THE CLINICAL AND BIOCHEMICAL EFFECTS OF RIBOFLAVIN AND NICOTINAMIDE SUPPLEMENTATION UPON BANTU SCHOOL CHILDREN USING MAIZE MEAL AS CARRIER MEDIUM*

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SUMMARY

A comprehensive clinical, biochemical and technological investigation was undertaken to establish the feasibility of enriching maize meal with riboflavin and nicotinamide. It was clear from the results that the addition of these vitamins to maize satisfied all the prerequisites of a scientifically sound enrichment scheme, as laid down by the NNRI. It was found that the addition of 1 mg of riboflavin and 10 mg of nicotinamide per 400 g maize meal was adequate to effectively reduce the incidence of subclinical deficiency of these two vitamins among Bantu schoolchildren. Since the enrichment was found to be effective, as well as technologically and economically feasible, it is strongly recommended that a compulsory national maize enrichment scheme be introduced with the least possible delay.

Although the exact incidence of pellagra in South Africa is not known, it has been established that the disease occurs very frequently among the maize-eating Bantu population. During a recent Pretoria survey,[1] an analysis of the biochemical data indicated that there was a high incidence of subclinical deficiency of riboflavin and nicotinic acid among Bantu schoolchildren. A survey of rural and urban adult Venda males also showed biochemical evidence of subclinical nicotinic acid deficiency (unpublished data). It has, therefore, been proved that there is a need for an additional intake of riboflavin and nicotinamide.

Ideally pellagra should be prevented by improving the diet by means of natural foods. From a social, educational and economic point of view this would be virtually impossible to accomplish since this would entail drastic changes in the basic dietary habits of the Bantu population. It would, therefore, be much easier to add the two vitamins to a staple food such as maize which is known to be consumed in large quantities by the Bantu population.

The fortification of maize meal with vitamins is an unfamiliar procedure in the Republic of South Africa, but in the United Kingdom and the USA the enrichment of bread flour with certain vitamins and minerals is common practice.

In the United Kingdom the Bread and Flour Regulations of 1963[2] require all bread and culinary flours to contain a minimum of 1·65 mg iron, 0·24 mg vitamin B_1 (thiamine) and 1·60 mg nicotinic acid per 100 g flour. The main purpose of adding these vitamins and iron is to restore these particular nutrients to the level at which they occur in 80% extraction flour. Chalk is added to increase the total intake of calcium in the national diet.

Supplementation is not state-subsidized in the UK and the cost of supplementation is borne to some extent by the consumer in the form of an increased flour or bread price. The scheme is controlled by the state in that the Public Analyst collects samples from time to time for analysis to determine whether the fortified product complies with the regulations. Because the food consumption pattern in the UK has changed since the introduction of the enrichment scheme approximately 20 years ago,[3] the whole program is at present being reconsidered and reinvestigated.[4-6]

Bread enrichment was first introduced in the USA in 1941 and nearly 90% of all white bread sold in the USA is vitamin enriched.[7] Apart from being enriched with vitamins, bread is also fortified with amino acids.[8] It is generally accepted that flour enrichment has had a most beneficial effect on American health since the program was started.[9]

Besides the UK and the USA, other countries also follow the practice of enriching their bread with vitamins. Among these countries are Australia, the Philippines, Sweden, Switzerland, Denmark, Canada, Puerto Rico and Peru,[10,11] and it is believed that more than 250 million people in the western world are today eating vitamin-enriched bread.[11]

The bread and flour enrichment program in the USA was followed by programs to enrich maize (corn) meals and grits, rice and other products. In the south-eastern states, where a high consumption of corn meal and grits was accompanied by a high incidence of pellagra, methods were developed to enrich locally ground corn products as well as those brought in from other parts of the United States.[12,13] Maize grits and maize meal are enriched by the admixture of rinse-resistant particles containing vitamins or a powdered vitamin-iron mixture.

The Federal Food and Drug Regulations define enriched maize meals as containing in each pound: not less than 2·0 mg nor more than 3·0 mg of thiamine; not less than 1·2 mg nor more than 1·8 mg of riboflavin; not less than 16 mg nor more than 24 mg of niacin or niacinamide; and not less than 13 mg nor more than 26 mg or iron. Calcium and vitamin D may or may not be included in the enrichment.[14]

In a publication *Food Enrichment in South Africa*[15] by the National Nutrition Research Institute, the basic principles which will ensure that an enrichment scheme rests on a sound scientific basis, are summarized as follows: evidence that there is a need for the nutrients to be added by enrichment; evidence that the enrichment of a staple food has advantages over the use of natural foods as a means of improving the diet; evidence that the amount of the enriching substance is sufficient to satisfy the proved need; proof that the enrichment medium is regularly consumed in significant quantities by those in need of an enriched diet; evidence that enrichment can be easily carried out in practice; evidence that the enriching

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substances or preparations do not appreciably alter the appearance, taste or physical characteristics of the medium.

