

## Double salt fortification with iodine and iron: An *in vivo* study on albino rat

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### Abstract

Iron, iodine and vitamin A deficiencies are major clinical conditions affecting 33% of global population. Dual fortification of salt with iron and iodine could be a sustainable approach to fight against Iron Deficiency Anaemia (IDA) and Iodine Deficiency Disorders (IDD). The present work was designed to analyse the bioavailability of iron and iodine fortificants. For this reason, common table salt (NaCl) was subjected to various analyses like moisture, ash and minerals followed by double salt fortification with iron compounds like sodium iron ethylene diamine tetra acetate (NaFeEDTA), iron(II) sulphate (FeSO<sub>4</sub>) and potassium iodate (KIO<sub>3</sub>) at two different levels. Bioavailability of iron and iodine fortificants was analysed by feeding albino rats with fortified salt at 3.5%. Serum thyroxin (T4) level increased significantly from day 0 to 28 (26.72 ± 0.2 to 31.41 ± 0.4 nmol/L). Haemoglobin (Hb) and serum ferritin levels also depicted significant increase with the passage of time. However, whole blood zinc protoporphyrin, serum transferrin and serum triiodothyronine significantly decreased. As a conclusion, the present work revealed that double fortification of salt with iron and iodine along with a suitable stabiliser could be an effective strategy to control the two major micronutrient deficiencies.

### Keywords

Iron deficiency anaemia

Iodine deficiency disorders

Micronutrient malnutrition

Double salt fortification

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### Introduction

Micronutrient deficiencies are usually referred to as hidden hunger. Although they are less obvious, they are acute and could be detrimental to human health in long term. Micronutrients including vitamins and minerals are essential for optimal growth and development of the human body. Micronutrient malnutrition is a global health concern affecting developing countries as well as the developed ones (Iahtisham-Ul-Haq *et al.*, 2017). Pre-school children and pregnant women are among the most vulnerable groups (WHO, 2001). Among the micronutrient insufficiencies, iron, iodine and vitamin A deficiencies affect about 33% of the global population (≈7.4 billion) with iron deficiency affecting two billion people worldwide. Similarly, 2 billion people are

iodine deficient and 254 million children aged under five are deprived of proper vitamin A (WHO and FAO, 2006). Overall, one-third of global population is afflicted by iron deficiency anaemia and iodine deficiency disorders (Fiedler, 2009). Pakistan is also facing micronutrient insufficiencies especially in children, pregnant and lactating women (NNS, 2011). While 50% of the children aged five or less are underdeveloped, more than 38% are underweight, and about 25% of all births are below the normal birth weight (Bhutta, 2000). Moreover, it is claimed that 95.4% of South Asian population is deficient in zinc (Iahtisham-Ul-Haq *et al.*, 2015).

Iron is required for various metabolic functions in the body, and its deficiency causes anaemia. Iron deficiency anaemia reduces person's ability to perform physically-demanding tasks thereby

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affecting overall productivity (Clark, 2008). Pakistani women and children have an alarmingly high level of iron deficiency. Statistical results highlighted that 48.7% mothers and 29% children aged under five as iron deficient (NNS, 2011). Furthermore, iodine deficiency is also a major public health problem worldwide including in Pakistan (Delange, 2001). WHO categorised Pakistan as “severe iodine deficient region” where 135 million people are deprived of proper iodine nutrition. About 76% Pakistani women were estimated to be moderately iodine deficient while about 36.5% mothers were reported to have severe iodine deficiency (NNS, 2011). Similarly, 25% pregnant women in Lahore are reported to be moderately iodine deficient. Consequently, these women and their neonates are at increased risk of getting iodine deficiency disorders (Elahi *et al.*, 2009). Low dietary supply of iodine is the main factor responsible for iodine deficiency (Linhares *et al.*, 2015; Ershow *et al.*, 2018). This is common in populations living in areas where the soil is drained off iodine content as a result of past glaciation or the repeated leaching effects of snow, water and heavy rainfall (WHO, 2004).

