IRON FORTIFICATION TRIAL : RESULTS OF A TWO YEAR TRIAL IN NORTHEAST THAILAND*

การควบคุมโรคซีดจากการขาดธาตุเหล็ก : ผลการศึกษาติดตามเป็นเวลาสองปี ในภาคตะวันออกเฉียงเหนือของประเทศไทย

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ABSTRACT

Iron fortification trial was undertaken for a duration of two years in Amphoe Uthumphon Phisai, Changwat Si Sa Ket A total population of 4,242 in 6 villages was included in the study and a group of females age 15-45 years of which the subgroups were 124 placebo, 129 fortified salt and 165 combined fortified salt and fortified fish sauce, was selected for paired study. The salt was fortified at a concentration of 1 mg Fe/g with iron complex ($FeSO_4$:SHMP : $NaHSO_4$ at 5:4:3) and 12 months later at 1 mg Fe/g with FeNaEDTA while the fish sauce at 0.25 mg Fe/ml with FeNaEDTA. After a continuous and regular administration and periodic

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observations throughout the two years the paired results were assessed by changes in haemoglobin levels, and group comparison by changes in serum ferritin values.

Analysis of co-variance and regression lines showed significant rise of mean changes of haemoglobin concentrations in the two treated groups. The effects of iron fortification as calculated at 25th percentile of the population from cumulative frequency distribution of haemoglobin at pre-and post-treatments of the two treated groups were 0.6 and 0.8 g/dl while those of the efficacies of fortified salt and fortified salt plus fortified fish sauce were 37.1 and 34.8% respectively. The slopes and intercepts of the regression lines fitted for each of the two treated groups were similar so that it could be concluded in this trial that iron fortification of salt or of both salt and fish sauce were effective and that the effects of fortification by salt alone or combined fortified salt and fortified fish sauce were not significantly different either expressed as slopes or as the elevation of haemoglobin concentrations. The findings were substantiated by the improvement of body iron stores in the two treated groups as assessed by serum ferritin determinations.

บทคัดย่อ

การศึกษาเพื่อควบคุมโรคซีดจากขาดธาตุเหล็ก ใน 6 หมู่บ้านของอำเภออุทุมพรพิสัย จังหวัดศรีสะเกษ ซึ่งมีอุบัติการซีดจากขาดธาตุเหล็ก ประมาณร้อยละ 50 และ 40 ในเพศหญิงและชาย ตามลำดับ ภายหลังการสำรวจภาวะทางเศรษฐกิจของประชากรกลุ่มนี้ซึ่งปรากฏว่ามีฐานะค่อนข้างยากจนแล้ว จึงศึกษาวิเคราะห์ตัวอย่างอาหารในหมู่บ้าน พบว่า มีปริมาณของธาตุเหล็กต่ำ ชาวบ้านติดเชื้อพยาธิปากขอในระดับไม่รุนแรง และระดับปานกลางประมาณร้อยละ 50 และพบว่าการติดเชื้อพยาธิปากขอในระดับนี้ไม่มีอิทธิพลต่อภาวะขาดธาตุเหล็กของประชากรในหมู่บ้าน เพราะพวกมีพยาธิหรือไม่มีพยาธิก็ขาดธาตุเหล็กเช่นเดียวกัน

