Efficacy trial of multiple micronutrient food supplement on children

Vinodkumar M.[1] , **Rajagopalan S.**[2]
[1] Managing Trustee, Sundar Serendipity Foundation, Chennai, India
E. Mail: vinodkumar_m_k@hotmail.com
[2] Distinguished Fellow, M S Swaminathan Research Foundation, Chennai, India

INTRODUCTION

Multiple Micronutrient deficiencies occur in many developing countries. It is very difficult to have multiple micronutrients in a single product as the multiple micronutrients interact with each other and there is a loss in potencies. This study overcomes these problems by encapsulating the multiple micronutrients so that they can be in a asingle product.

The aim of this study is to test the efficacy of a multiple micronutrient food supplement (MMFS) on the nutrition status of school children.

MATERIALS AND METHODS

In this study, we have tested the stability of the Multiple Micronutrient Food Supplement(MMFS) during storage, and during cooking. We have then tested the efficacy of the MMFS by using it in all the meals cooked for children in a residential school for a period of one year and tested the biochemical parameters like hemoglobin, red cell count and hematocrit.

Process of manufacture of the MMFS: When multiple micronutrients are used, they interact with each other leading to losses in potency. Iron is stable and best bioavailable in an acidic pH whereas Vitamin A is unstable and looses its potency in an acidic environment. To prevent such interactions, the micronutrients are microencapsulated to prevent interactions with each other. All ingredients used were of food grade.

Ferrous sulphate is the most bioavailable form of iron. However it is well known to interact with many vehicles of fortification or with other food ingredients during cooking by producing discolorations. Therefore ferrous sulphate (Heina Pharma, Mumbai, India) was chelated by us in our laboratory with chelating agents and the absorbtion promoter was further added to enhance the iron absorbtion. Ferrous sulphate was chelated with malic acid (Thirumalai Chemicals Ltd, Ranipet, Tamilnadu) and sodium hexameta phosphate (Sulux Phosphates, Mumbai). The acidic pH was maintained by sodium dihydrogen phosphate (Sulux Phosphates, Mumbai) which served as an absorbtion promotar. The resultant chelated iron complex was white in color. Vitamin A (Nicholas Piramal, Mumbai, India) which was supplied microencapsulated in gumaccacia and sugar was coated with butylated hydroxy anisole (P.D Fine Chem, Bangalore, India), butylated hydroxy toluene (P.D Fine Chem Bangalore, India) and tocopherol (Merck Ltd, Bangalore, India) to enhance the stability of vitamin A. Thereafter, it was encapsulated in cellulose acetate phthalate (GM.Chemicals, Mumbai, India) to protect it form the acidic environment (which is necessary for iron bioabsorbtion) and coated with a layer of silicone to provide heat resistance during cooking. The B complex vitamins B2 , B6 ((Romeda Chemicals Ltd , Mumbai) and B12 (Wockhardt Ltd Mumbai,India), niacin (Lasons India P Ltd, Mumbai, India) and folic acid (Shree Krishna Pharmaceuticals Ltd, Hyderabad) were coated together with glyceryl stearate (Om Corporation, Mumbai, India). Coated vitamin C (Amoli organics, Mumbai, India) was used for the study. Vitamin E oil (Merck Ltd, Bangalore, India) was adsorbed in silica and the powdered product was

used. Calcium pantothenate and lysine were used without any coating. All the above micronutrients were added to calcium carbonate (Ferrous Minerals and chemicals P Ltd, Mumbai) which served as the carrier. The nutrient composition of the MMFS is given in Table 1.

Ingredient	Nutrient composition	Nutrient status after cooking for 20 minutes	Loss in nutrient after cooking %	Nutrient status after one year of storage	Loss in nutrient on storage after one year %
Vitamin A IU/g	1500	1342	10.5	1099	26.7
Vitamin B2 mg/g	1	1	0	1	0
Calcium pantothenate mg/g	1	1	0	1	0
Niacin mg/g	15	15	0	14.94	0.4
Vitamin B6 mg/g	1	1	0	1	0
Folic Acid mcg/gm	100	99.49	0.51	99.5	0.5
Vitamin B12 mcg/gm	1	1	0	1	0
Vitamin E IU/g	30	29.93	0.23	29.9	0.33
Vitamin C mg/g	30	26.6	11.3	22.30	25.6
Iron mg/g	10	10	0	9.97	0.3
Lysine mg/g	250	241.9	3.24	249.56	0.18
Calcium %	15.63	15.63	0	15.63	0

Table 1: Nutrient composition of the multiple micronutrient food supplement (MMFS)and stability of the MMFS during cooking and storage.

Stability during cooking : A study was done to test the stability of all the micronutrients during cooking.(see table 1) The assays were carried out initially and after subjecting the micronutrients to typical Indian cooking conditions after adding them to an Indian dish- sambar(lentil soup) and cooking for 20 minutes. The required aliquotes from the lentil soup were taken for the analysis of micronutrients. Six samples were taken before and after cooking.

Samples of the MMFS were stored at 30 degrees C, humidity 45 %, for 12 months, (see table 1) and stability tests were done once in 3 months.

Bioefficacy Study: The Research Design was a pre- post test design with experimental (N=51) and control (N=72) groups. It was a randomized controlled trial. The children residing in the residential school constituted the experimental group. The children who lived in communities nearby and attended the day school constituted the control group. There was no intervention in the children belonging to the control group except deworming. The school where this study was conducted was chosen randomly from a list of schools that had residential school children and also admitted day scholars from communities around the school.