Stability of iodine in iodised and dual fortified salt (DFS) is also a crucial point to be considered as the environmental conditions of Pakistan are conducive to more iodine loss from these salts. Iodine deficiency disorders (IDD) impair thyroid metabolism and reduce the efficacy of iodine prophylaxis in areas of endemic goitre (Eastman and Zimmermann, 2017; Moleti *et al.*, 2017; Hess *et al.*, 2002). This might also result in impaired cognitive development, hypothyroidism, congenital abnormalities, cretinism and goitre (WHO and UNICEF, 2007).

Food fortification is one of the most significant developments in recent years to control micronutrient deficiencies and to improve the health and nutritional status of people in an economical way (Högler *et al.*, 2016). The efficacy of salt iodisation, the preferred strategy for IDD control, might be influenced by multiple nutritional factors, including goitrogenic foods, protein-calorie malnutrition and selenium deficiency (Zimmermann *et al.*, 2002). Iron supplementation of goitrous, iron-deficient children improves their response to oral iodised oil and iodised salt. Therefore, dual fortification of salt with iodine and iron could be a sustainable approach to prevent both insufficiencies. However, selection of suitable iron and iodine fortificants with good stability and bioavailability is of cardinal importance (Yusufali, 2001). Nevertheless the dilemma is, highly bioavailable iron compounds like iron(II) sulphate might cause undesirable changes in the food affecting

colour, taste and flavour, and are poorly absorbed in human system (Van Stuijvenberg *et al.*, 2008). In this context, the major goal of the present work was to evaluate suitable levels of fortificants and their interaction to assess their bioavailability. The effect of these fortificants to ameliorate IDA and IDD was also one of main objectives.

## Materials and methods

Common table salt (NaCl) was procured from National Foods Ltd. (Pakistan). Potassium iodate (KIO<sub>3</sub>) and sodium iron ethylene diamine tetra acetate (NaFeEDTA) were provided by the Micronutrient Initiative, Canada, while food-grade iron(II) sulphate (FeSO<sub>4</sub>) was supplied by registered scientific chemical supplier at local market.

### Chemical characterisation of salts

The moisture content of salt samples was determined gravimetrically according to AOAC (2007). Briefly, 5 g sample, was oven-dried (Model: DO-1-30/02, PCSIR, Pakistan) at 110°C for 12-24 h. The difference in weight exhibited the moisture loss. The moisture content (%) in the table salt sample was calculated using the following formula.

$$\% \text{ Moisture} = \frac{\text{Weight of sample after drying(g)}}{\text{Weight of sample before drying(g)}} \times 100$$

Ash content of salt samples was determined according to method No. 08-10 (AOAC, 2007). Briefly, salt sample (2 g) in a pre-weighed crucible was ignited in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550°C for 24 h or until constant weight was obtained. The ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of sample after ashing(g)}}{\text{Weight of sample before ashing(g)}} \times 100$$

Minerals (i.e., Na, K, Fe, I, Ca, Mg) were determined by methods No. 40-70 and 40-71 (AOAC, 2007). Briefly, the salt sample (0.5 g) was digested with concentrated nitric acid (HNO<sub>3</sub>) at low temperature (about 85°C) and then with perchloric acid (HClO<sub>4</sub>) at high temperature (about 180°C). The digested sample was then filtered and volume was made up to 250 mL. The mineral contents were then measured with Atomic Absorption Spectrophotometer (Varian AA240, Australia).

### Formulation of fortified salts

Different combinations of dual fortified salt (Table 1) were prepared by using different levels of FeSO<sub>4</sub>, NaFeEDTA and KIO<sub>3</sub> by following the

protocols (Wegmüller *et al.*, 2003; Andersson *et al.*, 2008; Van Stuijvenberg *et al.*, 2008).

Table 1. Treatments plan

Combination	Treatment	Level of Both Salts
T <sub>0</sub>	Control	-
T <sub>1</sub>	KIO <sub>3</sub> - NaFe EDTA	40 µg/g - 1 mg/g
T <sub>2</sub>	KIO <sub>3</sub> - NaFe EDTA	40 µg/g - 2 mg/g
T <sub>3</sub>	KIO <sub>3</sub> - NaFe EDTA	30 µg/g - 1 mg/g
T <sub>4</sub>	KIO <sub>3</sub> - NaFe EDTA	30 µg/g - 2 mg/g
T <sub>5</sub>	KIO <sub>3</sub> - FeSO <sub>4</sub>	40 µg/g - 1 mg/g
T <sub>6</sub>	KIO <sub>3</sub> - FeSO <sub>4</sub>	40 µg/g - 2 mg/g
T <sub>7</sub>	KIO <sub>3</sub> - FeSO <sub>4</sub>	30 µg/g - 1 mg/g
T <sub>8</sub>	KIO <sub>3</sub> - FeSO <sub>4</sub>	30 µg/g - 2 mg/g