ได้แบ่งกลุ่มทดลองเป็น 3 กลุ่ม คือ กลุ่มควบคุม กลุ่มได้รับเกลือเสริมธาตุเหล็กเพียงอย่างเดียว และกลุ่มได้รับเกลือเสริมธาตุเหล็กร่วมกับน้ำปลาเสริมธาตุเหล็ก ภายหลังการแจกเกลือและน้ำปลาให้กับกลุ่มทดลองติดต่อกันเป็นเวลา 2 ปี การประเมินผลด้วยการวัดระดับความเข้มของเฮโมโกลบินและระดับเฟอร์ริติน พบว่า กลุ่มได้รับอาหารเสริมธาตุเหล็กทั้ง 2 กลุ่ม มีการตอบสนองต่อปริมาณธาตุเหล็กที่เพิ่มขึ้นในอาหาร ทำให้มีระดับเฮโมโกลบินและระดับเฟอร์ริตินเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม การศึกษาผลของการเสริมธาตุเหล็กจากกราฟความถี่สะสมของเฮโมโกลบินพบว่า กลุ่มได้รับเกลือเสริมธาตุเหล็กเฮโมโกลบินเพิ่มขึ้น 0.6 กรัมเปอร์เซ็นต์ และกลุ่มที่ได้รับเกลือและน้ำปลาเสริมธาตุเหล็กเพิ่มขึ้น 0.8 กรัมเปอร์เซ็นต์ เมื่อคิดประสิทธิผลของการศึกษาครั้งนี้จะได้เป็นร้อยละ 37.1 และ 34.8 สำหรับกลุ่มเกลือเสริมธาตุเหล็กและกลุ่มเกลือ–น้ำปลาเสริมธาตุเหล็ก ตามลำดับ อย่างไรก็ตาม การทดสอบทางสถิติพบว่า กลุ่มได้รับการเสริมธาตุเหล็กทั้ง 2 กลุ่มไม่มีความแตกต่างกัน แต่โดยการวิเคราะห์ข้อมูลเฉพาะในกลุ่มผู้ที่มีค่าเฮโมโกลบินเพิ่มขึ้นที่สนองตอบการเสริมธาตุเหล็กเท่านั้น พบว่า มีค่าเฉลี่ยเฮโมโกลบินเพิ่มขึ้นถึง 1.16 กรัมเปอร์เซ็นต์ ในกลุ่มเกลือ–น้ำปลาเสริมธาตุเหล็กเมื่อเทียบกับกลุ่มเกลือเสริมธาตุเหล็กอย่างเดียวสูงขึ้นเพียง 0.71 กรัมเปอร์เซ็นต์ การประเมินผลของการเสริมธาตุเหล็กในเวลา 2 ปีนี้ได้ผลดี ทำให้ซีรัมเฟอร์ริตินในกลุ่มทดลองทั้งสองกลุ่มนี้ทวีขึ้นถึงเท่าตัว

INTRODUCTION

Iron deficiency with and without anaemia is widely prevalent in developing countries.[7,24,25] In Thailand the presence of anaemia varied from 30 to 60 % depending on localities, groups of target population and in particular economic status. The intervention programme of iron fortification preferably performed in the high risk population can be evaluated by studying the rise of haemoglobin concentration as well as replenishment of body iron stores as assessed by the measurement of serum ferritin in the target group and the results are to be compared to the placebo group.[18] This report presents preliminary findings on the effects and efficacies of a two year fortification trial after distributing iron fortified salt and combined iron fortified salt and iron fortified fish sauce in groups of females in villages of Changwat Si Sa Ket, with low income populations in the country. The present study involves the evaluation of changes in haemoglobin and serum ferritin concentrations prior to and two years after commencement of the field trial. The results of these studies showed that salt, combined salt and fish sauce fortified with iron were effective in increasing mean haemoglobin and serum ferritin levels of the population under study.

MATERIALS AND METHODS

Experimental design

Fortification trial was conducted for a duration of two years in subdistrict of Ban Kae, Amphoe Uthumphon Phisai, Changwat Si Sa Ket, northeastern Thailand. The subjects were 418 females, age 15 to 45 years, drawn from a total population of 4,242 from 6 villages of similar geographical, socio-cultural and economic status. The placebo and the treatment groups of population were selected from separate clusters of individuals after preliminary surveys to exclude subjects with haemoglobin concentration less than 8 g/dl, iron overload (serum ferritin more than 350 μg/l) as well as pregnant women and lactating mothers.[18] The initial sample size was more than 600 females or approximately 200 in each of the three study groups to allow for dropouts due to lack of co-operation, mobility to other places, being pregnant or lactating during the course of intervention.

By the end of the two year trial, the groups could be finally classified as 124 placebo, 129 and 165 treatment groups. The first treatment group had been given iron fortified salt in a concentration of 1 mg Fe/g of salt as iron complex (Fe (II) SO_4:SHMP:$NaHSO_4$ at 5:4:3) and, the second treatment group, – iron fortified salt at the same level of iron concentration and iron fortified fish sauce at a concentration of $\frac{1}{4}$ mg Fe/ml of fish sauce as ferric monosodium ethylene diamine tetra-acetic acid (Fe (III) NaEDTA). The iron fortified salt in the form of iron complex had to switch to FeNaEDTA at $\frac{1}{4}$ mg Fe/g of salt after 6 months of distribution due to development of dark color of food during cooking process which was unacceptable. The distribution of iron fortified products was under constant supervision of local health personnel.

