.153 I.U. and 1 g. contains 247 I.U. of vitamin D.

Higher level of cacao shell fat. Ratio of doses $\frac{0.48}{0.44}$. Therefore 1.24 mg. of fat contain 0.45 I.U. and 1 g. contains 367 I.U. of vitamin D.

The agreement is not very good but differences of this magnitude are to be expected when relatively small numbers of animals are used. By averaging the above two values a figure of 307 I.U. per g. of cacao shell fat is obtained. This is about three times the vitamin D content of an average cod-liver oil. From this value and from the fat content of cacao shell it may be calculated that the extractable fat carries about 40% of the total activity of cacao shell.

The feeding of cacao shell to cows and the estimation of its effect on the vitamin D content of butter (milk).

The feeding of the cacao shell was carried out during the winter stall feeding period of 1935. Four Shorthorn cows were used for this experiment. They were kept in the same cow-byre and were not allowed out to grass. From January 8th, 1935, all four cows received the following mixture of concentrates:

3 parts dried grains,
2 parts maize germ cake,
1 part soya bean meal.

In addition each cow was given daily:

2 lb. of hominy chop,
7 lb. of hay,
45 lb. of mangels.

The cows were kept on this ration till February 8th, 1935. The milks yielded by each cow between the evening milking on February 5th and the morning milking on February 8th (both dates inclusive) were separated and butters were churned from the unpasteurised and unripened creams. The butters were melted at 50°, filtered and the butter fats stored at -2° until required for tests. On February 8th two cows were given $\frac{1}{2}$ lb. of cacao shell, this was increased to 1 lb. on February 9th, $1\frac{1}{2}$ lb. on February 12th and on February 13th to 2 lb., fed invariably in the coarse flaky form in which it occurs in commerce. The feeding of cacao shell was continued at this level until March 17th. The cacao shell was taken into consideration when calculating the amount of concentrates fed daily to each cow according to her milk yield as is usual in our dairy herd. The remaining two cows were kept on the original ration throughout the experiment. Butters were churned again as described above from milks collected between the evening milking on March 10th and the morning milking on March 13th (both dates inclusive), that is after the experimental cows had received 2 lb. of cacao shell daily for 4 weeks. In this way eight samples of butter fat were obtained. Aliquots of each two samples representing one treatment were then pooled, the blending being done on the basis of the fat yields of the cows. The four samples so obtained, representing the four treatments (control cows—pre-experimental and experimental periods, "cacao shell cows"—pre-experimental and experimental periods), were then tested in protective experiments on rats at one level of intake, 0.3 g. of butter fat per rat per day (with the exception of Sundays), and compared with three levels of the international standard for vitamin D with the following results.

Table III. *The vitamin D content of butter from cows receiving cacao shell in their diet and of butter from control cows.*

Dose	Rat	Litter	Ash %			Dose	Rat	Litter	Ash %		
			Indi- vidual	Av.					Indi- vidual	Av.	
0.3 g. cacao	4637	1	40.8			0.3 g. control	4638	1	31.4		
shell butter fat	4645	2	39.1			butter fat	4644	2	30.4		
experimental	4647	3	46.0			experimental	4651	3	29.2		
period	4658	4	40.6			period	4657	4	31.8		
	4667	5	41.4				4668	5	31.3		
	4679	6	38.9				4680	6	32.9		
	4687	7	37.3				4688	7	34.2		
	4699	9	41.3	40.7			4700	9	31.3	31.6	
0.3 g. cacao	4634	F	31.1			0.3 g. control	4635	F	30.3		
shell butter	4646	3	28.0			butter fat	4672	5	33.1		
fat pre-experi-	4669	5	30.8			pre-experi-	4681	6	32.7		
mental period	4695	7F	30.9	30.2		mental period	4694	7	29.9	31.5	
0.05 unit inter-	4639	1	31.2			0.1 unit inter-	4640	1	36.3		
national	4643	2	28.5			national	4642	2	34.3		
standard for	4652	3	30.6			standard for	4648	3	35.1		
vitamin D	4656	4	29.9			vitamin D	4655	4	39.8		
	4670	5	27.8				4671	5	36.2		
	4683	6	29.7				4684	6	38.6		
	4689	7	32.8				4690	7	39.5		
	4701	9	29.8	30.0			4702	9	41.7	37.7	
0.2 unit inter-	4641	1	39.4			Negative con-	4636	F1	29.4		
national	4650	3	47.6			trols	4649	3	28.5		
standard for	4654	4	41.5				4653	4	28.1		
vitamin D	4673	5	40.7				4666	5	32.5		
	4685	6	47.5				4692	7	29.1		
	4691	7	44.7				4696	8	29.0		
	4698	8	38.4				4704	9	28.4		
	4703	9	40.8	42.6			4754	10	26.3		
							4755	10	28.9		
							4604	12	31.3	29.2	

