

Preparing and investigating the impact of iron and zinc fortified parboiled puffed rice bars on the nutritional status of school-aged children (9–12 years)

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Abstract: Parboiled rice is produced by steaming, soaking, and drying paddy grains, which helps retain nutrients. The parboiled rice has more nutrients and wholesome grains as compared to white (polished) rice. Parboiled rice has more nutrients than white (polished) rice and can be used as nutritional intervention in communities with nutrient discrepancies. The current project was designed to study the effect of parboiled puffed rice bars fortified with iron (Fe) and zinc (Zn) on the nutritional status of school-aged children. The iron and zinc contents of parboiled fortified rice were increased by adding salts to soaking water. Three treatments were tested: T_0 – puffed rice bars made from parboiled white rice, T_1 – puffed rice bars made from brown rice fortified with 200 mg Na-FeEDTA and 100 mg $ZnSO_4$ per litre and T_2 – puffed rice bars made from white rice fortified with 200 mg Na-FeEDTA and 100 mg $ZnSO_4$ per litre. The highest energy (391.1 kcal), Fe (71.8 mg·kg⁻¹), Zn (64.8 mg·kg⁻¹) and water activity (0.65) values were observed in T_1 . Three hundred children were randomly assigned to three groups: control group G_0 given T_0 treatment containing 32.5% and 29.5% recommended dietary allowance (RDA) of iron and zinc, respectively, group G_1 given T_1 treatment made from fortified brown puffed rice containing 89.8% and 81.1% RDA of iron and zinc, respectively whereas group G_2 was served T_2 treatment made from white rice containing 77.8% and 56.8% of the RDA for iron and zinc, respectively. After 45 days of the intervention, the highest changes in haemoglobin (Hb) level (8.9%), serum ferritin (7.7%) and serum zinc (13.9%) were observed in G_1 and lower values of Hb (4.6 %), serum ferritin (4.1 %) and

serum zinc (8%) were observed in control group G_0 . Means of Hb, serum ferritin and zinc increased from 1.24 to 1.33 g·L⁻¹, 33.5 to 35.1 µg·L⁻¹ and 10.2 to 10.8 µmol·L⁻¹, respectively, which showed a positive effect of iron and zinc fortified bar on health. The consumption of 100 g puffed rice bar fortified with 100 mg·kg⁻¹ Fe and 200 mg·kg⁻¹ can achieve approximately 89% RDA of iron and 81.1% RDA of zinc in school-aged children.

Keywords: fortified rice bars; glutinous rice; complete blood count; haemoglobin; parboiled puffed rice; serum ferritin

Rice is a staple food for more than 50% of the global population, regardless of lower micronutrient contents (especially deficiency in iron and zinc) (Fukagawa and Ziska 2019). Around 80% of the caloric intake of three billion people is provided by rice. Puffed rice is a product obtained from pre-gelatinised milled parboiled rice and it is a popular food in Asian countries, especially in Pakistan, Bangladesh and India. In the food industry it is also incorporated into fatty pastes, chocolate or boiled sugar confections such as ice cream and chocolate candies. During roasting the rice starch gets damaged, gelatinised and part of it is subsequently retrograded, which eventually leads to the development of resistant starch that may thus escape digestion in the small intestine and fulfil the nutritive requirement of the gut microflora (Kumar and Prasad 2013). Considering the facts, the present study was carried out to prepare fortified puffed rice bars by a modified parboiling method and use them for an efficacy trial to determine the impact of fortification on the iron (Fe) and zinc (Zn) status of children.

The micronutrient malnutrition is extensively prevailing among developing nations, especially in Pakistan, India, Sri Lanka and Bangladesh (Akhtar et al. 2013). According to the WHO, approximately 60% of deaths among children under five years of age in developing countries are attributed to undernourishment (Hansen et al. 2004). According to the National Institutes of Health (NIH), the recommended dietary allowance (RDA) of iron and zinc for children aged 9 to 13 years is 8 mg per day regardless of the gender. Iron deficiency anaemia (IDA) is the most widely prevalent nutritional disorder mainly affecting pre-schoolers, young girls, lactating and pregnant women (Bashir et al. 2013). Similarly, zinc deficiency (ZD) has been estimated to affect about 95.4% of the population in South Asia, where people mostly consume rice as a dietary staple food (Hettiarachchi et al. 2004).

