

Efficacy of Micronutrient fortified Extruded Rice in Improving the Iron and Vitamin A status in Indian Schoolchildren

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Abstract

The efficacy studies conducted for six months revealed a significant increase in Haemoglobin and serum ferritin and a significant decrease in total iron binding capacity in the subjects consumed iron-fortified rice, but more so in the subjects consumed rice fortified with iron along with either vitamin A or beta-carotene. Likewise, the serum retinol was significantly increased in children received beta-carotene or vitamin A fortified meals and more so was in the children received rice fortified with beta-carotene or vitamin A along with iron. In sensory evaluation the micronutrient fortified rice (after blending with natural rice in the ratio of 1: 100) was almost indistinguishable from natural rice after cooking except beta-carotene fortified rice which could not match the white colour of natural rice. The cost analysis revealed that cost to fortify rice can have a very small impact on rice consumption. From the present study, it can be concluded that providing micronutrient fortified rice in school feeding programme could reduce the large burden of ID, IDA and VAD among children in developing countries.

Keywords: Fortified rice, Haemoglobin, Serum Ferritin, Serum retinol, Iron deficiency anemia, Vitamin A deficiency.

1. Introduction

The nutrition indicators compiled for 34 rice-consuming countries indicate that the incidence of low birth weight, infant mortality, mortality of children under five years

of age and prevalence of underweight children are considerably higher in these countries (UNICEF 1991). The highest prevalence of anemia and vitamin A deficiency are seen in South Asian countries, such as India. These deficiencies are the result of strict vegetarian diets, lack of diversity in the diet, high rates of infection, and unfavourable socio-economic conditions (Mason *et al* 1999). Mineral deficiencies can cause a number of adverse effects. Severe nutritional anemia from iron deficiency can increase material mortality, reduce resistance to infections, alter mental function, and reduce productivity and scholastic performance. Iron deficiency anemia (IDA) and iron deficiency (ID) are terms not to be used as synonyms; in a sense IDA is an advanced stage of iron deficiency, it occurs when oxygen delivery to body tissues becomes impaired due to lack of iron available for adequate hemoglobin formation. IDA is the main cause of microcytic anemia (small red blood cells), and it is identified with fatigue and weakness. IDA can go unnoticed for a long time, and when severe enough it can strain several organ systems, also leading to breathing problems, hair loss, and depression (Drake & Higdon 2009). Vitamin A deficiency is widespread in rice-consuming countries of tropical Asia and is most serious in Bangladesh, India, Indonesia, Myanmar, Nepal, Philippines, Srilanka and Vietnam. Vitamin A deficiency in children can lead to corneal lesions which can result in partial or total blindness. Of the total three million children worldwide estimated to be suffering from Xerophthalmia, one third live in India (Juliano 1993).

Supplementation (i.e. iron and vitamin A tablets), food fortification and nutrition education, are three strategies that can reduce the prevalence of micronutrient deficiency in developing world.

Rice is most popular cereal worldwide, serving as a staple food for 39 countries and nearly half of the world's population (Juliano 1993). Micronutrient fortified rice can play a major role in improving the diet and meeting the micronutrient needs of the population (Vinodini, 2003). Previously we have optimized the extrusion conditions for development of re-fabricated rice by studying the effect of extrusion conditions on pasting behaviour and microstructure of re-fabricated rice (S.Z. Hussain and Baljit S 2013). The viscous and thermal behaviour of vitamin A and iron fortified re-constituted rice was also studied (S.Z.Hussain *et al* 2013). The primary aim of the study was to know whether the micronutrient fortified rice could reduce the prevalence of ID, IDA and VAD among the school children. The effect of vitamin A and beta-carotene on iron absorption was also studied.

2. Material and Methods

2.1 Study Method

The study was conducted in a school where a subsidized lunch feeding programme was placed providing the students with a 200-250g meal of cooked rice daily. Informed, written consent was obtained from the parents of the children and oral consent was obtained from children. The study took place from March 2012 to October 2012 under the auspices of the public health agencies. In a double-blind, 6-month school based feeding trial. Iron and vitamin A depleted 5-8-y-old school children (n= 222) were randomly assigned to receive either a rice-based lunch meal

fortified with micronutrients (n= 185) or an identical but unfortified control meal (n= 37). In the fortification group, 37 children were assigned to 5 sub-groups based on the ID, IDA and VAD. The sub-group 1, 2, 3, 4 and 5 were given the rice meals fortified with iron, beta-carotene, retinyl palmitate, iron + retinyl palmitate and iron + beta-carotene respectively. The meals were consumed under the direct supervision, and the daily left overs were weighed. All the children were dewormed at baseline and Haemoglobin, Serum ferritin, Serum retinol and Total iron binding capacity were measured at baseline and after 6 months.