#### Experimental design for in vivo study

Young albino rats (both male and female) were purchased from the National Institute of Health, Islamabad. During experiment, temperature ( $23 \pm 2^\circ\text{C}$ ) and relative humidity ( $55 \pm 5\%$ ) were controlled for 12 h of light and dark periods. For efficacy trials, nine groups of rats were made five in each as mentioned in Table 2. At the initiation of trial, few rats were sacrificed to develop baseline for results. Biological assay was conducted according to the model adopted by Nair *et al.* (1998) (Table 2). Diet and water intake and body weight were recorded on daily basis. Blood and urine samples were collected at 0 day, 14<sup>th</sup> day and at the end of biological assay. Blood samples were taken in heparin-coated as well as in non-heparin tubes for different analysis. Urine samples were collected following the method devised by Fenske (1988). Aluminium sheets were placed below cages equipped with stainless steel mesh floors and 24-hour urine samples were quantitatively collected from these sheets. Dried urine was effectively removed by single washing step. Urinary iodine, serum thyroxin, serum triiodothyronine, haemoglobin, serum ferritin, whole blood zinc protoporphyrin and serum transferrin receptor of each sample was analysed (Zimmermann, 2008).

#### Statistical analysis

The results obtained from different parameters were subjected to statistical analysis using 2-factor factorial completely randomised design (CRD). Significant ranges were interpreted according to Duncan's Multiple Range Test at 5% level of significance ( $p \leq 0.05$ ) described by Steel *et al.* (1997).

Table 2. Composition of rat's diet and salt used for fortification

Composition of diet		Composition of salt	
Ingredients	g/100 g	Parameter	Amount
Skimmed milk powder	54.45	Moisture (%)	0.20
Sucrose	35.00	Ash (%)	0.51
Groundnut oil	5.00	Ca (g/100 g)	0.09
Vitamin mix	1.00	Mg (g/100 g)	0.06
D.L. methionine	0.30	K (g/100 g)	38.50
Fiber (cellulose)	0.50	Na (g/100 g)	3.00
Choline chloride	0.25	Fe (mg/100 g)	9.00
Double fortified salt	3.50	I <sub>2</sub> (µg/100 g)	0.09

## Results

#### Composition of salt used for fortification

Moisture content of salt samples was found to be 0.2% which is in agreement with the range given by Sivakumar *et al.* (2001). Similarly, there was 0.062 g magnesium (Mg) and 0.094 g calcium (Ca) present in 100 g of salt. Moisture contents and Ca and Mg impurities were the key factors that caused iodine and iron losses and colour development in dual fortified salt, DFS (Diosady *et al.*, 2002; Zimmermann *et al.*, 2004). Magnesium content in salt must not exceed 0.05 g/100 g (Sivakumar *et al.*, 2001). Levels of iron and iodine of salt samples are presented in Table 2.

#### Biological assay of different parameters

Since more than 90% of consumed iodine is excreted in the urine, urinary iodine (UI) is a perfect measure of recent iodine consumed (Knudsen *et al.*, 2001). The UI values for albino rats varied significantly over the 28 days trial period. The mean UI values for different groups improved with time displaying an upsurge in iodine status of animals. The variation was statistically alike at baseline analysis with T<sub>5</sub> showing the highest UI value (1.5667 µg I/d) and T<sub>3</sub> the lowest (1.2667 µg I/d). At day 14, results indicated significant variations among the treatments where T<sub>1</sub> yielded the highest UI value (3.1 µg I/d) and T<sub>6</sub> and T<sub>8</sub> the lowest (2.80 µg I/day). At day 28, T<sub>4</sub> yielded the highest value for UI (5.1467 µg I/d) and T<sub>3</sub> the lowest UI value (4.4733 µg I/d) as compared to all other treatments (Figure 1a).

