Iron bioavailability, issues in food matrixes

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Iron bioavailability, issues in food matrixes

- Background

- Studies in RUTF

- Studies in rice
  - soluble FePP-CA/TSC complexes
  - starch matrix and coating
  - a multiple meal study in Ghana

- Conclusions
Chemistry of iron: solubility and redox equilibrium

Fe^{2+} \quad \text{(Partly soluble)}

Fe^{3+} \quad \text{(Virtually insoluble)}

\text{Fe(OH)}_2 \quad (0.1 \text{ M})

\text{Fe(OH)}_3, \quad (10^{-18} \text{ M})
Iron fortification

Water soluble

Food iron Fortification

Joins the common non heme iron pool

Inhibitors and enhancers

Food iron absorption

Water insoluble

Contamination iron Fortification compounds

Ligands (CA/TSC) (PP) (Others?)

Food iron excretion
4.1 mg Fe/meal
80 g wheat flour extrinsic tag radio iron

Exponential increase at PA:Fe < 1 (molar ratio)

(Hallberg et al. 1989)
RUTF

- What are RUTF’s?
  - High energy, protein, fat and carbohydrate concentrates.
  - Paste: peantus, skimmed milk powder, sugar.

- Hypothesis
  - Presence of calories (fat) affect gut transit time, thus possibly iron solubilization and bioavailability?

- In RUTF, with and without phytase

- In an emulsion model system
The effect of phytase when added to LNS/RUTF

- Phytase added at point of consumption in LNS
- Fat/calories would increase gastric transit time
- Phytase has an optimum at pH <3
- Hypothesis: interaction between phytase and fat in LNS/RUTF

![Fe absorption graph]

Effect of phytase $P<0.05$
Effect of LNS $P=0.06$
No interaction

Monnard et al, AJCN 2017
Iron fortification of rice

- Consumed as intact grains
- White color

FePP/FeOP only fortification compound

Low bioavailability
Compensated by adding higher levels of Fe

Specific matrix effects for rice?
In situ Generation of soluble Ferric Pyrophosphate Citrate Complexes in Rice

http://www.chemicalregister.com/Ferric_Pyrophosphate_Soluble

In situ generation of soluble FePP

Simultaneously extruded CA/TSC with FePP
→ higher iron bioavailability than addition of CA/TSC-solution

*P < 0.05; N = 20 healthy women

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Hackl et al. AJCN, 2016
Coating vs Hot extruded; Cold vs Hot extruded rice

**Cold/Hot** = Cold/Hot extruded \(^{57}\)FePP-fortified rice
Paired samples t-test; N=19

**HOR/COR** = Hot extruded/Coated \(^{57}\)FePP-fortified rice
**Reference** = Hot extruded rice, FeSO\(_4\) prior consumption

Hack et al. Submitted for publication
Hot vs. cold extruded: starch polymorphism?

<table>
<thead>
<tr>
<th>Sample</th>
<th>c (%)</th>
<th>polymorphism</th>
<th>a (Å)</th>
<th>b (Å)</th>
<th>c (Å)</th>
<th>g (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>54</td>
<td>A (monoclinic)</td>
<td>20.5</td>
<td>11.4</td>
<td>11.9</td>
<td>120</td>
</tr>
<tr>
<td>Cold Extruded</td>
<td>49</td>
<td>A (monoclinic)</td>
<td>20.4</td>
<td>11.4</td>
<td>12.0</td>
<td>120</td>
</tr>
<tr>
<td>Hot Extruded</td>
<td>21</td>
<td>V (orthorhombic)</td>
<td>12.8</td>
<td>28.9</td>
<td>8.9</td>
<td>90</td>
</tr>
</tbody>
</table>

Hack et al. Submitted for publication
Iron absorption from multiple meals

Study site: **Dungu primary school** – Tamale, Ghana

Assessment of iron bioavailability from FePP-fortified extruded rice

+ ZnO or ZnSO$_4$
+ ZnO or ZnSO$_4$ and CA/TSC
+ ZnO + CA + EDTA on Fe bioavailability

- Condiments of moderate PA : Fe
## Iron absorption from multiple meals

<table>
<thead>
<tr>
<th>Week</th>
<th>Meal&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Meal composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Screening</td>
<td>Each meal</td>
</tr>
<tr>
<td>1</td>
<td>57FePP + ZnSO₄</td>
<td>2 mg Fe + micronutrients + 3 mg Zn*</td>
</tr>
<tr>
<td>2</td>
<td>54FePP + ZnO</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>58FeSO₄ (Reference)</td>
<td></td>
</tr>
<tr>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57FePP + ZnSO₄ + CA/TSC</td>
<td>0.63 mg CA +18.1 mg TSC</td>
</tr>
<tr>
<td>7</td>
<td>54FePP + ZnO + CA/TSC</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>58FePP + ZnO + CA + EDTA</td>
<td>0.63 mg CA+2 mg EDTA</td>
</tr>
<tr>
<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Endpoint</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Meal assignment partially randomized by Venipuncture

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**Optimal formulation for fortification program?**
Results

All rice meals [except FeSO₄ (contained micronutrient mix only)] additionally contained iron as Ferric Pyrophosphate and a micronutrient mix.