2.2 Iron fortification Level

The micronized ferric pyrophosphate was used as iron source. The target Iron concentration of the rice-premix was set at 0.4g/100g of rice. The concentration in the final product, mixed at a ratio of 1:100 with natural rice grains would be 4 mgFe/100g. This amount would provide 8mg of additional Iron to a child consuming 200g of rice per day. Assuming 5% absorption in Iron-deficient subject, this fortification level would provide an additional 0.4 mg of absorbed Iron per day.

2.3 Vitamin A and beta carotene fortification level

The retinyl palmitate and beta-carotene fortification target level was set at 600IU/g and 2000IU/g respectively. The loss of retinyl palmitate and beta-carotene resulting in extrusion process was compensated in the final rice-premixes.

2.4 Sensory analysis

The rice-premixes after blending with natural rice in the ratio of 1 : 100 were cooked as per standard procedure and sensory characteristics such as kernel appearance after cooking, cohesiveness, tenderness on touching, tenderness on chewing, taste, elongation and overall acceptability were evaluated by a panel of semi trained Judges using the scale given by Directorate of Rice Research, Rajendranagar, Hyderabad, India as described by Subbaiah(2005).

2.5 Clinical Study

At baseline, height and weight were measured using standard technique. Z-scores for height-for-age (HAZ), weight- for- age (WAZ) and weight-for-height (WHZ) were calculated using EpiInfo programme (Version 6.0, CDC) and the NCHS/CDC/WHO growth reference data [WHO, 2006]. Haemoglobin was measured using Drabkins reagent (Dacie's method). Serum ferritin concentration was measured by solid phase, two site, and chemiluminescent immunometric assay (Alfrey 1978). Total Iron binding capacity (TIBC) and serum retinol were determined as per the method given by Teitz, 1976 and IVACG, 1982 respectively.

2.6 Statistical analysis and Economics

Two sample t- tests were used to compare changes in Haemoglobin, Serum ferritin, Total iron binding capacity and Serum retinol between the fortified and control group. The economics of the rice-premix manufactured by extrusion method was also studied.

3. Results

At baseline, we screened 250 children of 5-8 year of age for the study. The baseline characteristics of the two groups did not differ significantly after the randomization (Table 1). Out of 250 subjects enrolled, 222 completed the study. Twenty four of 28 subjects, who discontinued the study, were in micronutrient fortification group and 4 were in control group. After obtaining capillary blood for Hb analysis, we excluded 15 children for having Hb \geq 11g/dL and 5 as being severely anaemic (Hb $<$ 7.5 g/dL). The seven students moved away from the study after base line studies and one left the school. Each of the group was randomly assigned 37 children.

Table 1. Baseline characteristics of the children 5-8 years of age intended to receive the micronutrient fortified rice and unfortified rice meals for a period of six months

Characteristics	Micronutrient fortified group (n=200)	Control group (n=50)	t-value
Hemoglobin (g/dL)	10.2 \pm 0.15	10.4 \pm 0.12	0.26 ^{NS}
Serrum Ferritin (μ g/L)	16.4 \pm 0.26	17.1 \pm 0.17	0.01 ^{NS}
Total iron binding capacity (TIBC) (μ g/dL)	350 \pm 7.64	320 \pm 10.00	0.05 ^{NS}
Serum Retinol (mcmol/L)	0.76 \pm 0.04	0.82 \pm 0.02	0.18 ^{NS}
Prevalence of iron deficiency (ID) (%)	82 \pm 1.53	80 \pm 1.15	0.04 ^{NS}
Prevalence of iron deficiency anemia (IDA) (%)	31 \pm 2.08	33 \pm 1.15	0.26 ^{NS}
Prevalence of vitamin A deficiency (VAD) (%)	62 \pm 1.15	59 \pm 1.15	0.16 ^{NS}
Height-for-age z score (HAZ)	-1.30 \pm 0.15	-1.34 \pm 0.05	0.40 ^{NS}
Weight-for-age z score (WAZ)	-2.0 \pm 0.25	-2.09 \pm 0.05	0.37 ^{NS}
Weight-for-height z score (WHZ)	-1.74 \pm 0.03	-1.62 \pm 0.06	0.10 ^{NS}

Values are arithmetic means \pm SEM

NS there were no significant difference between groups.