This experiment was therefore undertaken to test the effectiveness and feasibility of riboflavin and nicotinamide supplementation through the medium of maize meal. Maize meal was chosen as the carrier medium since it is the staple food of the Bantu population.

MATERIALS AND METHODS

In order to carry out this experiment, an area had to be found where maize meal from a single mill was the only meal available. Such an area was found at Boyne, 25 miles from Pietersburg. The Boyne Roller Mills belong to the Zion Christian Church, as does the local shop. It may be assumed, therefore, that the vast majority of people living within the area served by the Moria Higher Primary School at Boyne will consume maize milled by the Boyne Roller Mills.

In a preliminary controlled experiment in which riboflavin and nicotinamide were administered to schoolchildren in tablet form, it was found that the daily supply of 1 mg of riboflavin and 10 mg nicotinamide effectively reduced the incidence of deficiency when judged by biochemical and clinical criteria (unpublished data).

In order to provide an additional 1 mg of riboflavin and 10 mg nicotinamide in 400 g maize meal, a premix containing 3·595 g riboflavin, 35·95 g nicotinamide and 960·45 g of maize meal was fed into the millstream at a rate of 2 oz (56·7 g) per bag (81·54 kg—180 lb). The premix was prepared and provided by Roche Products, Isando. In our investigation, premix was added by means of a Miag Micro-feeder while mixing was effected with the aid of a 10-ft-long screw conveyor. The conveyor had two screws, each 5 ft in length, turning in opposite directions; i.e. in the course of the first 5 ft of mixing, maize meal and supplement were turned in one direction while the operation was reversed in the course of the next 5 ft.

After written permission had been obtained from the Zion Christian Church Council and the Maize Board, a Miag Micro-feeder and mixing screws were installed in the Boyne Roller Mills. During the enrichment period of the experiment, the entire output of the mill was supplemented.

As a control area Sheshego Village, 53 km (33 miles) from Boyne, was chosen. A random sample of 70 children was drawn from the Standard 3 and 4 classes of both Boshego Higher Primary School (control group) and the Moria Higher Primary School (experimental group).

After written permission had been obtained from the parents, the children were clinically examined, their weights and heights recorded and a 3-hour urine specimen collected for biochemical analysis.

Immediately after this examination had been completed, the enrichment program at the mill was put into operation. The initial examination was carried out in October in order to allow at least two months to elapse after the babala crop had been consumed. The children were re-examined twice after the maize meal had been enriched for 95 and 137 days respectively. At this stage enrichment was stopped and the children were re-examined after a further 68 days had elapsed.

The urine specimens were assayed biochemically for riboflavin, N^1- methyl-2-pyridone-5-carboxylamide (2- pyridone), N^1-methyl-nicotinamide (N^1-Me) and creatinine content, using the same analytical techniques as those reported for the Pretoria surveys.[1] The weights and heights of both sexes were plotted against the Boston percentile ranges for boys.[16] The weights were also expressed as a percentage of the Boston 50th percentile for boys and girls separately in order to compare the percentage of expected weight with the Goméz classification.[17]

After the experiment had been completed, a small dietetic study was carried out to estimate the amount of maize meal consumed by the experimental group. A sequential random sample of 30 was drawn from the experimental group. Dietary intake was assessed by relating family size to the amount of maize meal bought monthly and according to the frequency and amount of maize meal cooked. As a rough means of correcting for age in a family, members above 10 years of age were taken to be adults. Those between 10 and 2 years were taken to be consuming half the adult quantity and children below 2 years of age were disregarded.

Statistical Methods

It is a known fact that when several statistical tests are carried out on variables which are interrelated, individual tests should be done at a significance level which is smaller than the level required for the over-all hypothesis. A result frequently used to approximate the individual levels which will guarantee an over-all α-level derives from the Bonferroni[18] inequality. It states that if there are k tests, each at level α/k, then the over-all level approximates α.

While this procedure ensures that a result will not be regarded as significant when in fact it is not, it is a conservative procedure and significant differences of a marginal nature could be overlooked as a result of its application. The control and experimental groups were compared statistically, in terms of the 4 biochemical measurements, using the Wilcoxon-Mann-Whitney two-sample rank test in order to determine whether the two groups differed significantly at any stage of the experiment.[19] To ensure an over-all significance level of 0·05, individual tests were, therefore, performed at a level of $0{\cdot}05/(4 \times 4) = 0{\cdot}003125$.

In order to determine whether changes observed in the biochemical parameters within the control and experimental groups during the different examinations were significant, the Wilcoxon matched-pairs signed-ranks test was used.[19] Since 32 individual tests were carried out, each individual test was performed at a 0·001562 level.