Consumption and acceptability of the fortified products

The iron fortified products were distributed to villagers for few months for testing

the acceptability and estimating the amount consumed for each individual family of average size of 7 persons. The average consumption of salt and fish sauce was approximately 10 g/head/day and 10 ml/head/day respectively. Since the products were distributed on free of charge basis, the amounts distributed were then limited to these figures to prevent from taking more than needed. During the trial the amounts of the product consumed by each family were recorded on a consumption card for final calculation of the actual amounts of salt consumed.

Monitoring of iron fortified products

Monitoring to control distribution of the fortified and unfortified products were carefully recorded at regular intervals throughout the whole study period. At the production centre in Bangkok, the products were periodically checked for total iron content after the method described by Hallberg and Rasmussen.[3]

Collection of specimens and laboratory analysis

In each subject the haemoglobin concentration was measured initially and finally at two years after commencement of the trial. Venous blood samples were collected for haemoglobin determination, serum ferritin by radioimmunometric assay[2,8,10] and abnormal haemoglobin by cellulose acetate elution technique.[15]

Statistical treatment

The regressions between initial and final haemoglobin values of each group were compared and tested for differences in their slopes and intercepts.[14, 16] The response to iron fortification was determined by measurement of haemoglobin changes above their initial values of both treatment groups and placebo group. The analysis of co-variance was applied to obtain more precise comparison between groups by removing the effects of differences in their initial haemoglobin concentrations.

Finally the treatment effects and efficacies of the trial were estimated by plotting the cumulative frequency distribution of haemoglobin concentrations when admitted to and at the end of the trial in comparison to those of apparently healthy subjects. The numerical index was obtained from the cumulative curves at a 25th percentile of the population.

Comparison of mean initial and final serum ferritin of the placebo and treatment groups were calculated by Student's 't' test after logarithmic transformation for normal distribution (geometric means).

RESULTS

Consumption of salt and fish sauce

Data obtained from a monthly record on salt and fish sauce consumption over the

period of 24 months were tabulated in Table 1. The average consumption of unfortified and fortified salt was 10 g/head/day based on average family members of 7, and 6 to 8 ml/head/day of fish sauce either fortified or unfortified.

Hookworm infestation

The prevalence of hookworm infection was mild to moderate. The faeces were positive for hookworm ova in 61% of 118 villagers randomly sampled for Stoll's egg count[17] ; of this 50% had marginal infection having 1-1,000 eggs/g faeces and 11% moderate infection having 1,000-4,000 eggs/g faeces. There was no evidence of differences in haemoglobin concentrations between the normal cases with negative egg counts and the marginal or moderate hookworm infected groups (Table 2).

Haematological characteristics

Complete blood profile of Si Sa Ket villagers as compared to apparently healthy subjects in Bangkok were shown in Table 3. These values were obtained from preliminary survey for haematological characteristics.[4,5,6] The overall incidence of anaemia in 6 villages were shown in Table 4.

In comparison with Bangkok subjects, the blood profile of Si Sa Ket subjects showed no definite decrease of the parameters in males. In women, there was a definitive decrease in haematocrit, haemoglobin and transferrin saturation levels, while the total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and free erythrocyte protoporphyrin (FEP) were elevated. The serum ferritin values in Si Sa Ket subjects were extremely low, – about one third of the Bangkok levels. However, all parameters except most of serum ferritin values were within normal limits.

Evaluation of fortification trial

Table 5 showed mean initial and final haemoglobin concentrations of the three study groups. In testing for differences in mean initial values of haemoglobin concentration of the three groups, the results showed significant difference by one way analysis of variance ($F > 1$). The results of the trial were then compared by analysis of co-variance to remove the effects of differences in the initial haemoglobin concentrations (Figure 1).