3.1 Sensory evaluation

Twenty five panellists evaluated the cooked fortified rice (after blending rice- premix with ordinary rice in the ratio of 1:100) in comparison to control for different sensory attributes before the start of feeding trial. Summary of the data obtained from the panellists is shown in figure 1. The overall acceptability of fortified rice was in the range of 3.2 to 3.6, whereas that of natural rice was 4.0 (overall acceptability score 4, 3, 2 and 1 indicates excellent, good, acceptable and undesirable respectively). The small difference in the overall acceptability score indicated that fortified and natural rice were perceived as almost same. As expected, the cooked kernel appearance score for the rice fortified with beta-carotene was lesser. The distinguished colour of beta-carotene fortified premix could not match the white colour of natural rice which also affected the taste and elongation score of such samples. Due to low amylose content of rice-premixes, the fortified rice-premixes were slightly sticky, whereas natural rice was found to be partially separated. However, no differences in tenderness on chewing were noticed. Because of low power of the test, it cannot be concluded that

fortified and natural rice are perceived as identical. However, low proportion of the panellists (less than are third) could notice any difference between fortified and natural rice during sensory evaluation and suggested that differences were minimal.

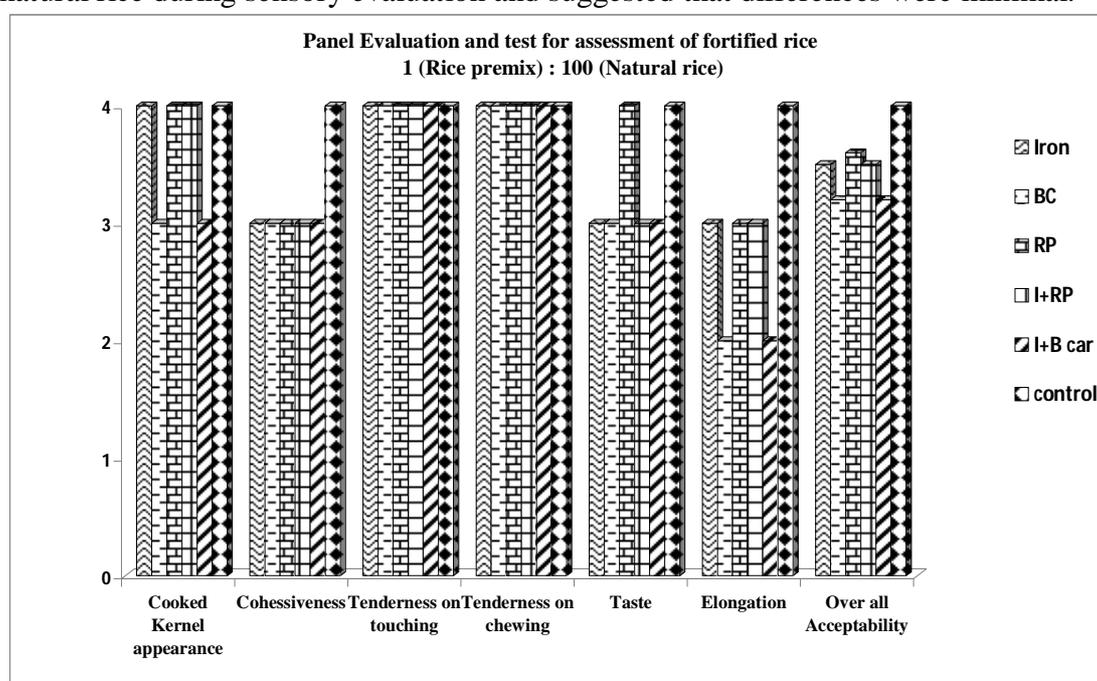


Figure 1. Sensory evaluation of fortified rice

3.2 Efficacy studies of fortified rice

The intervention groups did not differ significantly in base line characteristics for Hb, SF, TIBC, SR, ID, IDA and VAD. At baseline 6.4 and 9.7 percent children were mildly stunted ($HAZ < -1.0$), 3.3 and 6.8 percent were mildly under weight ($WAZ < -1.0$) and 2.8 and 1.4 percent were mildly wasted ($WHZ < -1.0$) in fortified and control group respectively.