Study Procedure: The MMFS was used in all the meals cooked for the children in the experimental group for a period of one year. Deworming was done in both experimental and control children by giving a tablet of albendazole 400 mgs at baseline, after 6 months of intervention and

after one year of intervention (end of study).Deworming was done to ensure that there are no worms which compete for the micronutrients and ensures the intestinal tract is clear for bioabsorbtion of the micronutrients as in other studies (Olsen A., 2003 ; Taylor M., 2001).

The MMFS was supplied to the school every month and the continuous use of the MMFS in all the meals prepared everyday was monitored. The dosage of the MMFS was one gram per child per day. Since the number of children was known the MMFS was weighed and packed in packets which had to be cut and added to the meals cooked for the day. The MMFS was used in all the food preparations. It was dissolved in water and added to liquid food preparations in the final stages of cooking. It was sprinkled as such on solid food preparations. The MMFS did not change the color or taste of any food preparation. *Biochemical parameters:* Nutritional status was assessed by estimation of hemoglobin, hematocrit and red blood cell count. Hemoglobin estimation was carried out before the start of the study, six months after the commencement and one year after commencement (end) of the study. The hematocrit and red blood cell count were done before the study began (base line) and at the end of one year of the study (end).

Blood collection, storage and laboratory analysis : 2 ml of venous blood was drawn from each child. The blood was transferred into vials which had EDTA as anticoagulant. The tests for hemoglobin, hematocrit and red cell counts were carried out in the sample within a few hours of blood collection. The blood samples were transferred to the laboratory within 2 hours of collection at the school. Hemoglobin was estimated by cyanamethemoglobin method (Dacie J.V., 1995) Hematocrit was estimated by centrifuging blood in wintrobe tubes (Dacie J.V., 1995). Red blood cell count was done by counting the cells using the neubauer counting chamber (Dacie J.V., 1995). Hemoglobin was done in duplicate for all the samples. In hematocrit and RBC count estimations, in 10% of the samples the test was done twice for validation.

Statistics : Statistical analysis was done using SPSS 11.0 (SPSS Inc., Chicago IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle WA, USA). Repeat measures Analysis of variance was done to compare the effects of group*time for hemoglobin, hematocrit, and Red blood cell count . Students t test was done to analyze the effects between groups and paired students t tests was done to analyze the effects within groups.

RESULTS AND DISCUSSION

Baseline characteristics of the experimental and control groups: The experimental and control groups of children were similar in age, intelligence, nutrient intake and socioeconomic status.

Efficacy study-Biochemical parameters: There was a significant ($P < 0.05$) improvement in the experimental group in hemoglobin, hematocrit and red cell count whereas in the control group there was a statistically significant decline($P<0.05$) in hemoglobin and red cell count.(**See table 2**)

Cost versus benefit: The cost of the multiple micronutrient food supplement is Rs 225 (5.6 US $) for 1 kg. Each child was given 1 gram per day. So the cost per gram works out to 22.5 paise (0.0056 US $). Therefore the cost of delivery of the micronutrients through the food supplement costs 22.5 paise per child per day. The cost of the amino acid lysine is the major cost in this costing 10 paise per child per day. Without lysine the cost of delivery of the rest of the micronutrients per child per day works out to 12.5 paise (0.0031 US $). At a mere cost of 12.5 paise (0.0031 US $) per day, the

child gets 11 micronutrients. We feel that this is one of the most economical ways of delivery of multiple micronutrients.

Experimental group n=51			Control group n=72	
	Base line	Endline after one year	Base line	Endline after one year
Hemoglobin gms/dl*	9.98 +-0.75[a] (9.77-10.19)	10.23 +-0.60[a] (10.07-10.4)	10.43 +-0.83[b] (10.23-10.63)	10.13 +-0.74[b] (9.95-10.31)
Hematocrit l/l*	0.2872 +-0.018[a] (0.2823-0.2922)	0.3000 +-0.021[a] (0.2941-0.3059)	0.3062 +-0.022 (0.3010-0.3115)	0.3013 +-0.023 (0.2958-0.3069)
Red blood cells million/cmm	3.24+-0.22[a] (3.18-3.30)	3.43+-0.27[a] (3.35-3.50)	3.62+-0.49[b] (3.5-3.74)	3.47+-0.28[b] (3.4-3.53)

Table2: Biochemical status of the experimental and control groups over a period of one year.
a: significant improvement(P<0.05) from baseline to endpoint
b: significant decrease(P<0.05) from baseline to endpoint
Data given as mean+-SD
*** ANOVA repeat measures Significant (P<0.05) group*time. 95% Confidence interval for mean with lower bound and upper bound values given in brackets.**

CONCLUSION

The Multiple Micronutrients are stable in the MMFS because they have been encapsulated and the micronutrients are, therefore, stable during cooking and storage and bio-available as seen in the efficacy study.

REFERENCES

- Olsen A. et al. (2003) *Effects of multimicronutrient supplementation on helminth reinfection: a randomized, controlled trial in Kenyan schoolchildren* Trans R Soc Trop Med Hyg (97) 109-114.
- Taylor M. et al. (2001) *The effect of different anthelmintic treatment regimens combined with iron supplementation on the nutritional status of schoolchildren in KwaZulu-Natal, South Africa: a randomized controlled trial* Trans R Soc Trop Med Hyg (95) 211-216.
- Dacie JV, Lewis SM (1995) *Practical Haematology 8th edition*. Churchill Livingstone Edinburgh