After fitting regression lines to the three groups, – placebo (Group 1), and the two treatment groups (Group 2: iron fortified salt and Group 3: iron fortified salt plus iron fortified fish sauce), a common slope of each regression line was calculated and tested for statistical differences among these three groups. From the results of analysis of co-variance, it could be seen that the slopes differed significantly from each other ($F = 19.1443$, $p < 0.001$). After adjusting means for the differences in initial haemoglobin concentrations for each individual, the intercepts of regression lines were then compared. The results showed that the intercepts of the two treatment groups differed significantly from the placebo ($F = 12.0837$, $p < 0.001$) but there was no difference between the two treatment groups ($t = 0.163$) nor the intercepts ($F < 1$). It could be con-

cluded that in this trial iron fortified salt and iron fortified salt plus iron fortified fish sauce were equally effective in increasing haemoglobin concentrations.

The analysis of co-variance for group 1, 2 and 3 was tabulated in Table 6 and , for group 1 and pooled data of group 2 and 3 in Table 7. The estimated regression equations were $y = 3.159 + 0.736x$, $y = 5.670 + 0.5608x$ and $y = 5.726 + 0.5512x$ for groups 1, 2, and 3 respectively, and $y = 5.704 + 0.552x$ for pooled data from group 2 and 3.

Body iron stores of the placebo and treatment group

Body iron store of the placebo and the two treatment groups at pre-test and 2 years after the iron fortification were shown in Figure 2. Improved body iron status of paired subjects as determined by immunoradiometric assay of serum ferritin was evidenced by increase in mean serum ferritin values after 2 – year trial; 8.62, 71.5 and 89.1 % for groups of placebo, iron fortified salt and combined iron fortified salt and iron foritfied fish sauce respectively. By Student's 't' test, the mean increase in serum ferritin levels was highly significantly different from the placebo ($p < 0.001$).

Effects and efficacies of the iron fortification trial

Figure 3a, 3b and 3c showed cumulative frequency distribution curves of treatment population as compared to the normal before and after the intervention programme. The effects (B-A g/dl) and efficacies (B-A)/(C-A) × 100% as determined at the 25th percentile of the population were shown in Table 8. The treatment effects in groups receiving iron fortified fish sauce were 0.65 and 0.8 g/dl and the efficacies were 37.1 and 34.8% respectively. The individuals who showed good response to the iron fortification or "responders" were 61.4 and 69.1% and the mean haemoglobin increase in responders were 0.71 and 1.16 g/dl for group 2 and 3 respectively.

DISCUSSION

The actual consumption of salt and fish sauce both fortified and unfortified obtained from the family record cards during the whole study period were tabulated in Table 1. The figures were quite similar to those obtained from the short term test for their acceptability in adults; on the average of 10 g of salt/head/day. As for the fish sauce, the actual consumption as recorded in the family consumption cards was 7 to 9 ml/head/day vs 10 ml/head/day by the acceptability test.

The daily iron intake was 7 mg/head/day which is half of those found among subjects in central region.[13] The high prevalence of anaemia of 56.2% and depleted body iron stores evidenced by low serum ferritin of less than 15 μg/l and high absorption of iron from standard reference dose in accompany with mild hookworm infection (Table 2) confirmed that these low economic population groups were at risk for iron deficiency with and without anaemia. The haematological characteristics of the population under study (Table 3) were also substantiated that the most prominent finding was serum ferritin values of about one third of the Bangkok levels and suggested that this population was suitable for the field trial. However, it should be

noted that the mean initial value of haemoglobin in females was less than the level for normal healthy females of 12 g/dl.[21,22]

The present study was designed to conform to human nature as much as possible in order to create no disturbances of usual daily life and environments by giving adequate education transfer with minimal incentive to enable re-applicability if feasible. However, we have found that during the first 9 to 12 months of distribution of the fortified products, villagers were enthusiastic and very co-operative and then become less so during the rest of the following years. The results as previously evaluated in the first 9 months in a limited number of subjects were encouraging, the mean increases in haemoglobin and serum ferritin concentrations were significantly higher than the initial values especially in females.[22]

The achievement of any field trial depends very much on the consumer acceptability. It was found from the surveys that in order to prevent unacceptable darkening of its color, the fortified dose for fish sauce had to be reduced to $\frac{1}{4}$ mg Fe/ml of its original concentration of 1 mg Fe/ml at the 6^{th} month of fortification. For salt we had to switch from iron complex to FeNaEDTA because of the resulting smell of sulphur and development of dark color when salt fortified with iron complex was used to preserve food and vegetables and again the fortifying dose had to be reduced to $\frac{1}{4}$ mg Fe/g of salt.