After six months of feeding trial, a significant ($P < 0.05$) increase in Haemoglobin (Hb), Serum Ferritin (SF) and a significant ($P < 0.05$) decrease in total iron binding capacity (TIBC) was observed in groups received iron fortified meals from their baseline levels, but more so in the groups received either iron and retinyl palmitate or iron and beta-carotene fortified meals. A significant ($P < 0.05$) increase in Hb was observed even in groups received retinyl palmitate or beta-carotene fortified rice only (Table 2). The serum retinol was significantly ($P < 0.05$) increased in groups, received beta-carotene and retinyl palmitate fortified meals, but a highly significant ($P < 0.01$) increase was observed in groups received iron and retinyl palmitate or iron and beta-carotene fortified meals (Table 2). The data depicted in table 2 also revealed that increase in serum retinol was more in the groups received either retinyl palmitate or retinyl palmitate and iron fortified meals than in the groups received beta-carotene or beta-carotene and iron fortified meals.

Table 2. Iron status indexes and serum ferritin level in children fed the micronutrient fortified and unfortified rice-based lunch meals during the fortification trial.

Indicators	Sub-Group	Baseline	Final (After 6 months)	Mean difference	t-value
Hemoglobin (g/dL)	1	10.5±0.17	12.3±0.15	1.8±0.03	3.402**
	2	10.4±0.26	12.1±0.17	1.6±0.02	2.103*
	3	9.9±0.21	11.6±0.31	1.8±0.03	1.963*
	4	10.0±0.21	11.9±0.30	1.9±0.04	4.1794**
	5	10.2±0.15	12.0±0.21	1.9±0.02	6.00**
	Control	10.4±0.10	10.6±0.21	0.3±0.004	1.00 ^{NS}
Serum Ferritin (µg/L)	1	17.3±0.67	24.7±0.98	7.2±0.13	5.692**
	2	16.8±0.42	19.5±1.31	2.8±0.05	1.445 ^{NS}
	3	15.5±0.29	17.8±0.90	2.1±0.04	1.377 ^{NS}
	4	16.0±0.25	27.4±0.20	11.6±0.19	14.596**
	5	16.4±0.26	32.8±0.31	16.5±0.37	37.624**
	Control	17.1±0.25	19.2±0.76	2.0±0.03	1.419 ^{NS}
Total iron binding capacity (µg/dL)	1	328±2.08	297±1.53	31±0.66	5.976**
	2	330±5.13	313±2.52	17±0.33	1.485 ^{NS}
	3	374±2.52	362±4.36	12±0.25	1.288 ^{NS}
	4	368±2.08	296±2.65	72±1.45	15.306**
	5	350±5.29	266±2.65	84±2.15	9.916**
	Control	320±5.29	306±4.00	14±0.31	1.308 ^{NS}
Serum retinol (mcmmol/L)	1	0.84±0.02	0.87±0.02	0.03±0.0006	0.605 ^{NS}
	2	0.71±0.03	0.90±0.06	0.18±0.003	1.923*
	3	0.69±0.04	1.00±0.12	0.32±0.005	2.0485*
	4	0.76±0.03	1.15±0.03	0.37±0.008	4.875**
	5	0.80±0.04	1.06±0.04	0.25±0.005	2.4678**
	Control	0.82±0.03	0.83±0.02	0.03±0.0006	0.378 ^{NS}

*Significant at 5% level of significance

**Significant at 1% level of significance

NS = Non significant

Group 1 : Children receiving iron fortified meals

Group 2 : Children receiving beta-carotene fortified meals

Group 3 : Children receiving retinyl palmitate fortified meals

Group 4 : Children receiving iron + retinyl palmitate fortified meals

Group 5 : Children receiving iron + beta-carotene fortified meals

Control: Children receiving non-fortified meals

3.3 Cost analysis

The cost of producing extruded rice-premix is about Rs. 97.56/kg (Table3) whereas increase in cost after fortification (after blending rice-premix with natural rice in the ratio of 1:100) is only Rs. 0.97/kg. These fortification costs are very small, and very little added investment is needed in the rice consuming areas as a whole to accomplish fortification.