Serum triiodothyronine concentration (T3) usually increases or remains unchanged in iodine-deficient populations while serum thyroxin (T4) concentration usually decreases (De Escobar *et al.*, 2004). The results for T4 demonstrated a significant variation during efficacy study. At day 0, T<sub>8</sub> yielded the highest T4 production (27.667 nmol/L) while T<sub>3</sub>

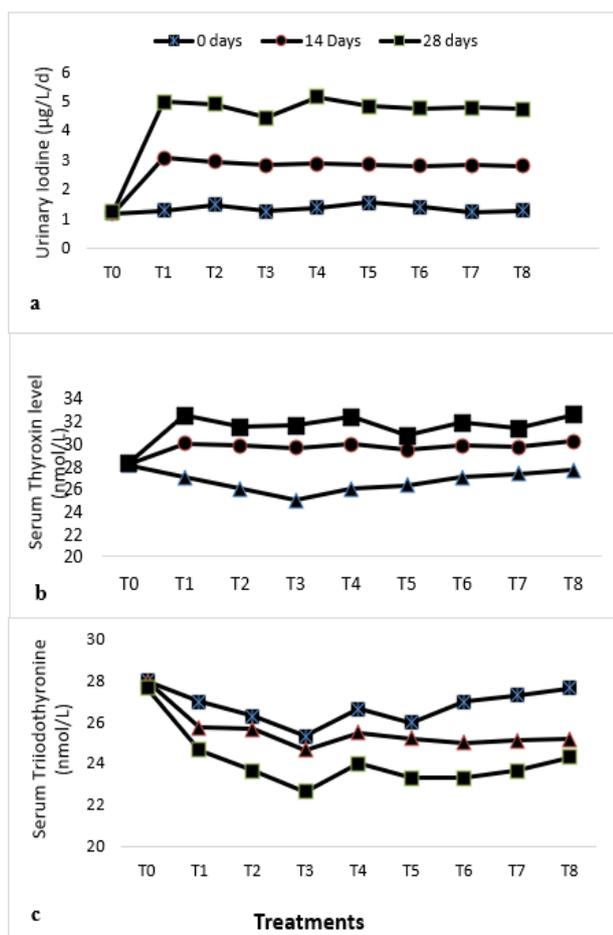


Figure 1: (a) Urinary iodine ( $\mu\text{g/L/d}$ ) of DFS-fed albino rats, (b) serum thyroxin level (T4) ( $\text{nmol/L}$ ) of DFS-fed albino rats, and (c) serum triiodothyronine (T3) ( $\text{nmol/L}$ ) of DFS-fed albino rats

yielded the lowest (25.000  $\text{nmol/L}$ ). Consumption of iodine-enriched diet by rats not only boosted their iodine level but also their serum T4 production. After 14 days, T<sub>8</sub> yielded the highest (30.21  $\text{nmol/L}$ ) and T<sub>5</sub> (29.45  $\text{nmol/L}$ ) the lowest. After 28 days, T<sub>8</sub> yielded the highest (32.567  $\text{nmol/L}$ ) and T<sub>5</sub> the lowest (30.683  $\text{nmol/L}$ ) (Figure 1b & 1c).

T<sub>3</sub> also demonstrated a significant variation over the trial period. At day 0, T<sub>8</sub> yielded the highest (27.667  $\text{nmol/L}$ ) and T<sub>3</sub> the lowest (25.333  $\text{nmol/L}$ ). After 28 days, T<sub>3</sub> yielded lowest (22.667  $\text{nmol/L}$ ) and T<sub>1</sub> yielded the highest (24.000  $\text{nmol/L}$ ) (Figure 1c).

Haemoglobin (Hb) is most commonly used when screening iron deficiency. However, it has a low specificity and sensitivity if used alone (Cook, 2005). The results clearly indicate a significant variation among Hb levels in rats after treating them with DFS. At day 0, T<sub>4</sub> yielded the highest (10.767  $\text{g/dl}$ ) and T<sub>6</sub> and T<sub>7</sub> the lowest (10.33  $\text{g/dl}$ ). At day 14, T<sub>7</sub> yielded the highest (13.2  $\text{g/dl}$ ) and T<sub>3</sub> the lowest (11.1  $\text{g/dl}$ ). At day 28, T<sub>6</sub> yielded the highest (15.90  $\text{g/dl}$ ) and T<sub>1</sub> the lowest (12.6  $\text{g/dl}$ ) (Table 3).