Non-paired comparison showed only 12.8% reduction of overall prevalence of anaemia as assessed by haemoglobin concentration (Table 4). The lower the haemoglobin values at pretest the better response were obtained as can be seen more obviously at the haemoglobin levels of less than 8 and between 8 to 10 g/dl. The assessment by serum ferritin concentration showed 40.8 % reduction of iron depleted cases having serum ferritin values less than lower limits for normal females (<13 µg/l). However, at least the iron repletion accomplished in this fortification study for iron depleted individuals was significantly convincing.

Statistical treatment of the fortification trial by analysis of variance and by fitting a regression line of the final haemoglobin concentration on the initial haemoglobin concentration of the placebo and tested groups shown in Tables 6, 7 and Figure 1. The non-parallel regression lines of the tested groups to the placebo group indicated that the effect of treatment were different at different levels of initial haemoglobin concentration. Both iron fortified salt and combined iron fortified salt plus iron fortified fish sauce were equally effective in increasing haemoglobin concentration as shown by a definite elevation of the regression lines over that of the placebo (Figures 1a and 1b). The estimated effect of the trial (Table 8 and Figure 3) as shown by the shift of haemoglobin concentration toward the normals at 25^{th} percentile of the population were 0.65 and 0.8 g/dl for groups received iron fortified salt and combined iron fortified salt and iron fortified fish sauce respectively. The numerical indices of efficacy were 37.1 and 34.8 % accordingly. Among the subjects who received iron fortified salt and/or fish sauce, the number of responding individuals were 61.4 and 69.1 % and, the mean haemoglobin increase was 0.71 and 1.16 g/dl respectively.

Serum ferritin values appeared to be a strong confirmatory parameter in showing the significant response to iron fortification. The evaluation of the trial confirmed improvement by 71.5 and 89.1 % of the originally existed body iron store in the two tested groups ($p<0.001$). However, after the trial, mean serum ferritin values in these groups of population were still in the

low normal range approximately half of those found in Bangkok females while the values at pre-test were only one third of the Bangkok level.

Nevertheless, it might be concluded that the trial produced moderately effective results for the selected target group.[20]

Although the mean increase in haemoglobin concentration was not impressive, the repletion of body iron stores or levels of serum ferritin were significantly convincing. The degree of response depends on many factors, the most important of which are in order: how high the initial haemoglobin and serum ferritin values, how regular the administration of the fortified products at consistently high enough fortifying dose of iron or the constant acceptance of the consumers, and how high the bioavailability of iron compounds mixed in a definite recipe of food ingredients.[1,9,12,19] Recent studies in India indicate that the fortification of common salt is technically feasible and field trial has shown a good haematological response.[11,26] Similarly, preliminary analysis of an extensive field trial by fortifying refined sugar with NaFeEDTA in Guatemala has shown significant improvement in iron status.[23] However, there are many factors that may contribute to fail to demonstrate a beneficial effect of fortification for a variety of reasons as previously mentioned.[1] Field trial is the more costly and a long term effective program. The trial may fail because the fortified product is not well accepted by the population as well as pitfalls related to the design itself. We realized from this intervention ourselves the facts that to obtain the results of the trial as evaluated in 2 years takes time, expenditure and much efforts until the time that the effects of the fortification would be demonstrable. In this particular case, many variables mostly unanticipated had repeatedly arisen.

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Table 1. Average consumption of salt and fish sauce both fortified and unfortified as obtained from a monthly record over the period of 21 to 24 months in the villages under studies.