Table 3. Economic analysis of rice-premix manufactured by extrusion method (based on production of 250 tons per year)

Capital costs	(Rs. in Lakhs)
Hammer mills	0.90
Mixers	1.00
Extruder	8.50
Dryers	2.0
Stabilizers, weighing balances, stitching machines and other electrical items	0.65
Total equipment	13.05
Building	5.0
Total capital	18.05
Annual Costs	
*Capital	0.9025
**Interest	0.722
	1.6245
Labour (4 @ Rs. 200/day)	2.40
Utility (Electricity, water etc.)	3.0
Repairs	2.0
Broken rice (275 tons @ 15,000/tonne)	41.25
Packages along with labels	0.30
Management (@ 20,000/month)	2.4
Fortificant mix	191.00
Total annual cost	243.9745

Total cost per kg of = Rs. 97.589/ kg
rice-premix

Increase in cost after fortification = Rs. 0.97 / kg

(The rice-premix is to be blended with national rice in the ratio of 1:100)

*Assumed 20 years of useful life

**Assumed that the average cost of financing over the 20 year period is 4% of the total capital value.

4. Discussion

The results of our study showed the highly significant increase in Hb, SF and a highly significant decrease in TIBC in groups received iron and retinyl palmitate or iron and beta-carotene fortified meals than in groups received only iron fortified meals. This is because the consistent intake of vitamin A and beta carotene increase the iron absorption. Both of these compounds prevent the inhibitory effect of phytates and polyphenols on iron absorption. Panth et al (1990) and Angeles et al (2008) reported that combining vitamin A with iron has been shown to be more effective in increasing red blood cell production than giving iron alone in girls and in pregnant women. Suharno et al (1993) conducted the eight-week double-blind, placebo-controlled study on 251 anaemic pregnant women and observed that combination of 8,000 IU of

vitamin A (retinol palmitate) and 60 mg of iron was effective in eliminating anaemia in 97% of the women compared with only 68% for those women who were supplemented with 60 mg of iron alone. Roodenburg *et al* (1996) reported that Vitamin A deficiency may also impair mobilization of iron, allowing it to accumulate in the liver and spleen and suggested that vitamin A status should also be evaluated for anyone if there is excess iron storage. In our study it was also observed that beta-carotene had more effect on iron absorption than vitamin A. This might be due to the fact that vitamin A can increase iron absorption up to two fold, whereas beta-carotene can increase by three fold. This was in concomitance with the results already established by Maria *et al* (1998). The Hb was significantly increased even in groups received retinyl palmitate or beta-carotene fortified meals only. As, the feeding was strictly supervised, we do not feel this was due to exchange of rice meals. The Mohanram *et al* (1977) has stated that children who were deficient in vitamin A were more likely to be anaemic. Another study conducted by Freudenheim *et al* (1986) showed that vitamin A or beta-carotene supplementation can reduce some types of anaemia. As stated earlier also by Mejia *et al* (1976) and Roodenburg *et al* (1996) that vitamin A is critically involved in production of red blood cells and the mobilization of iron. Woede *et al* (1993) and Palafox *et al* (1996) conducted many studies and have shown a significant correlation between vitamin A or beta-carotene and red blood cell production.

The vitamin A and beta-carotene may form a complex with iron, keeping it soluble in the intestinal lumen, which could have resulted in increased absorption of retinol in groups received iron and retinyl palmitate or iron and beta-carotene fortified meals in our study. However, more increase in serum retinol was observed in groups received retinyl palmitate fortified meals than the groups receiving beta-carotene fortified meals. Olson (1999) reported that, for many years beta-carotene has been assumed to convert into 1/6th as much vitamin A (retinol) in healthy people. This means 10,000 IU of beta-carotene would convert to 1,666 IU of vitamin A. But, now researchers have questioned the estimate of the 6-to-1 conversion ratio of beta carotene to vitamin A. The newer studies have suggested that the beta-carotene conversion for many people may be as low as 29 to 1 (Solomons 1999; Tang 2000; Hickenbottom 2002). This means that 10,000 IU of beta-carotene would convert to only 344 IU of retinol. Nierenberg *et al* (1997) conducted a four years study of high dose (41,000 IU/day) beta-carotene supplementation with a variety of people and observed 151% average increase in blood beta carotene levels, but no significant increase in blood vitamin A (retinol) levels, confirming that poor conversion of beta-carotene to vitamin A is common. Hickenbottom *et al* (2002) evaluated the vitamin A activity of beta-carotene in 11 healthy, well fed men living in a controlled environment and found that five of the eleven men (45%) did not experience increased vitamin A blood levels after beta-carotene consumption. The authors concluded that Vitamin A activity of beta-carotene, even when measured under controlled conditions, can be surprisingly low and variable. Lin *et al* (2000) conducted a study on 11 healthy well fed women living in a controlled environment and found similar results as was reported by Hickenbottoms (2002). Five out of 11 (45%) did