Multiple micronutrient enrichment or fortification has proven an effective strategy to reduce morbidity and mortality among the target groups (Akhtar et al. 2013). The WHO has suggested four simple strategies for the control and prevention of ID (iron deficiency) and ZD, which include increasing iron and zinc intake, infection control, supplementation, and enrichment or fortification of food (Prentice et al. 2017). Iron and zinc successful enrichment and fortification stories have been described in research or literature, i.e. the regions like Mongolia, Tajikistan, Azerbaijan, Kyrgyz Republic, Middle East, Uzbekistan, Kazakhstan and North Africa successfully executed iron and zinc fortification in maize and wheat flour (McLean et al. 2008).

The project of food fortification and enrichment on either voluntary or mandatory basis should be pre-planned and carefully monitored to attain optimum results and to avoid side effects. Additionally, a lot of study should be done to maintain food colour, flavour, and overall appearance (Kuong et al. 2016). Important restrictions for any fortification include types and suitable knowledge of fortificants, fortified meals, and plan feasibility. According to studies (Hurrell 2002), the absorption of iron from given NaFeEDTA was shown to be at least three times better than that from other ways of iron utilisation. The iron profile of children aged 11 to 17 years was significantly improved by a NaFeEDTA enriched soy sauce at a dose level of 5 and 20 mg iron per day (Huo et al. 2002). NaFeEDTA maintains the superior geometric mean bioavailability (9%) over ferrous sulphate (5.5%) according to Davidson et al. (2002) evaluation of the iron bioavailability in meals prepared by using maize tortillas and paste of black beans that was enriched with different iron sources including ferrous fumarate, ferrous sulphate and NaFeEDTA. Bouhouch et al. (2016) looked at the effects of iron-fortified wheat flour cookies and found that while they considerably improved the iron status,

they had no beneficial effect on cognitive scores. In another study, three- to eight-year-old Kenyan students were given electrolyte iron ($56 \text{ mg}\cdot\text{kg}^{-1}$) and maize grain fortified with iron (e.g. NaFeEDTA) at higher and lower concentrations of 56 to $28 \text{ mg}\cdot\text{kg}^{-1}$, respectively. The findings demonstrated that the children's iron status was only enhanced by large doses of NaFeEDTA (Andang'o et al. 2007).

Moretti et al. (2006) promoted the use of ferric pyrophosphate-enriched extruded rice in Indian school-children, demonstrating that iron deficiency anaemia decreased by 15 to 30% in the research group that consumed the fortified meal, whereas there was no significant difference observed in the control group ($P > 0.05$). According to Davidsson et al. (2002), the chemical composition of the meal and the presence of stimulators and inhibitors (positive and negative effect on iron available in diet) during iron absorption affect the bioavailability of iron. In addition, choosing the right vehicles is essential when fortifying meals. Cereal products have long been used as fortification vehicles in various nations. According to Eyenga et al. (2020), Abedini et al. (2023) and Sari et al. (2001), fish sauce, sugar, common salt, chocolate, biscuits, and cookies are additional carriers. However, children from all socioeconomic backgrounds prefer fortified parboiled puffed rice bars, which is why they were chosen. At the moment, food products are fortified with ZnSO_4 and NaFeEDTA. Since the iron and zinc component stability, traceability, and bioavailability of final products are crucial issues that require further research.

Iron and zinc bioavailability in rice is affected by the presence of naturally occurring phytic acid that binds these minerals and reduces their bioavailability in the body. While rice is a good source of iron and zinc, the higher phytic acid content in certain rice varieties can hinder the absorption of these nutrients. However, zinc bioavailability is less affected by phytic acid compared to iron, as zinc is more evenly distributed within the rice grain. Fortifying rice with iron and zinc compounds can increase the availability of these minerals. The main objective of the study was to make and analyse the cheaper fortified parboiled puffed rice bars and to elucidate its bioavailability in the body and its influence on the haemoglobin, iron and zinc status of school going-children.