Village	Food vehicle	Number of families*	Consumption ** mean ± SD	Range
6	Unfortified salt	98	10.7 ± 2.85	7.9 – 13.6
1,3,4,5	Iron fortified salt ($\frac{1}{4}$mg Fe/g)	181	10.1 ± 3.36	6.8 – 13.5
2	Iron fortified salt ($\frac{1}{4}$mg Fe/g)	76	10.2 ± 3.32	6.8 – 13.5
	Iron fortified fish sauce ($\frac{1}{4}$ mg Fe/ml)	72	8.1 ± 3.04	5.1 – 11.1
2	Unfortified salt	76	8.6 ± 2.81	5.8 – 11.4
	Unfortified fish sauce	74	6.9 ± 2.35	4.5 – 9.2

* Average family member of 7

** g/head/day for salt and ml/head/day for fish sauce

Table 2. Hookworm infection and haemoglobin concentration in 118 villagers.

Group	Sex (M,F) (number)	Haemoglobin (g/dl) mean ± SD	P-value *
Negative egg counts	F(19)	11.71 ± 1.91	–
	M(24)	13.26 ± 1.64	–
Marginal infection	F(33)	11.90 ± 1.38	NS
	M(19)	13.36 ± 1.33	NS
Moderate infection	F(9)	12.16 ± 1.01	NS
	M(5)	13.92 ± 1.21	NS

*P-values were obtained by comparison to group with negative egg counts using the unpaired Student's 't' test.

NS = not significant

Table 3. Blood profile of 189 Bangkok subjects and 138 villagers in Changwat Si Sa Ket (Mean ± SD).

Parameter	Bangkok		Si Sa Ket	
	Male (n = 45)	Female (n = 144)	Male (n = 37)	Female (n = 101)
Age (years)	27.7 ± 6.36	23.3 ± 6.58	37.0 ± 6.8	35.2 ± 6.8
Haematocrit (%)	44.4 ± 2.20	41.0 ± 2.46	40.2 ± 3.5	35.4 ± 3.5
Haemoglobin (g/dl)	14.8 ± 0.87	13.8 ± 0.90	13.4 ± 1.35	11.9 ± 1.33
Serum iron (μg/dl)	107.0 ± 27.96	107.9 ± 21.97	125.5 ± 63.4	105.6 ± 59.9
UIBC (μg/dl)	251.3 ± 36.27	260.3 ± 34.57	265.5 ± 65.2	286.7 ± 87.7
TIBC (μg/dl)	365.0 ± 50.58	369.1 ± 46.47	391.3 ± 97.3	394.0 ± 99.0
Transferrin saturation (%)	29.7 ± 5.57	29.1 ± 6.20	29.6 ± 10.1	25.5 ± 11.0
Red Cell protoporphyrin (μg/ml)	–	22.8 ± 7.09	33.9 ± 15.1	37.6 ± 16.3
Serum ferritin* (μg/l)	81(21-314)	48 (13-173)	32 (5-192)	15 (3-80)

*Geometric mean values (±2SD given in parenthesis) from 209 male, 503 female villagers in Si Sa Ket and 45 male, 200 female Bangkok subjects

Table 4. Haemoglobin concentrations, serum ferritin values and prevalence of anaemia in non-paired population from 6 villages at pre-test and 2 years after the trial.

	Pre-test		2 years	
	n	%	n	%
Haemoglobin (g/dl) Female				
<8	26	5.27	9	1.80
8 - 10	87	17.65	49	9.88
10 - 12	164	33.30	185	37.30
>12	277	56.19	243	48.99
Total	493	100.0	496	100.0
Serum ferritin (μg/ l) <13	503	41.9	496	24.8

Table 5. Mean initial and final haemoglobin concentrations of the three study groups.

Groups	Initial Hb (g/dl) mean ± SD	Final Hb (g/dl) mean ± SD
1. Placebo	12.32 ± 1.31	12.23 ± 1.11
2. Fortified salt	11.65 ± 1.60	12.20 ± 1.16
3. Fortified salt + Fortified fish sauce	11.50 ± 1.69	12.06 ± 1.16

Table 6. Analysis of covariance (group 1, 2 and 3), comparison of regression lines.