A test for differences in proportion[20] was used to ascertain whether the incidence of clinical signs found during the various examinations in the control and experimental groups differed significantly. Since 20 individual tests were carried out, each test was performed at a level of 0·0025.

RESULTS

Technological Results

No serious technological problems were encountered in the course of the experiment. The Miag Micro-feeder was initially tested in the laboratory and found to be capable of delivering 2 oz of premix in 7½ minutes with an accuracy of ± 5%. After it had been installed in the mill the instrument was recalibrated and tested. Subsequently 15 samples were drawn from the millstream at various intervals before they were put into the bags and they were then assayed for riboflavin and nicotinic acid.[21,22]

The results of these assays are given in Table I and a

TABLE I. NICOTINIC ACID AND RIBOFLAVIN ASSAY OF SUPPLEMENTED MAIZE MEAL (WET BASIS)

Sample No.	Riboflavin mg/400 g meal	Deviation from mean %	Nicotinic acid mg/400 g meal	Deviation from mean %	Ratio riboflavin: nicotinic acid
1	0·642	16·6	8·54	22·6	1 : 13·3
2	0·870	13·0	10·87	1·5	1 : 12·5
3	0·877	13·9	11·36	2·9	1 : 12·9
4	1·023	32·9	16·00	44·9	1 : 15·6
5	0·601	21·9	10·75	2·6	1 : 17·9
6	0·599	22·2	9·22	16·5	1 : 15·4
7	0·828	7·5	11·16	1·1	1 : 13·5
8	0·801	4·0	10·79	2·3	1 : 13·5
9	0·716	7·0	9·54	13·6	1 : 13·3
10	0·745	3·2	8·83	20·0	1 : 11·9
11	0·748	2·9	11·49	4·1	1 : 12·2
12	0·681	11·6	11·46	3·8	1 : 17·5
13	0·720	6·5	11·48	4·0	1 : 10·4
14	0·769	0·1	12·52	13·4	1 : 14·9
15	0·927	20·4	11·55	4·6	1 : 10·6
Mean	0·770	12·2	11·04	10·5	1 : 13·7
SD	0·120		1·785		

TABLE II. DEVIATION FROM MEAN IN VITAMIN ASSAY

Deviation %	No. of samples	
	Riboflavin	Nicotinic acid
0 - 5	4 (26·7%)	9 (60·0%)
5 - 10	3 (20·0%)	0 (0%)
10 - 15	3 (20·0%)	2 (13·3%)
15 - 20	1 (6·7%)	2 (13·3%)
20 - 25	3 (20·0%)	1 (6·7%)
>25	1 (6·7%)	1 (6·7%)

frequency distribution of deviation from the mean in Table II. Preliminary experiments indicated that maize meal, owing to its superior free-flowing properties, was a better excipient and also more economical than starch in the premix. After its preparation, samples of the premix were also assayed for the two vitamins by Roche Products (Pty) Ltd, Isando, and found to contain between 100 and 103% of the intended quantities.

It may, therefore, be assumed that a reasonably homogeneously enriched maize meal was produced during the experiment.

The dietetic investigation showed that estimates of maize meal intakes based upon the amount bought monthly are much more reliable than by weighing the amount of maize meal cooked for a single meal. The results obtained by weighing were, therefore, disregarded. The mean intake was found to be 474 ± 127 g per adult per day. This result confirms the initial assumption that the maize intake of the Bantu is in the vicinity of 400 g per head per day.

Weights and Heights for Age

After the first examination of the children an error was found in the scales used. Weights were, therefore, measured with different scales at the second examination. These individual weights were plotted against the Boston percentiles and are given separately for the control group (Fig. 1) and the experimental group (Fig. 2). Twenty-three and 33% of the control and experimental groups, respectively, fell below the 3rd percentile. The mean weights were 85·9 ± 14·2% of the expected weight for the control and 83·2 ± 14·0% for the experimental group.

The mean age for the control group was 13·2 years and 13·8 years for the experimental group, with an age range

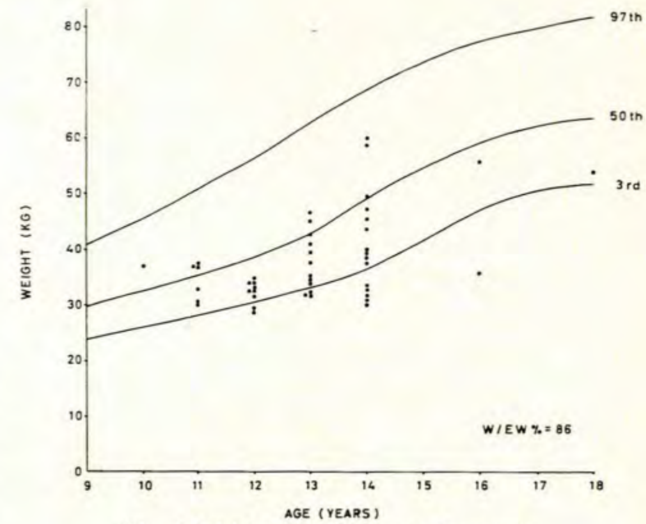

Fig. 1. Weight for age (control group).