Boxplots for fractional iron absorption from six different meals (n=26 or *n=25)

Crosses indicate outliers, **outlier not shown (16.7%)

Different letters indicate significant differences (p<.05), Bonferroni corrected repeated measures ANOVA

Fractional iron absorption

- Lowest from ZnO
- Similar from ZnSO₄ and ZnO+CA/TSC
- Highest from ZnSO₄+CA/TSC; EDTA+CA and FeSO₄
Summary and Conclusions

- Phytase is effective in enhancing Fe absorption from LNS/RUTF. Fat may have a role as well, but further studies needed

- Use of «chelators» for FePP fortified rice may allow to reduce Fe level in iron fortified rice in the future

- In rice starch structure may affect Fe release from rice kernels, but bioavailability is high with the use of CA/TSC or EDTA

- Coating is comparable to extrusion as a fortification technique for rice w.r.t. Fe and Zn bioavailability

- Single and multiple meal studies are consistent
Thank you
Stable iron isotopes in human nutrition

oral isotope (dietary absorption)
Labeled test meal (2-4 mg $^{57}\text{Fe}$, $^{58}\text{Fe}$, $^{54}\text{Fe}$)

Blood sample after 14 days

Shift in enrichment ratios of the $^{57}\text{Fe}$ and $^{58}\text{Fe}$ into the ‘natural’ $^{56}\text{Fe}$ in the red blood cells

Fe absorption + Fe utilization = Fe bioavailability

Naturally abundant in the body ($^{56}\text{Fe}$)

Stable Isotope Abundance

- $^{54}\text{Fe}$ 5.8%
- $^{56}\text{Fe}$ 92%
- $^{57}\text{Fe}$ 2.1%
- $^{58}\text{Fe}$ 0.28%
Ferric phosphates

- Compound of choice for rice fortification (along FeOP)

- Heterogeneous class of compounds with varying purity and own color

- Ligands can strongly affect its solubilitization (CA/TSC, PP, others ?)

- CA/TSC appears to need to be co-localized and possibly cooked with the FePP to exert its enhancing potential.
Ferric orthophosphosphate

- Heterogeneous class of compounds
  - (like FePP)
- Different sources vary in
  - Amorphous content
  - Surface area
  - Solubility
- Good predictor for bioavailability is solubility
  - In turn dependent more on amorphous content than particle size
### Rat repletion assay, Solubility of FeOP

<table>
<thead>
<tr>
<th>FePO₄ Source</th>
<th>Iron Dissolved (mg)/Total Iron (g) a,b</th>
<th>%RBV ± SD c,d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.02N HCl</td>
<td>0.05N HCl</td>
</tr>
<tr>
<td>Source 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lot 1 (n = 1)</td>
<td>99 ± 9 a</td>
<td>4.44</td>
</tr>
<tr>
<td>lot 2 (n = 3)</td>
<td>-</td>
<td>8.13 ± 0.06</td>
</tr>
<tr>
<td>Source 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lot 1 (n = 3)</td>
<td>78 ± 7 b</td>
<td>0.65 ± 0.21</td>
</tr>
<tr>
<td>Source 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lot 1 (n = 3)</td>
<td>51 ± 5 d</td>
<td>0.12 ± 0.009</td>
</tr>
<tr>
<td>lot 2 (n = 3)</td>
<td>-</td>
<td>0.16 ± 0.005</td>
</tr>
<tr>
<td>lot 3 (n = 2)</td>
<td>-</td>
<td>0.10 ± 0.002</td>
</tr>
<tr>
<td>Source 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lot 1 (n = 3)</td>
<td>83 ± 7 b</td>
<td>1.28 ± 0.03</td>
</tr>
<tr>
<td>Source 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lot 1 (n = 2)</td>
<td>60 ± 6 c</td>
<td>0.10 (n = 1)</td>
</tr>
<tr>
<td>pooled variance</td>
<td>-</td>
<td>8.588</td>
</tr>
<tr>
<td>pooled SD</td>
<td>-</td>
<td>0.093</td>
</tr>
<tr>
<td>pooled assay CV</td>
<td>-</td>
<td>5.00%</td>
</tr>
</tbody>
</table>

Best predictors of RBV:
- Surface area ($R^2=0.83$)
- Amorphous content ($R^2=0.91$)

Particle size was less good as a predictor ($R^2=0.91$)


Wide variation in solubility and RBV between different sources of FeOP
In vitro results

Highest iron solubility for extruded FePP + CA/TSC

→ Confirmed in vitro

Hackl et al. AJCN, 2016

P<0.05; N = 3
Screened participants

- Anemia = Hb < 11.5g/dl
- ID = Zinc Protoporphyrin > 43 (μmol/mol heme)

<table>
<thead>
<tr>
<th>Hemoglobin, g/L(^1)</th>
<th>115 (113,118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnPP, μmol/mol heme(^1)</td>
<td>36.1 (31.8,40.8)</td>
</tr>
</tbody>
</table>

\(^1\)geomeans (95% CI); N=67 (33F/34M)

<table>
<thead>
<tr>
<th>Prevalences (%) in study population</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>40%</td>
</tr>
<tr>
<td>Anemia</td>
<td>33%</td>
</tr>
<tr>
<td>ID Anemia</td>
<td>19%</td>
</tr>
</tbody>
</table>

Inclusion of 30 ID and/or anemic children 5 – 9 years

Day 1: Drop out: 5 participants; 1 re-recruitment

→ 26 children completed the study
### Anthropometrics and iron status parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>7 (7.8)</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>114.7 ±9.3</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>22.1 ±3.6</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>117 ±2</td>
<td></td>
</tr>
<tr>
<td>ZnPP, µmol/mol heme</td>
<td>51.5 (44.6,59.5)</td>
<td></td>
</tr>
<tr>
<td>Positive Malaria microscopy, N</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Plasma ferritin</td>
<td>42.1 (30.9,57.3)</td>
<td></td>
</tr>
<tr>
<td>Transferrin receptor</td>
<td>8.8 (7.8,10.1)</td>
<td></td>
</tr>
<tr>
<td>CRP&gt;5, N</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ±SD or geomeans (95% CI); $^1N=26$ (11F/15M)