Serum ferritin (SF) levels indicated significant variation during the study trial before and after treatments. Interactive effect between days and treatments was found to vary significantly showing a regular sufficient iron intake by the test rats. At day 0, T<sub>6</sub> yielded the highest SF (74.367  $\text{ng/mL}$ ) and T<sub>4</sub> the lowest (73.967  $\text{ng/mL}$ ). After 14 days, T<sub>8</sub> yielded the highest (82.71  $\text{ng/mL}$ ) and T<sub>3</sub> the lowest (80.01  $\text{ng/mL}$ ). After 28 days, T<sub>8</sub> yielded the highest (90.200  $\text{ng/mL}$ ) and T<sub>4</sub> the lowest (87.300  $\text{ng/mL}$ ) (Table 3).

For zinc protoporphyrin, at day 0, T<sub>2</sub> yielded the highest level (64.66  $\mu\text{g/dl}$ ) and T<sub>1</sub> and T<sub>3</sub> yielded the lowest. After 14 days, T<sub>1</sub> yielded the highest (43.15  $\mu\text{g/dl}$ ) and T<sub>8</sub> the lowest (38.95  $\mu\text{g/dl}$ ). After 28 days, T<sub>1</sub> yielded the highest (35.067  $\mu\text{g/dl}$ ) and T<sub>8</sub> the lowest (28.300  $\mu\text{g/dl}$ ) (Table 4).

For serum transferrin, at day 0, T<sub>7</sub> yielded the highest level (72.0%) and T<sub>2</sub> the lowest 68.83%. After 14 days, T<sub>2</sub> yielded the highest (57.73%) and T<sub>5</sub> the lowest (46.34%). After 28 days, T<sub>6</sub> yielded the highest (43.33%) and T<sub>3</sub> the lowest (32.33%) (Table 4).

## Discussion

All the tested rats revealed an increase in UI, T<sub>4</sub>, Hb and SF levels and a decrease in zinc protoporphyrin, serum transferrin and T<sub>3</sub> levels as compared to baseline. These results are in agreement with previous studies which stated that fortification is a promising tool to tackle hidden hunger (Gera *et al.*, 2012; Das *et al.*, 2013; Sultan *et al.*, 2014, Haas *et al.*, 2014). In another investigation, Andersson *et al.* (2008) found a significant increase in the levels of UI, T<sub>4</sub>, Hb and SF and a decrease in zinc protoporphyrin, serum transferrin and T<sub>3</sub> levels of a population group feeding on salts fortified with KIO<sub>3</sub> and two iron compounds, a ferrous iron and ferric one. Their results showed a noteworthy growth in UI for group feeding on DFS having ferrous iron but there was a negligible increase in case of ferric iron when compared to baseline. Similarly, a significant increase in T<sub>4</sub>, Hb and SF levels and decrease in iron deficiency of population was observed. However, the results were more pronounced for ferrous iron as compared to ferric. Likewise, in the present work, FeSO<sub>4</sub> revealed significant variation from NaFeEDTA containing treatments especially in the case of Hb and serum ferritin levels. For SF, animals feeding on FeSO<sub>4</sub> fell in one homogenous group showing slight variation between them while NaFeEDTA containing treatments segregated into two homogenous groups each with same level of NaFeEDTA used for fortification (Table 3).

