Original Article

The effectiveness of fortified flour on micro-nutrient status in rural female adults in China

Junsheng Huo PhD[1], Jing Sun BD[1], Jian Huang BD[1], Wenxian Li BD[1], Lijuan Wang MD[1], Lilian Selenje MD[2], Gary R Gleason BD[3], Xiaodong Yu BD[4]

[1]*Institute of Nutrition and Food Safety, China Center for Disease Control and Prevention, Beijing, China*
[2]*UNICEF Office for China*
[3]*Iron Deficiency Project Advisory Service, Boston, MA, USA*
[4]*Public Nutrition and Development Center, Beijing, China*

This research was designed to evaluate the effectiveness of fortified flour on micronutrient status in poor rural adult women. A total of 4,700 farmers as the intervention group were supplied with multi-nutrient fortified wheat flour for three years, while 2750 farmers as the control group were supplied with unfortified wheat flour. Wheat flour was fortified with vitamins A, B-1, B-2, niacin, folic acid, iron and zinc in mg/kg at 2, 3.5, 3.5, 35, 1, 20 and 25, respectively. Blood samples were taken at baseline and annually from about 300 volunteer adult females aged 20-60 years in each group. Hemoglobin (Hb), serum retinol, serum iron, free erythrocyte protoporphyrin, serum zinc were measured annually and a dietary survey conducted every 6 months. Average intakes of fortified micronutrients increased to adequate levels with average consumption of fortified flour at 117 g per person per day. Intervention groups showed a significant increase in terms of Hb levels from 24 m to 36 m and anemia rate decreased from 15.1% at baseline to 10.8% at 36 m. Serum iron levels of the intervention group significantly increased from 12 m to 36 m and erythrocyte protoporphyrin decreased from 24 m to 36 m respectively. Serum retinol and serum zinc of intervention group improved significantly from 12 m to 36 m compared with baseline and control group. The results showed that the fortified flour could improve micronutrient status of adult females in poor rural region.

Key Words: wheat flour, fortification, intervention, micronutrients, anemia

INTRODUCTION

Micronutrient deficiency remains a serious problem for various reasons such as: low family income and limited food choices.[1] Flour fortification has been suggested as a strategy to prevent and control vitamin and mineral deficiencies.[2] However, more scientific data are needed from flour fortification in the Chinese population, especially in high risk populations in poor rural areas. Wheat flour is usually cooked, steamed, or boiled in southeastern countries. This is distinct from baking in most flour fortification experienced countries, therefore, the effectiveness of flour fortification needs to be determined in relation to nutrition improvement.[3] This research is designed to evaluate the effectiveness of flour fortification on micronutrient status in poor rural adult females in China.

MATERIALS AND METHODS

Pilot villages and subjects

Weichang, a county in Hebei province, located in the northwest of China, was selected as the trial site. A total of 4,700 farmers in 1233 households were supplied with multi-nutrient fortified wheat flour for three years from 2004 to 2007, while 2750 farmers in 751 households were supplied with unfortified wheat flour. Adult women were studied in this study as they were considered the most vulnerable group to micronutrient deficiency. A total of 309 volunteers aged 20-60 years selected in the intervention villages participated in the measurement and blooding sampling, not all of the women attended all of the surveys because of personal reasons. A total of 302 volunteers in the control villages were surveyed as well, but again not all of them attended all of the surveys. A questionnaire was used to record personal and family information including medical history. Individuals with any chronic diseases according to their medical history were excluded.

Wheat flour compensation

In 2002, Chinese state council inaugurated the "Reforestation and Regrass Statute" for environmental protection in west provinces. Farmers in west provinces can voluntarily reforest or regrass their land with very low production for grain while central government compensates grain to families of farmers according to their reforested or

Corresponding Author: Dr Junsheng Huo, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, 29 Nanwei Road, Xuanwu District, Beijing 10050, China.
Tel: 0086-10-83132383; Fax: 0086-10-83132317
Email: jshuo@263.net.cn
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regrassed land. In some areas, wheat flour was compensated for the convenience of farmers. To fortify the compensation flour is considered an approach to improve nutrition status as the farmers in the area are at risk of micronutrient deficiency. Weichang county, Chende city, Hebei province as one of the wheat flour compensation county chosen for this observation.[4,5] The amount of compensated wheat flour can vary widely because of the variation of reforested or regrassed land in each family. The compensated flour was produced in a Weichang based mill named Baixue mill. Flour formulation of the fortified flour was developed by the research team based on existing micronutrient deficiencies in China (Table 1).[6] Microdoser was used to add nutrient premix into flour.[7] Spot tests, as a standard semi-quantitative method was used as quality control in Baixue mill. Premix used for flour fortification was from Sight and Life.[8] Quality control was conducted by sampling by China CDC for measurement of micronutrients fortified in the flour and by Baixue mill for quality measurement of flour according to the requirements of Chinese wheat flour standards.