MATERIAL AND METHODS

Procurement of raw materials. Paddy samples of the high-yielding Pakistani cultivar 'Shadab' were

procured from the Rice Research Institute, Kala Shah Kaku (KSK). Food grade fortificants, i.e. NaFeEDTA, zinc sulphate (ZnSO_4) and other materials, essential for the preparation of crispy rice bars, were bought from local markets. All reagents and chemicals used in this research were of analytical grade.

Preparation of Fe and Zn fortified parboiled rice. The fortified parboiled puffed rice (FPPR) samples were prepared by following the modified methods of Prom-u-thai et al. (2009). Paddy samples of the coarse Pakistani cultivar 'Shadab' were fortified with 200 mg NaFeEDTA and 100 mg ZnSO_4 per litre. Paddy (mature, healthy, dried, cleaned and sieved) samples were soaked in the prepared solution for 5 h with mechanical stirring to keep the grains moving (to ensure uniform suspension and equal contact of the paddy with the nutrient solution and avoid clumping or layering) followed by resting for 6–7 h before dry roasting in sand until a few grains started to pop. Just after roasting, paddy samples were washed with fresh tap water followed by sun drying. After sun drying, the moisture content was measured to be about 13% before dehusking and polishing.

Preparation of sweet crispy puffed rice bars. Puffed rice bars were prepared by mixing sugar [$20 \text{ g}\cdot(100 \text{ g})^{-1}$], corn syrup [$19 \text{ mL}\cdot(100 \text{ g})^{-1}$], vegetable oil [$1 \text{ mL}\cdot(100 \text{ g})^{-1}$], puffed rice [$60 \text{ g}\cdot(100 \text{ g})^{-1}$], and a small amount of water, followed by heating at 100°C for 7–8 min. The 70° Brix sugar solution was poured quickly into the measured amount of puffed rice and mixed quickly with the help of a mixer for the uniform and smooth coating of sugar on individual kernels. The mixture was transferred onto a specially designed flat table and sheeted with a roller. Subsequently, bars of specified dimensions were cut, individually wrapped in moisture-impermeable polyethylene polymeric films, sealed and stored at room temperature for further analysis.

Energy value. Gross energy of puffed rice bars was determined by taking a 0.5 g sample using an oxygen bomb calorimeter (C2000 Basic, IKA®-Werke GMBH and Co., Germany) as described by Azeem et al. (2021).

Mineral analysis. Mineral analysis of powdered rice samples was conducted according to AOAC Method No. 975.03B using flame photometer-410 (Sherwood Scientific Ltd., UK) and atomic absorption spectrophotometer (Varian AA240, Varian Medical Systems Australasia Pvt. Ltd., Australia). The detailed procedure was previously explained by Verma and Srivastav (2017).

Water activity. Water activity of fortified puffed rice samples was measured using a Hygropalm water activity meter measured using a Hygropalm water activity meter by following AOAC Official Method 978.18 (AOAC 2000).

Efficacy trial. Three hundred twenty school-going children (9–12 years) were selected for an efficacy trial. The study was approved by the Institutional Bioethics Committee (IBC) of the University of Agriculture, Faisalabad, Pakistan with registration No. AG-58763. The informed written consent for participation in the study was given by the families. Twenty children with acute illness or severe malnutrition were excluded. Finally, 300 healthy kids free of acute and chronic illnesses were divided into three groups using randomly generated computer numbers. Fortified parboiled puffed rice snacks were supplied to 100 members of each group during playtime, and this process continued for 45 days. The distribution and consumption of fortified parboiled puffed rice bars were monitored by the researchers and educators.

G₀ – children served puffed rice bars made from non-fortified parboiled white rice control; G₁ – children served puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre; G₂ – children served puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre. The first group was considered a placebo group. Parents were counselled to limit their children's use of supplements and to maintain consistency in their eating practices during the intervention period. Pretrial pupils who complained of diarrhoea were treated by bringing their iron and zinc concentrations down to the amount that was necessary. Parents knew their children were receiving fortified parboiled puffed rice bars because the placebo group was regarded as a control group, but they were unaware of whether the fortified parboiled puffed rice bars contained iron and zinc.