Table 3. Haemoglobin and serum ferritin of DFS-fed albino rats

Treatments	Haemoglobin (g/dl)			Serum Ferritin (ng/mL)		
	0 days	14 Days	28 days	0 days	14 Days	28 days
T <sub>0</sub>	10.56 ± 0.57	10.60 ± 0.59*	10.70 ± 0.46*	74.33 ± 0.40	74.00 ± 0.55*	74.16 ± 0.49*
T <sub>1</sub>	10.40 ± 0.56	11.40 ± 0.49**	12.60 ± 0.40***	74.06 ± 0.50	80.07 ± 0.41**	86.06 ± 0.46***
T <sub>2</sub>	10.43 ± 0.44	11.90 ± 0.41	13.20 ± 0.52***	74.06 ± 0.31	80.25 ± 0.67**	87.36 ± 0.54***
T <sub>3</sub>	10.53 ± 0.52	11.10 ± 0.44	12.70 ± 0.48***	74.13 ± 0.69	80.01 ± 0.60**	86.30 ± 0.48***
T <sub>4</sub>	10.76 ± 0.40	12.00 ± 0.62	13.40 ± 0.47***	73.96 ± 0.52	80.22 ± 0.56**	87.30 ± 0.65***
T <sub>5</sub>	10.50 ± 0.41	12.50 ± 0.55	14.67 ± 0.54***	74.30 ± 0.62	81.55 ± 0.87**	89.70 ± 0.85***
T <sub>6</sub>	10.33 ± 0.48	12.90 ± 0.47	15.90 ± 0.45***	74.36 ± 0.50	82.45 ± 0.72**	90.00 ± 0.79***
T <sub>7</sub>	10.33 ± 0.40	13.20 ± 0.37	14.57 ± 0.44***	74.30 ± 0.63	81.62 ± 0.80**	89.63 ± 0.53***
T <sub>8</sub>	10.56 ± 0.42	13.00 ± 0.44	15.63 ± 0.51***	74.06 ± 0.50	82.71 ± 0.64**	90.20 ± 0.58***

T<sub>0</sub>=Control, T<sub>1</sub>=KIO<sub>3</sub>-NaFe EDTA (40 µg/g – 1 mg/g), T<sub>2</sub>=KIO<sub>3</sub>-NaFe EDTA (40 µg/g – 2 mg/g), T<sub>3</sub>=KIO<sub>3</sub>-NaFe EDTA (30 µg/g – 1 mg/g), T<sub>4</sub>=KIO<sub>3</sub>-NaFe EDTA (30 µg/g – 2 mg/g), T<sub>5</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (40 µg/g – 1 mg/g), T<sub>6</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (40 µg/g – 2 mg/g), T<sub>7</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (30 µg/g – 1 mg/g), T<sub>8</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (30 µg/g – 2 mg/g). Values are mean ± S.D of three independent measurements. Asterisks indicate that the differences (\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001) between the means of different diets are statistically significant as determined by one-way ANOVA with Duncan's multiple range test.

A decrease in zinc protoporphyrin, serum transferrin and T3 levels was also recorded by Gera *et al.* (2012) who reported the effect of iron-fortified foods on haematological and biological indicators. Previously, Wegmüller *et al.* (2006) also found that ferric is better as compared to ferrous when they used ferric iron in combination with the KIO<sub>3</sub>. SF and Hb levels increased remarkably in population feeding on diet containing DFS. Since KIO<sub>3</sub> oxidised ferrous iron and affected its bioavailability, thus ferric iron performed comparatively better than ferrous in the presence of KIO<sub>3</sub>. Micronutrient fortification and its impact has been comprehensively reviewed in women and children by Das *et al.* (2013). Earlier, Zimmermann *et al.* (2003) also observed a significant upturn in levels of aforementioned parameters of individuals feeding on DFS for 10, 20 and 40 weeks.

While a decrease in zinc protoporphyrin, serum transferrin and T3 levels was also observed in that study. In another study, Zimmermann *et al.* (2004) found results comparable to that found in the present work. Wang *et al.* (2008) considered the effect of NaFeEDTA on different blood analysis including zinc protoporphyrin and they found no noteworthy variation.

Slight variations of the present work as compared to others mentioned above might be due to type and quality of fortificant used and the environmental conditions under which the experiment was conducted. Different iron compounds depict significant variation among their bioavailabilities and stabilities at different environmental conditions. Maintaining iron bioavailability and stability while avoiding colour changes is a difficult task to achieve