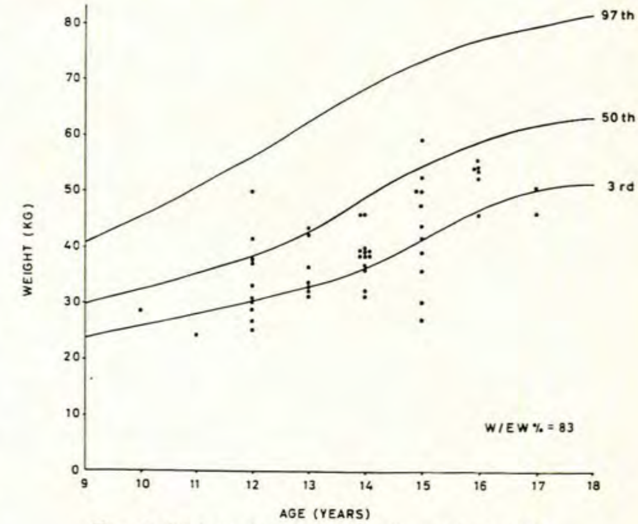

Fig. 2. Weight for age (experimental group).

TABLE III. APPLICATION OF THE GOMÉZ CLASSIFICATION OF MALNUTRITION BY WEIGHT

	W/EW %	*Control group*	*Experimental group*
Normal	>90	34·8%	33·3%
1st degree	90 - 76	43·5%	41·2%
2nd degree	75 - 60	21·7%	21·6%
3rd degree	<60	0%	3·9%

The mean W/EW % was 85·9 ± 14·2% for the control group and 83·2 ± 14·0% for the experimental group.

of 10 - 18 years.

The results obtained by applying the Goméz classification of degree of malnutrition by weight are given in Table III. The results obtained for the two groups were very similar, with 33 - 35% in the normal range and 22 - 25% being classified as suffering from 2nd and 3rd degree malnutrition. The height for age, in relation to the Boston percentiles, is given in Figs. 3 and 4. Twenty-nine and 45%

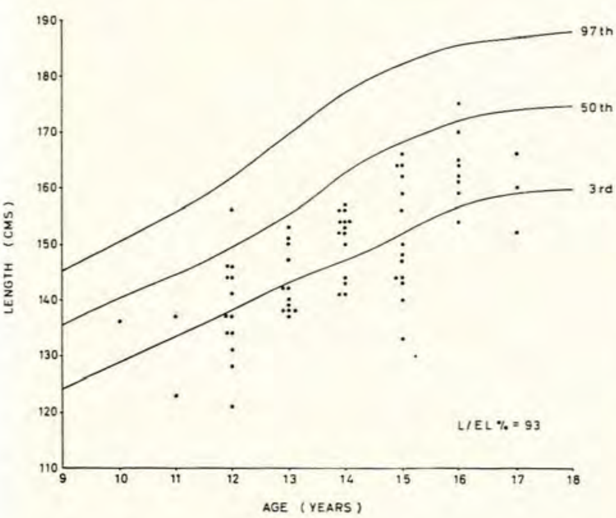

Fig. 3. Height for age (control group).

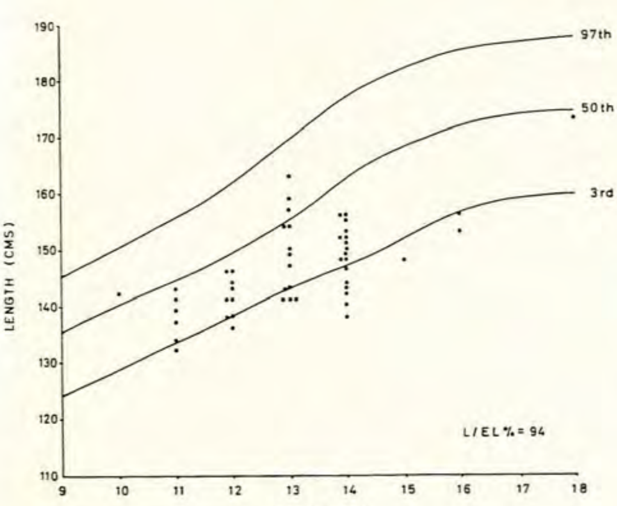

Fig. 4. Height for age (experimental group).

of the control and experimental groups, respectively, fell below the Boston 3rd percentile for height. The mean values for height as a percentage of expected height were respectively 94·1 ± 4·4 and 92·9 ± 5·3 for the control and experimental groups.

The control and experimental groups were, therefore, comparable in terms of age, weight and height.