Local grain department distributed flour through state grain bureau owned grain stores in the villages. All farmers received wheat flour from the local grain department twice a year, with distribution scheduled for January and July. Households could purchase extra flour from the local market if the amount of compensated flour was not enough for family consumption or for other reasons, i.e. villagers may purchase better flour than supplied. According to the national flour standard, flour has four grades of which grade 1 is considered the highest quality.[9] People in the villages usually consume grade 2, and sometimes other grades. Because of this, grade 2 flour was used as the trail flour. A record note book was used to record flour sources and amount of consumption in a household.

Surveys and measurements

Surveys were carried out from baseline and at 6, 12, 18, 24, 30 and 36 months. Every six months, a survey was conducted on about 300 subjects in each group to collect information on dietary intake, anthropometry and hemoglobin (Hb) measurements. In addition, blood samples were taken for serum retinol, serum iron (SI), serum zinc and free erythrocyte protoporphyrin (FEP) in every annual survey. Serum folic acid and serum ferritin were only measured at baseline and at the final survey.

Anthropometric measurement

Height/Weight Scale - RGT-140/160 and RGZ-120 manufactured by Wuhan Scale Equipment Plant was used to measure the body weight and height. Subjects were asked to take off their shoes when weighed. Total weight of subjects with clothes was measured, and the net weight was then calculated by reduction of average cloth weight from total weight. Average cloth weight was estimated by weighing clothes from 20 subjects. Bio-electrical Body Composition Analyzer Quartu Ⅱ RJL (manufactured by US RJL System Inc.) was used to measure the electrical resistance and reactance; and to calculate body fat composition.[10]

Dietary survey

A food frequency questionnaire was used for the dietary survey.[11] Each subject was interviewed for 40 minutes regarding their intake of food products made from wheat flour and their consumption practices for various kinds of vegetables, meat, eggs, milk, and other foods. Nutrient intakes were estimated by calculation based on the Chinese food composition table.[12]

Blood collection, storage and analysis

A 5 mL fasting blood sample was taken from each subject early morning before 7 o'clock. Of that, 20 μL venous blood was placed on filter paper, dried at room temperature, transferred from field to China CDC's laboratory within the same day, stored in a refrigerator at -80^{o}C, and later used for FEP measurement.[13] The remaining specimen was naturally cooled for 20 minutes and then centrifugalize for 15 minutes at 3000 rpm. The serum was separated, transferred to China CDC's laboratory at the same day and stored under -80^{o}C for measurement of serum Zn, serum iron and serum retinol. Serum retinol was measured with HPLC Waters-600 under the conditions: C18 reverse column, 4.6 mm×25 cm; methanol/water 98:2. Ultra-violet detector with wave length: 300 nm; injection amount: 200 μL and flow rate: 1.8 mL/min.[14] FEP was measured with Fluoro- spectrophotometer 970CRT with the following conditions: incitation wave length 403 mm, cranny 10 nm; emit wave length 605 nm, cranny 5 nm.[15] Serum zinc and iron were measured with the atomic absorption spectrophotometer, Varian SpectrAA-200.[16] Serum folic acid was measured with AXSYM kit produced by Abbott, Germany. Hemocue B-Hemoglobin testing system manufactured by Hemocue AB of Sweden was used to measure hemoglobin with the second drop of blood drawn from middle finger of the subjects.[17]

Statistical methods

Data was analyzed by SPSS Version 7 for Windows. Means and standard deviations of food consumption, nutrient intakes and the measurement data were calculated. Student *t*-test was used to test differences in food nutrient

Table 1. Nutrient formulation and selected fortificants in pilot flour fortification

Nutrients	Vit A	Vit B-1	Vit B-2	Niacin	Folic acid	Zn	Fe
Fortificant	Retinol acetate	Thiamin hydrochloride	Riboflavin	Niacin amide	Folic acid	Zinc oxidate	Electrolytic iron
amount of nutrient (mg/kg flour)	2	3.5	3.5	35	1	25	20†

†dosage of electrolytic iron should be 40 mg/kg as recommended, but it resulted in unacceptable grey color of compensated flour that induced refuse to accept by local people, thus the dosage was adjusted to 20 mg/kg flour.