Complete blood count (CBC). Blood samples (5 mL) were collected at baseline (0 day), in the middle (23rd day) and at the end of the trial (45th day) of intervention from each child through a venipuncture process by a trained phlebotomist. For the serum collection, each sample containing 5 mL was divided into two equal portions and shifted into ESR and gel tubes (anticoagulant tubes). Immediately after the collection of samples, the complete blood count (CBC) of samples was carried out using a CBC analyser (Sy-Bh192, Sunny Medical Equipment Limited, China), which analysed the following parameter such as haemoglobin that was estimated in ESR tubes separately. While the sample in a gel tube was centrifuged to isolate the serum that was placed in the refrigerator at –20 °C for further tests.

Serum ferritin and serum zinc. Serum ferritin and serum zinc in samples were measured by following the procedure described by Sari et al. (2001) one hour after the blood sample collection. Serum ferritin was measured using an enzyme immunoassay technique (IMx System; Abbott, USA).

Statistical analysis. Three separate analyses were performed on each sample, and the mean ± standard deviation was used to report the findings. By means of SPSS version 17.0 (SPSS Inc., USA), the statistical analyses were carried out using Tukey's HSD test to verify the changes between the mean values and the one-way analysis of variance (ANOVA) to assess the changes or variations between treatments. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Gross energy value

Means for gross energy content (Table 1) showed the values ranging from 387.8 to 391.1 kcal·(100 g)^{–1}. The highest energy value [391.1 kcal·(100 g)^{–1}] was observed in treatment T₁ (puffed rice bars made from

Table 1. Effect of fortified puffed rice made from brown and white fractions of Shadab cultivar on gross energy, Fe, Fe % recommended dietary allowance (RDA), Zn, Zn % RDA and water activity of fortified puffed rice bars

Treatments	Energy (kcal)	Fe (mg·kg ^{–1})	Fe % RDA provided	Zn (mg·kg ^{–1})	Zn % RDA provided	Water activity
T ₀	387.8 ± 0.54 ^a	26.4 ± 0.34 ^c	32.5 ± 0.91 ^c	23.6 ± 0.01 ^c	29.5 ± 0.01 ^c	0.58 ± 0.05 ^b
T ₁	391.1 ± 0.23 ⁺	71.8 ± 0.21 ^a	89.8 ± 0.26 ^a	64.9 ± 0.52 ^a	81.1 ± 0.68 ^a	0.65 ± 0.21 ^a
T ₂	389.8 ± 0.01 ^a	62.2 ± 0.44 ^b	77.8 ± 0.50 ^b	45.4 ± 0.71 ^b	56.8 ± 0.84 ^b	0.59 ± 0.01 ^b

^{a–c}different letters in a row indicate significant difference ($P \leq 0.05$); values are represented as mean ± SD of rice bars ($n = 3$); T₀ – puffed rice bars made from parboiled white rice control; T₁ – puffed rice bars made from brown rice fortified with 200 mg Na-FeEDTA and 100 mg ZnSO₄ per litre; T₂ – puffed rice bars made from white rice fortified with 200 mg Na-FeEDTA and 100 mg ZnSO₄ per litre

brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre) followed by 389.8 kcal·(100 g)⁻¹ in T₂ (puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre) and 387.8 kcal·(100 g)⁻¹ in T₀ (puffed rice bars made from parboiled white rice control). In a study, the physicochemical analysis of granola bars formulated with puffed glutinous rice and selected dried Sunnah foods revealed an energy value ranging from 322.1 to 373.4 kcal·(100 g)⁻¹ (Agbaje et al. 2016). In numerous studies, energy values of puffed rice ranging from 284.0 to 417.0 kcal·(100 g)⁻¹ have been reported (Mendes et al. 2013; Shaheen et al. 2013). These variations might be due to slight differences in the final compositions of bars. However, the main factor responsible for these differences was sugar, which is mostly used in the formulation for binding and sweetness purposes. Moreover, slight variations in the energy value of brown and white rice bars of the same cultivar might be due to the presence of bran on brown puffed rice which was high in fibre content.