Clinical Signs of Deficiency Disease

At the time of the first examination none of the children in the control group had overt pellagra. In the experimental group, however, 9 children (13%) were found to have obvious pellagrous lesions (Table IV). At the time of the second examinations these lesions had disappeared in all of them and did not recur during the rest of the experiment. Although the difference in incidence between the two groups and the change in incidence within the experimental group was not significant at a level of 0·0025, i.e. 0·25% (as tested), it was significant at a level of 0·01 (1%).

TABLE IV. INCIDENCE (%) OF CLINICAL SIGNS

	Examination No.	*Control group*	*Experimental group*
Pellagrous lesions	1	0·0	13·6
	2	2·2	0·0
	3	0·0	0·0
	4	0·0	0·0
Skin signs	1	13·5	18·2
	2	15·2	25·0
	3	20·0	12·2
	4	33·3	32·7
Lip signs	1	11·5	28·8
	2	30·4	35·4
	3	22·2	26·5
	4	28·2	44·2
Tongue signs	1	1·9	1·5
	2	2·2	0·0
	3	0·0	0·0
	4	0·0	0·0

A number of children showed clinical signs compatible with a lesser degree of deficiency, such as crazy-paving over the shin area, slight hyperpigmentation and desquamation of the skin, cheilosis and atrophy of the villi of the tongue. The incidence of these clinical signs is given in Table IV. At no stage of the experiment, however, did the control and experimental groups differ significantly at a level of 0·0025 (0·25%).

Biochemical Results

A summary of the means and standard deviations (found during the various examinations numbered 1 - 4) for the biochemical assays of the urine samples from the control and experimental groups is given in Table V. In considering these results, it should be borne in mind that enriched maize meal was available to the experimental group at the time of the second and third examinations.

Statistical analyses of the biochemical data are given in Tables VI and VII.

The incidence of low excretion values for riboflavin,[23] N^1-Me, 2-pyridone and the 2-pyridone/N^1-Me ratios[24] are given in Figs. 5 - 8.

All the biochemical variables showed essentially the same trends. Although certain parameters (e.g. 2-pyridone) in the

TABLE V. MEAN VALUES FOR THE BIOCHEMICAL PARAMETERS

Variable	*Examination No.**	Control group *Mean*	Control group *SD*	Control group *Sample size*	Experimental group *Mean*	Experimental group *SD*	Experimental group *Sample size*
Riboflavin (μg/g creatinine)	1	294	240	52	174	140	66
	2	313	170	46	754	554	48
	3	301	186	45	792	572	49
	4	233	202	39	214	181	52
N^1-Me (mg/g creatinine	1	4·8	1·6	52	4·7	1·6	66
	2	5·4	1·6	46	6·1	2·2	48
	3	5·9	2·2	45	7·0	2·2	49
	4	4·7	1·3	39	4·3	1·5	52
2-pyridone (mg/g creatinine)	1	1·77	2·77	52	0·54	1·42	66
	2	4·86	4·29	46	8·76	6·09	48
	3	4·56	3·14	45	7·58	4·30	49
	4	3·42	3·05	39	2·49	2·63	52
2-pyridone/N^1-Me ratio	1	0·33	0·46	52	0·10	0·30	66
	2	0·84	0·67	46	1·50	0·99	48
	3	0·78	0·50	45	1·30	1·53	49
	4	0·67	0·51	39	0·59	0·65	52

*Examination no.: 1 = before supplementation; 2 = after 95 days of supplementation; 3 = after 137 days of supplementation; 4 = 68 days after supplementation was stopped.

TABLE VI. STATISTICAL TESTS FOR DIFFERENCE BETWEEN CONTROL AND EXPERIMENTAL GROUPS

	Experiment No. 1	2	3	4
Riboflavin/g creatinine	*	*	*	—
N^1-Me/g creatinine	—	—	—	—
2-pyridone/g creatinine	*	*	*	—
2-pyridone/N^1-Me	*	*	—	—

*Significant at a level of 0·003125.

TABLE VII. STATISTICAL TESTS FOR DIFFERENCES IN TIME

		1:2	2:3	3:4	1:4
Riboflavin/g creatinine	Control	—	—	—	—
	Experimental	*	—	*	—
N^1-Me/g creatinine	Control	—	—	*	—
	Experimental	*	—	*	—
2-pyridone/g creatinine	Control	*	—	—	—
	Experimental	*	—	*	*
2-pyridone/N^1-Me	Control	*	—	—	*
	Experimental	*	—	*	*

*Significant at a level of 0·001562.