Table 2. average food consumption in the pilot areas in three years (mean±SD, g/d)

	Control	Intervention
Rice	169.9±47.4	165.4±55.5
Flour	160.0±27.3	164.2±33.9
Other staples	39.3±5.4	32.5±7.1
Potatoes	70.4±10.4	51.5±13.1
Stock meat	53.6±9.3	54.2±10.4
Eggs	58.8±13.5	59.4±16.6
Fish and shrimp	7.3±2.7	7.1±1.8
Milk	18.6±10.3	14.2±8.6
Beans	37.8±3.9	38.2±6.4
Vegetables	288.3±62.4	277.2±64.1
Fruits	123.2±37.6	145.4±52.7
Edible oil from plant sources	31.4±5.0	31.2±5.8
Edible from animal sources	15.9±2.3	16.0±2.6
Salt	11.8±0.9	11.6±1.0
Soy sauce	15.1±1.9	13.5±2.0
Vinegar	8.6±1.6	7.2±1.3

Table 3. Consumption of total flour and fortified flour in intervention groups in the pilot areas

	0 m	6 m	12 m	18 m	24 m	30 m	36 m
Flour (g/d)	160.2±107.6	164.1±85.1	161.8±99.9	155.3±108.7	171.4±87.5	172.8±89.5	163.6±74.6
Fortified flour (g/d)	100.6±86.7	93.3±50.6	153.7±90.9	117.2±82.5	123.6±69.3	97.2±138.7	87.8±75.7
Fortified flour/flour (%)	62.9	56.9	95.0	75.5	72.1	56.3	53.7

Table 4. Nutrient intakes in the intervention groups

	Vit A (mcg)		Vit B-1 (mg)		Vit B-2 (mg)		Niacin (mg)		Iron (mg)		Zinc (mg)	
	Intake	RNI%	Intake	RNI%	Intake	RNI%	Intake	RNI%	Intake	AI%	Intake	AI%
Nutrients intakes from baseline diet	495.8±230.6*	70	1.1±0.3	84	0.8±0.3	67	9.5±5.4	73	23.8±6.7	119	9.6±2.9	83
Average nutrients intakes from fortified diet	749.2±229.7**	107	1.5±0.4*	115	1.2±0.4**	100	13.8±6.2**	106	26.5±10.2*	133	12.5±4.5*	108

* $p<0.05$; ** $p<0.01$

Note: RNI % or AI %: Percentage of actual intake of a nutrient accounting for recommended nutrient intakes (RNI) or adequate intake (AI)

intakes and ANOVA were used to test differences in parameters of biochemical analyses and anthropometrics.[18]

RESULTS

Anthropometry of subjects

There were 302 subjects in the control group at baseline survey, aged 37.7±7.8 yrs, with height 155.0±7.8 cm, weight 55.3±7.8 kg,, body fat 25.7±7.0% and BMI 23.4±3.0; and 309 subjects in the intervention group with aged 37.8±7.5 yrs, height 155.3±5.4 cm, weight 55.8±7.3 kg, body fat 26.3±6.9% and BMI 23.5±2.8. Data of the two groups showed no noticeable differences at baseline and remained unchanged in the three years observation period. Data on anthropometric measurements of subjects also showed no differences to those of 20-60 years old women from the national nutrition survey.[1]

Food consumption

The average staple food consumption in the three observation years is 365 g per person per day in the trial area. Ratios of rice, flour and other staples i.e. corn are 46%, 45% and 9% respectively. The average consumption of fortified flour during three years intervention period was 117 g per person per day, and ranged from 87.8 to 154 g. There were no remarkable differences and changes in consumption patterns between two groups during the observation period. The supplied flour accounted for about 44% of total consumed flour in the pilot areas, but the amount of compensated flour for each family can be changed if the family wanted more of another compensated product, such as corn flour (Tables 2 and 3).