Fe and Zn

Means for Fe and Zn contents (Table 1) of puffed rice bars showed the values ranging from 26.4 to 71.8 mg·kg⁻¹ and from 23.6 to 64.8 mg·kg⁻¹, respectively. The highest Fe (71.8 mg·kg⁻¹) and Zn (64.9 mg·kg⁻¹) contents were observed in treatment T₁ (puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre) as compared to 62.2 mg·kg⁻¹ Fe and 45.4 mg·kg⁻¹ Zn contents in T₂. Fe and Zn contents were lower in bars made from fortified white rice than in those made from fortified brown rice. This might be due to the high initial mineral content of brown rice associated with the presence of bran, which is a rich source of minerals.

Water activity

Means for water activity (Table 1) for puffed rice bars showed the values ranging from 0.58 to 0.65. The high water activity (0.65) was observed in treatment T₁ (puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre) as compared to 0.59 in T₂ (puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre). Water activity was low in the white rice bars, whereas the highest value was found in brown rice bars. It might be due to the bran portion in the brown rice bar, which absorbs water from the environment due to its hygroscopic nature. The physicochemical analysis of granola bars formulated with

puffed glutinous rice and selected dried Sunnah foods revealed the water activity ranging from 0.56 to 0.61 (Agbaje et al. 2016). The preferable water activity for cereal bars should be between 0.45 and 0.57 for the longer shelf life (Ryland et al. 2010).

Efficacy trial

Haemoglobin level (Hb). Means for the effect of eating fortified puffed rice bar on the Hb level of school-going children in different groups showed the values ranging from 1.19 to 1.39 g·L⁻¹ (Table 2). The highest (8.9%) change of Hb was observed in group G₁ (served puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 89.8% RDA of iron) followed by 6% in group G₂ (served puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 77.8% RDA of iron) while the lowest change (4.6%) was observed in the control group given unfortified rice bar with 32.5% RDA of iron (Figure 1) when the influence of phytic acid is manifested with great probability. In one study, 115 primary school students (6–11 years old) were given iron-fortified biscuits for 43 weeks. Treatments had a significant impact on haemoglobin levels ($P < 0.0001$). Anaemia (haemoglobin < 120 g·L⁻¹) decreased from 29.6% to 15.6%, according to the findings of the study (van Stuijvenberg et al. 1999). As iron is the fundamental building block of haemoglobin, this increase was brought about by iron fortification; hence, a rise in iron intake is directly correlated

Table 2. Effect of fortified puffed rice bars on haemoglobin (Hb), serum ferritin, and serum zinc of children ($n = 100$)

Parameters	Study intervals (days)	Study		
		G ₀	G ₁	G ₂
Hb (g·L ⁻¹)	0	1.25 ± 0.88	1.27 ± 0.59	1.19 ± 1.02
	23	1.29 ± 1.14	1.32 ± 0.77	1.24 ± 0.95
	45	1.31 ± 1.25	1.39 ± 0.75	1.27 ± 0.88
Ferritin (µg·L ⁻¹)	0	34.1 ± 1.01	32.2 ± 1.28	34.1 ± 2.51
	23	35.0 ± 2.11	33.5 ± 1.04	35.2 ± 2.74
	45	35.5 ± 2.34	34.7 ± 1.29	36.0 ± 1.55
Zinc (µmol·L ⁻¹)	0	10.1 ± 0.86	10.2 ± 1.03	09.6 ± 0.73
	23	10.6 ± 0.93	10.5 ± 0.84	10.2 ± 0.84
	45	10.9 ± 0.74	11.6 ± 0.81	10.7 ± 0.79

Values are given as mean ± SD; G₀ – children served with puffed rice bars made from non-fortified parboiled white rice (control); G₁ – children served with puffed rice bars made from brown rice fortified with 200mg NaFeEDTA and 100 mg ZnSO₄ per litre; G₂ – children served with puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre