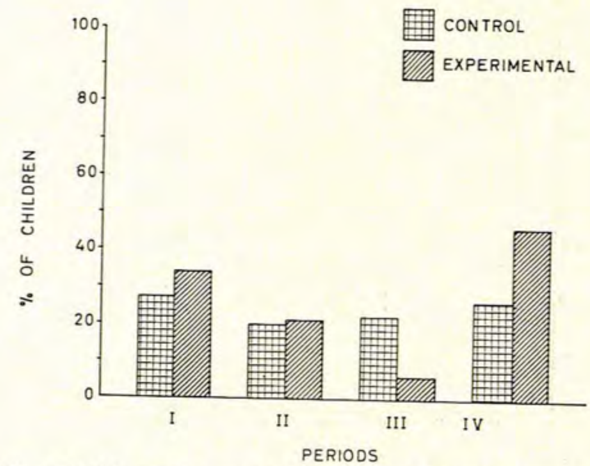

Fig. 6. Incidence of low N^1-Me excretion values (< 4 mg/g creatinine)

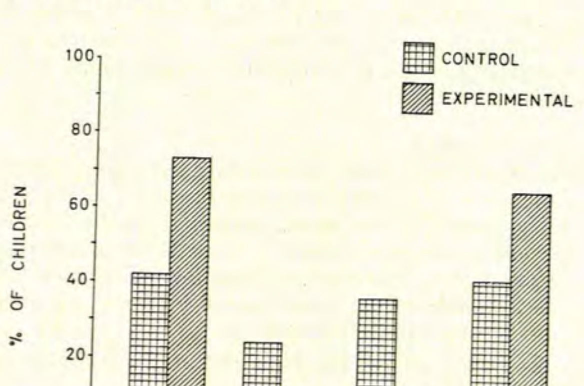

Fig. 5. Incidence of low riboflavin excretion values (< 200 μg/g creatinine).

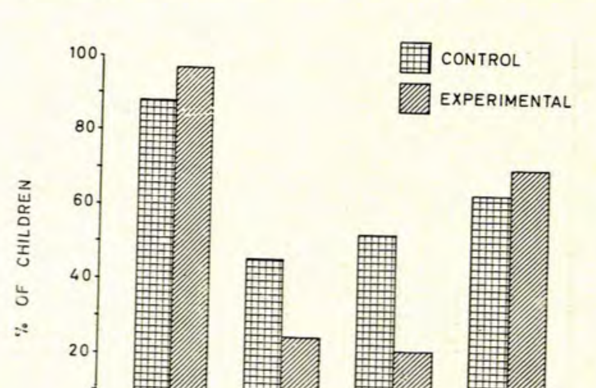

Fig. 7. Incidence of low 2-pyridone excretion values (< 4 mg/g creatinine).

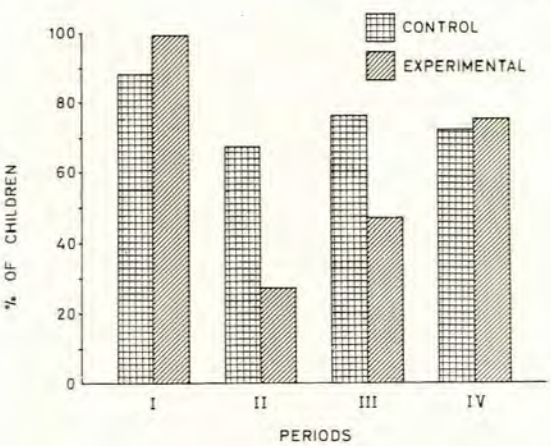

Fig. 8. Incidence of low 2-pyridone/N[1]-Me ratios (ratios <1).

control group also showed a fluctuation during the experimental period, the change that took place in the experimental group was much more pronounced (Table V). The fact that the experimental group did respond to the enriched maize meal is confirmed by the statistical analyses (Tables VI and VII).

In considering the statistical results given in Table VI, where the control and experimental groups are compared, the direction of the significant differences found should also be taken into account.

At the first measurement the experimental group had lower values than the control group; at the time of the second and third measurements, however, the values obtained for the experimental group were higher than those of the control group.

Economic Evaluation

The cost of the premix was calculated by Roche Products and each kilo of premix contained the following:

Riboflavin 3·600 g @ R17 per kg	= R0.0612
Nicotinamide 35·950 g @ R3 per kg	= R0.10785
Maize meal 960·450 g @ R4 per 81·54 kg	= R0.0525
	R0.2212

Import duty of 10% was included in the cost of the two vitamins.

In order to enrich 22·8 million 180-lb bags of maize meal *per annum*, approximately 1 300 metric tons of premix would be required.[25] It was estimated that, in providing such a large quantity of premix, labour, mixing costs, storage, etc., would add between 30 and 50% to the basic cost of 22·12 cent/kg. The cost of the premix could, therefore, be anything between 28·756 and 33·18 cent/kg (1·64 - 1·89 cent per 180-lb bag of enriched maize meal).