Nutrient intakes

Data from baseline survey showed intakes of micronutrients fortified into flour, except iron, were less than Chinese RNI or AI for local adult females (Table 4).[19] Intakes of vitamin A, B-1, B-2, niacin and zinc accounted for only 70%, 84%, 67%, 73% and 83% of Chinese female RNI, but after flour fortification the average intakes of these micronutrients reached 107%, 115%, 100%, 106% and 108% respectively. The data indicated apparent sufficient iron intakes, but it is recognized that an overestimation on the absorption rate of iron may explain that iron intake is actually not enough.[20,21] An increase of dietary intakes of nutrients in the intervention groups was a result of the supply of fortified flour.

Table 5. Changes in hemoglobin levels in adult women

Month	Control group			Intervention group		
	N	Mean±SD (g/L)	Anemia (%)	n	Mean±SD (g/L)	Anemia (%)
0	298	131.9±13.3	13.1	308	132.2±13.3	15.1
6	237	130.6±13.8	14.4	258	132.3±13.1	13.4
12	228	131.2±13.6	14	257	133.1±14.3	14.8
18	267	132.1±13.0	13.5	263	133.5±12.2	13.1
24	258	132.6±13.3	14.7	267	135.9±14.3†,‡,**	11.9
30	262	131.3±12.9	14.9	270	135.8±12.7†,‡,**	10.7
36	247	131.5±13.0	14.2	269	135.7±14.3†,‡,**	10.8

†Compared with baseline, *p<0.05; **p<0.01
‡Compared with control group, *p<0.05; **p<0.01

Table 6. Changes of FEP (please add full name under the Table), SI, serum zinc, serum retinol and folic acid levels (mean±SD)

Mo	FEP(μg/dl)		SI(mg/L)		Serum zinc(mg/L)		Serum retinol(μg/dl)	
	Control	Intervention	Control	Intervention	Control	Intervention	Control	Intervention
0	44.5±14.7 -295	42.6±13.7 -303	0.76±0.28 -284	0.75±0.27 -298	0.73±0.25 -284	0.75±0.27 -298	35.4±9.1 -296	33.9±8.8 -307
12	49.1±14.0 -235	44.7±11.6 -241	0.77±0.26 -235	0.87±0.21†,‡,** -241	0.72±0.24 -235	0.75±0.28 -241	34.2±9.9 -238	37.0±9.6†,‡,** -242
24	45.7±13.4 -258	39.7±10.1†,‡,** -267	0.75±0.25 -255	0.85±0.19†,‡,** -265	0.72±0.19 -255	0.78±0.16‡,** -265	34.8±9.3 -255	38.5±8.4†,‡,** -265
36	46.5±13.4 -240	38.9±7.5†,‡,** -276	0.76±0.26 -230	0.86±0.16†,‡,** -260	0.71±0.19 -229	0.79±0.16†,*,‡,** -260	34.4±8.7 -230	37.9±6.0†,‡,** -262

The numbers in the parenthesis are numbers of subjects
†Compared with baseline, *p<0.05; **p<0.01
‡Compared with control group, *p<0.05; **p<0.01
§FEP: free erythrocyte protoporphyrin. SI: serum iron

Hemoglobin and anemia

Hemoglobin levels of the intervention group increased significantly from the 24th month to the 36 th month compared with baseline and the control group (Table 5). Anemia rate of the intervention group decreased from 15.1% at baseline to 10.8% at 36 months, while anemia rate remained unchanged from 13.1% at baseline to 14.2% at 36 months in control group.

Changes of FEP, SI, serum zinc, serum retinol and folic acid

The effects of fortified flour on the serum nutritional parameters are shown in Table 6. Levels of serum iron of the intervention group showed significant increase from the 12th month to the 36th month and FEP decrease from 24th month to the 36th month respectively. Serum retinol and serum zinc of intervention group improved significantly from the 12th month to 36th month compared with baseline and control group.