Table 4. Serum transferrin and serum zinc level of DFS-fed albino rats

Treatments	Serum Transferrin (%)			Serum Zinc level (µg/dl)		
	0 days	14 Days	28 days	0 days	14 Days	28 days
T <sub>0</sub>	70.00 ± 0.95	69.90 ± 1.16*	70.00 ± 1.08*	64.63 ± 0.59	64.25 ± 0.51*	64.30 ± 0.55*
T <sub>1</sub>	67.79 ± 1.11	56.34 ± 1.06**	41.00 ± 1.02***	63.96 ± 0.50	43.15 ± 0.82**	35.06 ± 0.78***
T <sub>2</sub>	68.83 ± 1.47	57.73 ± 1.41**	43.33 ± 0.91***	64.10 ± 0.67	40.40 ± 0.72**	33.36 ± 0.55***
T <sub>3</sub>	71.00 ± 1.29	56.65 ± 1.10**	41.66 ± 1.35***	63.96 ± 0.63	41.86 ± 0.80**	34.33 ± 0.51***
T <sub>4</sub>	69.50 ± 1.15	57.33 ± 1.05**	42.66 ± 1.14***	64.13 ± 0.52	39.50 ± 0.70**	30.20 ± 0.72***
T <sub>5</sub>	70.40 ± 1.26	46.34 ± 1.16**	32.66 ± 1.13***	64.66 ± 0.62	39.34 ± 0.76**	29.46 ± 0.75***
T <sub>6</sub>	69.00 ± 1.06	48.20 ± 1.12**	36.33 ± 1.08***	64.63 ± 0.60	39.73 ± 0.82**	29.06 ± 0.81***
T <sub>7</sub>	72.00 ± 1.15	46.50 ± 1.09**	32.33 ± 1.04***	64.43 ± 0.59	40.00 ± 0.55**	29.63 ± 0.63***
T <sub>8</sub>	71.00 ± 1.09	49.86 ± 1.11**	38.66 ± 1.06***	64.30 ± 0.48	38.95 ± 0.61**	28.30 ± 0.44***

T<sub>0</sub>=Control, T<sub>1</sub>=KIO<sub>3</sub>-NaFe EDTA (40 µg/g – 1 mg/g), T<sub>2</sub>=KIO<sub>3</sub>-NaFe EDTA (40 µg/g – 2 mg/g), T<sub>3</sub>=KIO<sub>3</sub>-NaFe EDTA (30 µg/g – 1 mg/g), T<sub>4</sub>=KIO<sub>3</sub>-NaFe EDTA (30 µg/g – 2 mg/g), T<sub>5</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (40 µg/g – 1 mg/g), T<sub>6</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (40 µg/g – 2 mg/g), T<sub>7</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (30 µg/g – 1 mg/g), T<sub>8</sub>=KIO<sub>3</sub>-FeSO<sub>4</sub> (30 µg/g – 2 mg/g). Values are mean ± S.D of three independent measurements. Asterisks indicate that the differences (\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001) between the means of different diets are statistically significant as determined by one-way ANOVA with Duncan's multiple range test.

especially in developing countries where the salt used is usually of low grade. Water-soluble, highly bioavailable iron compounds react with moisture and impurities in salt, which causes colour changes and iodine losses. On the other hand, poorly soluble iron compound has relatively low bioavailabilities. Furthermore, it is important to note that the bioavailability of iron compounds used in food fortification programs is dependent on the presence of enhancers and inhibitors in the diet. In the case of iodine fortificants, iodate is better than iodide for their stabilities. Potassium iodide is extensively used in iodine fortification which is readily oxidised when comes in contact with oxygen, changes to elemental iodine and sublimes. In the presence of humidity, sunlight and high temperature, the process is even accelerated; hence using iodate can be another option. Unfortunately, iodated salt can lose most of added iodine in the presence of salt impurities at high temperature for extended period. Thus, the use of stabiliser or microencapsulation could be an option to curtail this loss.

## Conclusions

Iron and iodine deficiencies often coexist in different regions of the world especially in developing countries. The output of the present work recommends that double fortification of salt with suitable iron and iodine compounds could be an effective approach to prevent and control the two major micronutrient malnutrition prevailing globally. However, the performance of DFS might vary according to environmental conditions, salt quality and dietary habits of the population. The formulations assessed in the present work could be effective if the salt used is of high quality and precautions such as microencapsulation and the use of workable stabiliser like SHMP are taken to minimise iron and iodine losses. Furthermore, the present work also highlighted that ferrous iron is a better option for iron fortification because of its high bioavailability. To conclude, double fortification of salt with iodine and iron could tremendously improve the health and wellbeing of individuals. The benefits might not be obvious to the targeted population immediately. Consumer awareness and education is, therefore, an important part of fortification programs.

## Declaration of interest

The authors report no conflict of interest. The authors are solely responsible for the content and writing of the paper.

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