With the collaboration of the Division of Food Technology, National Institute of Food Research, CSIR, the Maize Board calculated the cost of adding the premix to the maize meal. The basis upon which cost was calculated for the operation of a feeder, is as follows:

Repayment over 10 years at 10% interest on a capital outlay of R785 (cost of feeder, installation and a 10-ft mixing screw)	R127.75
Running costs (¼ hp motor)	10.00
Maintenance	10.00
Labour costs (¼ hour per day) on basis of 8-hour shift per day and salary of R3 600 *per annum*	112.50
Total	R260.25

On an average, the mills work 2 shifts of 8 hours per day for 22 days a month, which is equivalent to 352 working hours per month. The fixed cost per hour per apparatus is thus $\frac{260{\cdot}25 \times 100}{12 \times 352} = 6.161$ cent.

Since the feeding apparatus has a capacity of more than 1 000 bags an hour and the milling rate in South Africa nowhere exceeds 200 bags per hour, the number of feeders required will be determined by the number of millstreams in each mill. Calculated on this basis, the maximum number of feeders required for mills of various sizes is given in Table VIII. Costs were calculated for 32 mills, selected so as to be representative of all commercial mills in the country. The results of this costing are given in Table IX.

TABLE VIII. NUMBER OF FEEDERS REQUIRED IN RELATION TO MILLING CAPACITY

Capacity of mill in bags per hour	*Maximum No. of feeders required*
1 - 25	1
26 - 50	2
51 - 100	3
101 - 150	4
151 - 200	5

TABLE IX. CALCULATED COST PER BAG IN RELATION TO MILLING CAPACITY

Capacity of mill in bags per hour	*No. of mills*	*Fixed cost in cents per bag*
1 - 50	17	0·317
51 - 100	9	0·229
101 - 200	6	0·205
	Average	0·242

The addition of these fixed costs per bag to the cost of the premix results in a minimum cost of 1.844 cent per bag and a maximum cost of 2.209 cent per bag. To these sums should be added the cost of transporting premix from the manufacturer to the various mills. It is, however, unlikely that, even in the remotest parts of the country, the total cost will exceed 2.500 cent per bag.

DISCUSSION

Technologically this experiment proved to be fairly simple. Once the feeder instrument had been calibrated and correctly set, it continued to run consistently well with the minimum of attention. Roche Products reported that no problems were encountered in preparing about 240 kg of premix at a time. The premix was found to be homogeneous and, when assayed, found to contain a quantity of vitamins very close to the correct amount. The premix was strongly orange-coloured, but this colour fortunately completely disappeared after admixture with maize meal.

Only the Zion Christian Church Council (as owners) and the manager of the mill at Boyne were informed of the purpose of the experiment and what the premix added to the maize meal contained. The manager of the mill reported that there was no fall-off in demand and it can, therefore, be assumed that the enriched maize meal was acceptable and that the colour and taste of the meal had not been affected.

Analyses of the enriched maize meal showed a certain amount of fluctuation in the added vitamin content (Tables I and II). These fluctuations were not considered to be excessive. However, it was strange that the ratio of riboflavin to nicotinamide (1:10 weight by weight) in the premix, was not reproduced upon analysis of the final product. The enriched maize meal, instead of containing an additional 1 mg riboflavin and 10 mg nicotinamide in 400 g maize, was found to contain only an additional 0·77 mg of riboflavin and 11·04 mg additional nicotinamide. This corresponds to a ratio of 1:13, weight by weight. Although riboflavin is the less stable vitamin of the two, a re-assay of the same samples after 4 - 5 months' storage showed no detectable decrease in riboflavin content. The instability of riboflavin cannot, therefore, explain why relatively less riboflavin was found to have been added than nicotinamide. The assays on the maize samples were carried out by two technicians working independently. It is possible, however, that there was an inherent error in the assay technique used. At this stage there is no conclusive explanation for this finding.

A comparison of weights and heights for age in relation to the Boston percentiles showed that the degree of malnutrition was very similar in the control and experimental groups (Figs. 1 - 4). This was confirmed by the results obtained from the application of the Goméz classification of the degree of malnutrition according to weight (Table III). It is clear, however, that the nutritional status of the children used in this experiment was substantially better than that of the children used in the preceding experiment.

The incidence of abnormal clinical signs recorded did not change significantly during the experiment, which was in contrast to the findings of the previous experiment. This may be partly due to a difference in intake. During the first experiment vitamin tablets were given, while in the second experiment intake would have varied according to the consumption of maize meal. It is also possible that the minor skin and mucous membrane lesions are partly acquired as a result of factors other than riboflavin and nicotinamide deficiency. Although the incidence of pellagrous lesions was not statistically significant at the level of 0·0025 (0·25%), it is nevertheless considered important to note that such lesions decreased from 13% to 0% in the experimental group when the enriched maize meal was introduced.