Source of Variation	df	$(x-\bar{x})^2$	$(x-\bar{x})(y-\bar{y})$	$(y-\bar{y})^2$	Regression coefficient	Deviation from regression		
						df	SS	MS
Group 1	124	212.2439	156.2939	152.6423	0.7364	124	37.5494	0.3053
Group 2	129	330.1986	185.1870	150.6970	0.5608	128	46.8441	0.3659
Group 3	165	472.5024	260.4555	223.2562	0.5512	164	79.6931	0.4859
Sum of deviation from regression						415	164.0866	0.3954
Pooled within group	418	1014.9449	601.9364	536.5955	0.5937	417	179.2259	0.4298
Comparison between the slopes F = 7.5696/0.3954 = 19.1443, P <0.001 Slopes are highly significantly different.								
Between group	2	51.3487	8.3313	2.2963				
Total	420	1066.2736	610.2677	538.8918		419	189.6131	0.4525
Difference between adjusted means F = 5.1936/0.4298 = 12.0837, P < 0.001 The difference is highly significant.								

Table 7. Analysis of covariance, mean haemoglobin differences between group 2 and 3.

Source of variation	df	$(x-\bar{x})^2$	$(x-\bar{x})(y-\bar{y})$	$(y-\bar{y})^2$	Regression coefficient	Deviation from regression		
						df	SS	MS
Pooled group 2+3 only	294	802.7010	445.6425	373.9532	0.5552	293	126.5420	0.43188
Between 2 groups	1	1.5640	1.1489	1.3423				
Total	295	804.2649	446.7914	375.2955		294	126.7571	0.43115
Difference between adjusted means F = 0.21511/ 0.43115, F<1 No significant difference								

Note : By Student's 't' test, the slopes of group 2 and 3 are not significant different (t = 0.163).

Table 8. The effects and efficacies of iron fortification trial.

Group of subjects	Effects (B − A) g/dl	Efficacies $\{(B-A)/(C-A)\} \times 100\%$	Responders (%)	Mean Hb increase (g/dl)
1. Placebo	0.15	12.5		
2. Iron fortified salt	0.65	37.1	61.4	0.71
3. Iron fortified salt + Iron fortified fish sauce	0.80	34.8	69.1	1.16

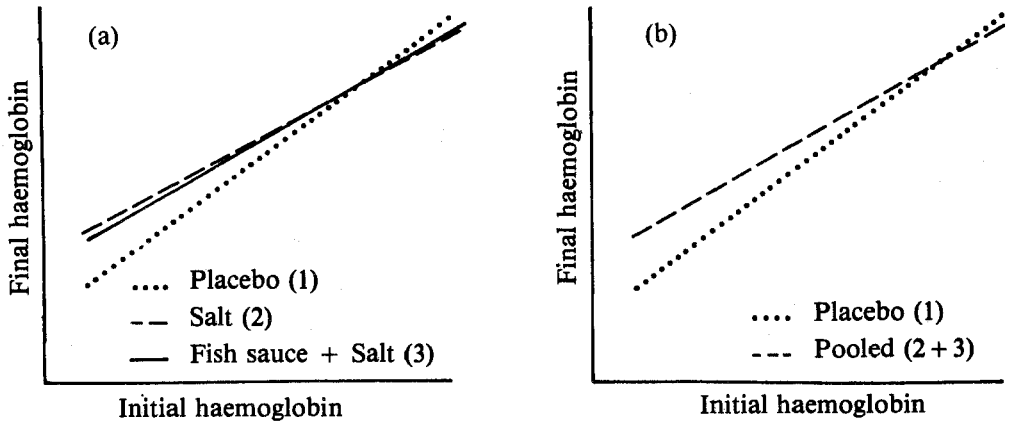

Fig. 1 Comparison of regression lines of the placebo and the two treatment groups (a) ...placebo, – – – fortified salt, — combined fortified salt and fortified fish sauce, (b)...placebo, – – – pooled data (fortified salt + combined fortified salt and fortified fish sauce).