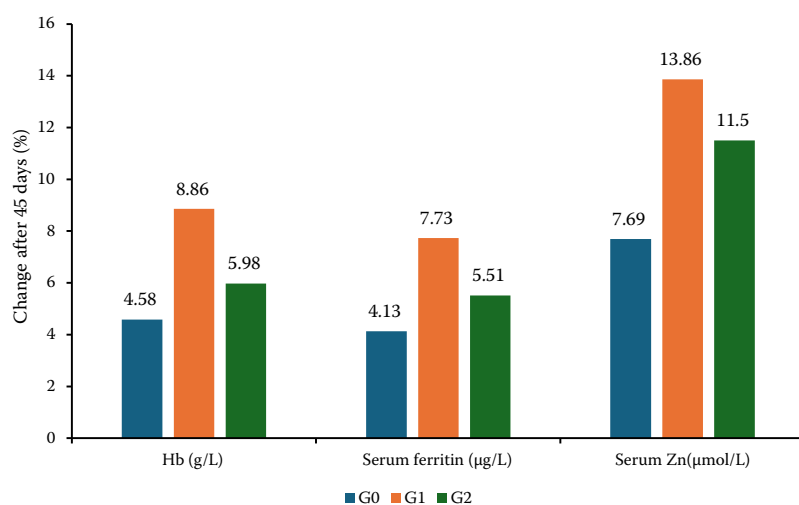


Figure 1. Percent change in haemoglobin (Hb), serum iron and serum zinc in children after completion of trial

G₀ – children served with puffed rice bars made from non-fortified parboiled white rice (control); G₁ – children served with puffed rice bars made from brown rice fortified with 200mg NaFeEDTA and 100mg ZnSO₄ per litre; G₂ – children served with puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100mg ZnSO₄ per litre

with an increase in serum haemoglobin levels (Longfils et al. 2008).

Serum ferritin. Means for the effect of eating fortified puffed rice bars on serum ferritin of children in different groups showed the values ranging from 32.2 to 36 µg·L⁻¹ (Table 2). The highest (7.7%) change in serum iron was observed in group G₁ (served puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 89.9% RDA of iron) followed by 5.5% in group G₂ (served puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 77.8% RDA of iron) while the lowest change (4.1%) was observed in the control group given unfortified rice bar with 32.5% RDA of iron (Figure 1) with great probability. The quantity of iron stored in the body is closely correlated with the amount of ferritin in the blood. Another study examined the effects of various iron fortifiers, including FeSO₄ (20 mg·kg⁻¹) and NaFeEDTA (60 mg·kg⁻¹), on wheat in anaemic school-aged children; it was found that the content of serum ferritin rose by 3% in comparison with the control group (Prom-u-thai et al. 2009).

Serum zinc. Means for the effect of eating fortified puffed rice bars on serum zinc of school-going children in different groups showed the values ranging from 9.6 to 10.9 µmol·L⁻¹ (Table 2). The highest (13.9%) change in serum zinc was observed in group G₁ (served puffed rice bars made from brown rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 81.1% RDA of zinc) followed by 11.5% in group G₂ (served puffed rice bars made from white rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre with 56.8% RDA of zinc) while the lowest change (8%) was observed in the control group given unfortified rice bar

with 26.5% RDA of zinc (Figure 1). In a previous study, the consumption of extruded rice grains fortified with zinc, iron and vitamin A resulted in an increase in the zinc status of Thai school children when incorporated into a school lunch program. There was a significant improvement in iron and serum zinc levels because of this intervention (Pinkaw et al. 2014).

CONCLUSION

According to the compiled data, eating fortified puffed rice bars can help improve the haemoglobin, serum ferritin, and serum zinc levels of school-age children. It is also a cheap solution to help people with low-to-middle incomes who are iron and zinc deficient. White rice fortified with 200 mg NaFeEDTA and 100 mg ZnSO₄ per litre obtained the highest sensory scores. This study suggests that 100 g of puffed rice prepared using fortified rice with 100 mg·kg⁻¹ Fe and 200 mg·kg⁻¹ Zn possesses a substantial potential to warrant food as well as nutritional safety and security. The cost of fortified parboiled puffed rice bars was calculated as 0.45 USD per child per day. Enteropathy, extensive malabsorption, and diarrhoea could be obstacles to this strategy for achieving the best possible outcomes. In order to achieve clinical and safety effectiveness, thorough randomised trials must be conducted in future, along with additional research to elucidate the mechanism underlying these outcomes.

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