not experience measurable increase in their blood vitamin A after consuming beta-carotene. The author's came up with a conclusion that vitamin A activity of beta-carotene is variable and surprisingly low in women. The reason for this are not well understood. The changes in the prevalence of iron deficiency (ID), iron deficiency anemia (IDA) and vitamin A deficiency (VAD) in the treatment and control groups observed in our study are shown in figure 2 and 3 respectively. Over the 6-months study, the prevalence of ID, decreased from 82% to 26% in the micronutrient fortified group and from 80 to 52% in the control group. However, the prevalence of IDA decreased from 31 to 13% in the micronutrient fortified group and remained almost unchanged in control group (33% and 31%). Similarly in case of VAD, a significant decrease from 62 to 20% was observed in micronutrient fortified group, whereas it was virtually un-changed in control group (59%). This was in accordance with the results already established by Diego *et al* (2006).

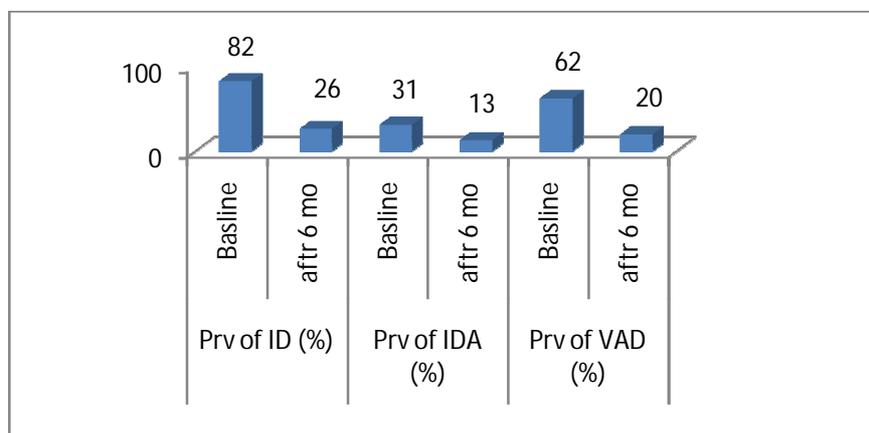


Figure 2. Prevalence of ID, IDA and VAD in children who received fortified rice for 6 months

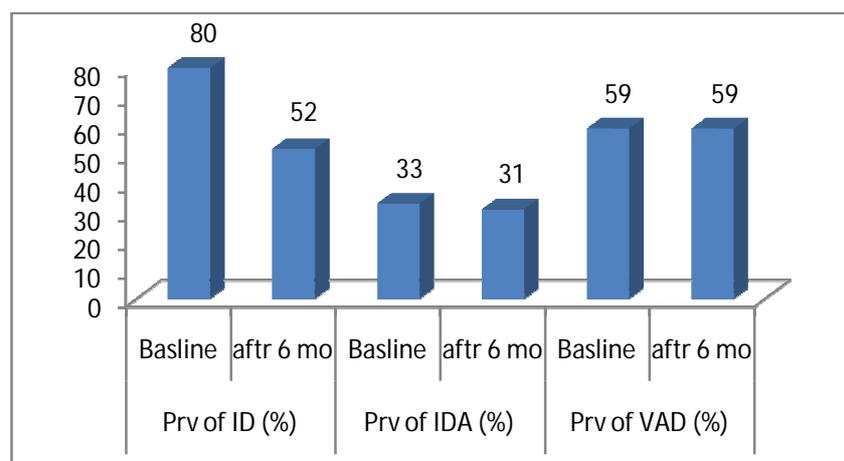


Figure 3. Prevalence of ID, IDA and VAD in children who received unfortified rice for 6 months

5. Conclusion

Micronutrient fortified rice obtained through extrusion (except beta-carotene fortified rice, which could not match the white colour of natural rice) has excellent sensory characteristics. Fed in a school lunch meal, it increases the haemoglobin, serum ferritin, serum retinol and is a logical solution to reduce the prevalence of ID, IDA and VAD in school children in developing countries.

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