Although the assay of the enriched maize meal showed less additional riboflavin than expected, the response of the children to riboflavin enrichment was more pronounced than it was to nicotinamide. Whereas the excretion of riboflavin in the control group remained more or less constant at approximately 300 μg/g creatinine throughout the experiment, the experimental group excreted roughly 550 - 600 μg/g creatinine more during the period of enrichment than before or after (Table V). This is also reflected by the decrease in the incidence of low excretion values, which dropped from 73% to 8 - 10% in the experimental group (Fig. 5). In the experimental group, however, a small proportion of the children were found who, throughout the experiment, showed no marked changes in riboflavin excretion. There can be no absolute certainty that all the children were consuming enriched maize meal; certain families could have been buying maize meal from Pietersburg or shops not obtaining their meal from Boyne Roller Mills and it is quite possible that the majority of the 8 - 10% of children who did not respond, belonged to such families. The quantities consumed might also have varied considerably. It must be borne in mind, moreover, that a deficiency of these two vitamins is basically a problem of high maize consumption and that, with enrichment, the quantity of additional vitamins will increase as maize consumption increases. From the biochemical results, it can be deduced that the level of riboflavin supplementation was adequate and most effective in reducing the incidence of biochemically detectable deficiency.

The results obtained for the excretion of nicotinic acid metabolites were not quite as clear-cut as they were in the case of riboflavin. Nevertheless, the incidence of low 2-pyridone values decreased from 97% to 22% in the experimental group while the incidence in the control group changed from 88% to 46% (Fig. 7). The incidence of low N^1-Me excretion decreased from 34% to 6% in the experimental and from 27% to 20% in the control group (Fig. 6).

After enrichment had ceased, the marked rise which occurred in the incidence of low values for both parameters in the experimental group strongly suggests that enrichment had a marked influence on the level of nicotinic acid nutrition. The mean excretion levels of the two metabolites changed much less markedly in the control group (Table V).

As in the preceding experiment, there was found to be a tendency for the incidence of a low 2-pyridone/N^1-Me ratio to decrease and subsequently to rise (Fig. 8). Although no explanation can be given for this, it is considered to be of minor importance, since the levels of both metabolites were much higher while the children were on the enriched maize meal (Table V).

The results for these metabolites further strengthened suspicions that a small number of children were probably not consuming enriched maize meal.

It may be concluded with a reasonable degree of certainty that the amount of nicotinamide in the enriched maize meal was adequate and effective in reducing the incidence of subclinical deficiency.

Statistics from the Maize Board show that 29·5 million 200-lb bags of maize were used for human consumption during the year 1968 - 1969.[25] During the same year 19 million 200-lb units of maize meal were produced by commercial millers and it was estimated that a further 1·5 million units[26] of maize which had been held back on farms, Bantu reserves, etc., were also processed at commercial maize mills. A total of 20·5 million 200-lb units (22·8 million 180-lb bags), or 70% of maize used for human consumption could, therefore, be supplemented at the milling point.

Throughout the country, with the exception of the Transkei, maize meal is the maize product most commonly used. In the Transkei, however, maize meal constitutes only 27% of maize used for human consumption, the balance mostly being sold as whole maize.[26]

If the proposed vitamin supplementation scheme is introduced as a compulsory scheme, on a nation-wide basis, the economic implications need to be considered. If the cost of supplementation were borne entirely by the consumer, on the basis of an estimated 2.5 cent per 180-lb bag of maize meal and a consumption of 400 g per day, it would mean an additional 4.47 cent per head per year. According to the Maize Board, 22·8 million 180-lb bags of maize meal were used for human consumption during the year 1968 - 69.[25] The total annual cost of supplementation would thus be R570 000. When the benefits likely to accrue to the national health by the introduction of such a scheme are taken into account, the costs, to the consumer or to the State or both, become negligible.

It is known that clinical pellagra has a high incidence among Bantu adults and children, and biochemical analyses show an even higher incidence of subclinical deficiency of both nicotinic acid and riboflavin, as might be expected. Although it is recognized that pellagra is not a condition caused merely by vitamin deficiency, it is nevertheless likely that vitamin supplementation would be of considerable value towards improving the present situation.

CONCLUSIONS AND RECOMMENDATIONS

After a comprehensive investigation of the possibility of enriching maize meal with riboflavin and nicotinamide, the following conclusions may be drawn:

(*a*) that there is a need for such supplementation;

(*b*) that the quantities of vitamins added to maize meal were sufficient to be effective at least according to biochemical evaluation;

(*c*) that, through enriching maize meal, that section of the population most in need will be effectively reached;

(*d*) that such a scheme is technologically and economically feasible;

(*e*) that such maize meal enrichment does not change the appearance or taste of the meal and that consumer resistance is unlikely to be encountered.

It is strongly recommended, therefore, that a compulsory national maize meal enrichment scheme should be introduced with the least possible delay. The costs involved in such a scheme can be borne by the consumer or by the State or shared between them.

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