The gains in percentage of ash over negative controls were as follows:

"Cacao shell" butter	Pre-experimental	+ 1.0
	Experimental	+ 11.5
Control butter	Pre-experimental	+ 2.3
	Experimental	+ 2.4
International standard for vitamin D (v.D. 10)	0.05 unit	+ 0.8
	0.10 unit	+ 8.5
	0.20 unit	+ 13.4

For the calculation of the ratio of dosages the response given by 0.05 unit of standard was used for the low potency butters whilst that given by 0.2 unit of standard was used for the experimental "cacao shell" butter. The calculations were carried out in the same way as described in the earlier part of this paper and the following values were obtained:

"Cacao shell" butter fat	Pre-experimental 1 g. contains 0.17 I.U. of vitamin D
	Experimental 1 g. contains 0.51 I.U. of vitamin D
Control butter fat	Pre-experimental 1 g. contains 0.20 I.U. of vitamin D
	Experimental 1 g. contains 0.21 I.U. of vitamin D

If the "cacao shell" butter is compared with the control butter then the feeding of cacao shell has resulted in an increase in potency of $\frac{0.51}{0.21}$ = approx. 2.5 times.

When comparing the butters yielded by the same cows before and after the feeding of cacao shell the increase is $\frac{0.51}{0.17} = 3$ times. In our experience and under our conditions winter Shorthorn butter fat contains about 0.20–0.25 I.U. of vitamin D per g., summer butter fat 0.50–0.60 [Kon]. It may be said therefore that the feeding of 2 lb. daily for a month of cacao shell, equivalent to about 32,000 I.U. of vitamin D daily, to cows on a winter ration has resulted in raising the vitamin D content of their butter fat (and therefore of their milk) from the winter to the summer level.

SUMMARY.

1. Cacao shell, a by-product of chocolate manufacture, was found in curative and protective experiments on rats to contain 35 I.U. of vitamin D per g., in good agreement with the findings of Knapp and Coward [1934] who reported for the product a content of 28 units per g.

2. Cacao shell fat contains about 300 I.U. of vitamin D per g. The extractable fat carries about 40 % of the total activity of the cacao shell.

3. The feeding to two Shorthorn cows under winter stall feeding conditions of 2 lb. of cacao shell daily for a month (equivalent to 32,000 I.U. of vitamin D daily) has resulted in increasing the vitamin D content of their butter fat (and milk) from the winter to the summer level.

The above report deals only with one aspect of the investigation of cacao shell as a food for cows. The effect of the cacao shell feeding on the health of the cows, the milk yield, fat percentage, solids-not-fat *etc.* has been investigated by Capt. J. Golding and other members of the staff of N.I.R.D. The results will be published in detail elsewhere. But it may be stated here that no untoward effects whatever have been noted in the present experiment.

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REFERENCES.

- Knapp and Coward (1934). *Analyst*, **59**, 474.
Kon. Unpublished work.