DISCUSSION

Data from the three year survey showed that the average consumption of fortified flour was 117 g (87.8 g-153.7 g) per person per day and this amount of fortified flour contributed to adequate intakes of fortified vitamins and minerals including: vitamins A, B-1, B-2, niacin, folic acid and zinc, which are widely deficient in China, especially in rural and poor areas.[1,2] The levels of serum retinol in the intervention group were significantly improved from 33.9 μg/dl at baseline to 37.0 μg, 38.5 μg and 37.9 μg at 12 months, 24 months and 36 months, respectively, while the levels of the control group remained unchanged. The result was confirmed by other large population intervention studies.[20] The levels of serum zinc in the intervention groups increased significantly from 0.75 mg/dl at baseline to 0.78 mg/ml and 0.79 mg/dl at 24 months and 36 months, respectively, but there were no remarkable change in terms of serum zinc levels in the control groups during this period. Since there is lack of evidences on zinc intervention in large populations, it could be assumed that zinc oxidation was effective on increasing zinc levels in adult women.[20] Significant change were observed for FEP and SI levels from the 24th month to the 36th month in the intervention group compared with baseline and the control group. Our findings were consistent with other researches and the increase of body iron level may explain the decrease of anemia rate in this study.[21-23] Sufficient data suggested iron fortification or iron supplement intervention is effective on anemia reduction in the Chinese population, although surveys still needed to define the iron deficiency rate.[13] It explains iron deficiency anemia may account for a high portion of anemia. Sodium iron ethylenediaminetetraacetic acid NaFeEDTA was reported as a suitable iron fortificant in other flour intervention studies and is recommended by the WHO guideline for flour fortification.[24,25] It is recognized that NaFeEDTA is less inhibited by phytate and polyphones as iron absorption inhibitors which are commonly high in grain based diet, but the high price of NaFeEDTA should be a barrier for it to be widely used in flour fortification.[26,27] The result suggested that NaFeEDTA at 20g iron/kg flour is effective in adult females in terms of levels of SI, Hb and FEP. The results were consistent with results of other studies, but a higher dosage may be needed for effectiveness within 24 months.[28,29] A longer time on improving zinc status of adult females with flour fortification intervention may also be required. Higher dosages of iron and zinc may be required for a better effectiveness in flour fortification. Although measurement are needed on vitamin B_1, vitamin B_2, niacin and folic acid for a complete analysis to further explain the bio-effects of fortified flour on the female adults in this intervention study, the results obtained have showed the values of flour fortification in rural Chinese population in terms of bio-effect and feasibility for target population intervention.

Reforestation and regrass are applauded by the farmers and effective for environmental protection, including water and soil loss. The evidence from this observation study should lead to strong consideration on integrating a nutrition improvement strategy into this national policy. International experiences on flour fortification should also be supportive on the consideration mass fortification of wheat flour in China. Further observations are necessary on the effect of fortified flour intervention on congenital development, work performance as well as cost-effectiveness.

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AUTHOR DISCLOSURES

Authors declare that there was not conflict of interest in this study and authors in this study are not directly and indirectly affiliated to any profit making units that may related to conflict of interest. This study was funded by UNICEF with support from the United States Center for Disease Control and Development.

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Original Article

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[1]*Institute of Nutrition and Food Safety, China Center for Disease Control and Prevention, Beijing, China*
[2]*UNICEF Office for China*
[3]*Iron Deficiency Project Advisory Service, Boston, MA, USA*
[4]*Public Nutrition and Development Center, Beijing, China*

营养强化小麦面粉对农村成年妇女微量营养素状况的影响

本研究的目的是评估营养强化面粉对贫困农村地区成年妇女微量营养素状况的作用。项目选择国家级贫困县围场进行 3 年强化面粉营养干预。干预组为 4,700 名农民，由项目组通过退耕还林补贴方式，提供多种微量营养素强化的小麦面粉，同时对照组 2,750 名农民食用未强化的面粉。面粉中强化的营养素及其在每公斤面粉中的用量为維生素 A 2 mg、維生素 B_1 3.5 mg、維生素 B_2 3.5 mg, 尼克酸 35 mg、叶酸 1 mg、铁 20 mg、锌 25 mg。从对照组和干预组各选择约 300 位妇女，年龄为 20-60 岁的自愿者，并在干预前及每 12 个月进行血液样品的收集，检测血红蛋白、血清视黄醇、血清铁、游离原卟啉、血清锌，且每 6 个月进行血红蛋白检测和膳食调查。平均每人每日强化面粉摄入量为 117 g，从而使强化的营养素之平均摄入增加并达到充足水平。干预组血红蛋白水平在 24-36 月间明显高于对照组，且贫血率从基线的 15.1%下降到 36 个月的 10.8%。与对照组和基线相比，干预组血清铁在 12-36 个月间显著增加，而游离原卟啉则从 24 至 36 月显著下降。干预组血清视黄醇和血清锌从 12-36 月显著比基线和对照组高。结果显示强化面粉对中国贫困地区成年妇女的微量营养素状况具有改善作用。

关键词：小麦面粉、营养强化、营养干预、微量营养素、贫血