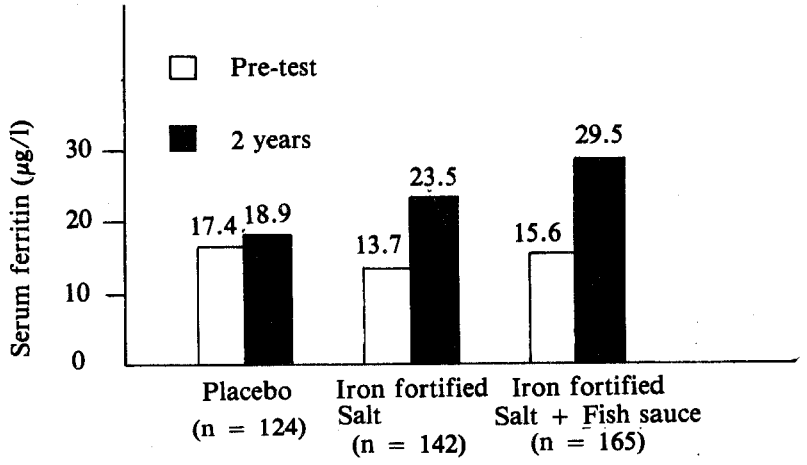

Fig. 2 Mean increase in serum ferritin levels in placebo and 2 treatment groups at 2 years after the trial (paired results).

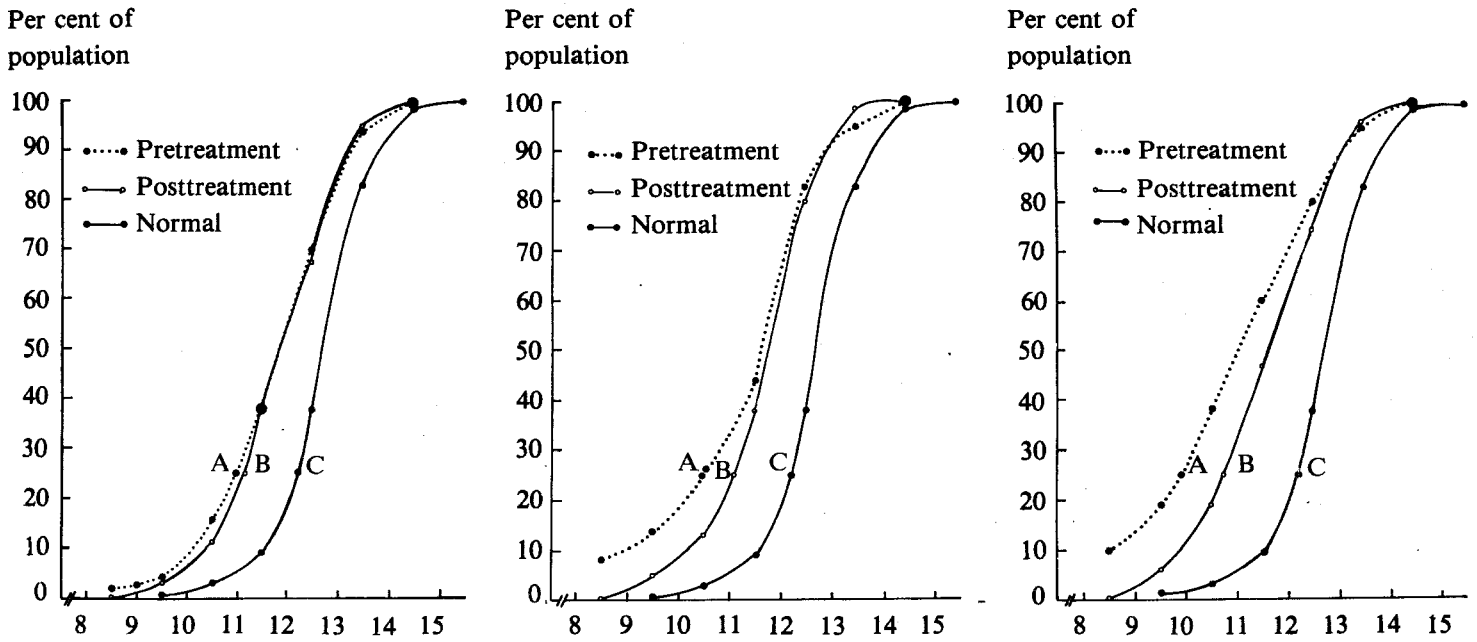

Fig. 3a : Placebo

Fig. 3b : Iron fortified salt

Fig. 3c : Iron fortified salt + Iron fortified fish sauce

Fig. 3 Cumulative frequency distribution curves of haemoglobin concentrations of the placebo and the two treatment groups before and after the intervention as compared to the normals (Curve C).