Sustainable foods for gastrointestinal health across the lifespan

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Microbiome mediated metabolism of whole foods and the components

Gastrointestinal Disease Control and Prevention

Daniell and Ryan (2012). The Nutrigenome and Gut Microbiome: Chronic Disease Prevention with Crop Phytochemical Diversity
Gut Health across the lifespan

Daniell and Ryan (2012). The Nutrigenome and Gut Microbiome: Chronic Disease Prevention with Crop Phytochemical Diversity
Rice bran: an agricultural by-product of whole grain rice

Contains about 20-27% of total dietary fiber, 12-20% of protein, and a rich source of iron, B vitamins, and small molecules (fatty acids & antioxidants)

Contains about 90-94% carbohydrate with very little fiber content, 6-10% protein, and no significant vitamins or minerals

Borresen, E.C. and Ryan, E.P. Rice Bran: A food ingredient with global public health opportunity (2014)
Nearly half of the people in the world eat rice

Each dot represents 100,000 people living on less than 1 dollar per day

- many live in rice-producing areas where malnutrition and diarrheal diseases are prevalent and chronic diseases are on the rise.
Rice Bran (1g)

Fat (0.21g)
- Saturated (g) 0.04
- Monounsaturated (g) 0.08
- Polyunsaturated (g) 0.07
- MFA 18:1, Oleic (g) 0.07
- PFA 18:2, Linoleic (g) 0.07

Protein (0.13g)

CHO (0.50g)
- Dietary Fiber, Total (g) 0.21
- Crude Fiber (g) 0.06
- Sugar, Total (g) 0.01
- Sucrose (g) 0.01

Ash (0.10g)
- Sodium (mg) 0.05
- Potassium (mg) 14.9
- Phosphorus (mg) 16.8
- Magnesium (mg) 7.81
- Selenium (µg) 0.16
- Calcium (mg) 0.57
- Iron (mg) 0.19

Vitamins
- Alpha-Tocopherol (mg) 0.05
- Thiamin (mg) 0.03
- Niacin (mg) 0.34
- Pyridoxine (vitamin B6) (mg) 0.04
- Folate (total) (µg) 0.63
- Folate (DFE) (µg) 0.63
- Pantothenic Acid (mg) 0.07
- Vitamin K (µg) 0.02

Essential Amino Acids (mg)
- Histidine 3.55
- Isoleucine 5.68
- Leucine 10.2
- Lysine 6.5
- Methionine 3.06
- Cystine 3.17
- Phenylalanine 6.35
- Tyrosine 4.11
- Threonine 5.55
- Tryptophan 1.08
- Valine 8.81

Non-Essential Amino Acids (mg)
- Alanine 9.7
- Aspartic Acid 13.08
- Glutamic Acid 18.54
- Glycine 8.75
- Proline 6.68
- Serine 6.62
- Arginine 10.58
Rice Bran promotes Growth and Colonization of Fecal *Lactobacilli* in vivo

Henderson et al. Journal of Medicinal Food. 2011
Kumar et al. BMC Microbiology. 2012
Rice bran modulation of Gut microbes in healthy adults

Sheflin et al., 2015. Pilot Dietary Intervention with Heat-Stabilized Rice Bran Modulates Stool Microbiota and Metabolites in Healthy Adults. Nutrients 7, 1282-1300
Changes in the Stool Metabolome Following 30g/day of Rice Bran Intake

Percent change from baseline relative to control

- Anthranilic acid
- Beta-sitosterol
- Phenylacetic acid
- Cycloartenol
- Benzoic acid
- Myo-Inositol-1,3,4,5,6P5
- Hydrocinnamic Acid
- Indole-2-carboxylic acid

Sheflin et. al, 2015. Pilot Dietary Intervention with Heat-Stabilized Rice Bran Modulates Stool Microbiota and Metabolites in Healthy Adults. Nutrients 7, 1282-1300
Rice Varietal Differences in Tocotrienol and Tocopherol Contents

The Vicious Cycle Between Enteric/Diarrheal Disease and Malnutrition

The need for novel and rapidly achievable intervention strategies remains high, and should address enteric dysfunction, impaired gut mucosal immunity AND malnutrition.
Enteric Pathogens Implicated in Diarrheal Disease

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Small bowel</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Salmonella, Escherichia coli, Clostridium perfringens, Staphylococcus aureus, Aeromonas hydrophila, Bacillus cereus, Vibrio cholerae</td>
<td>Campylobacter, Shigella, Clostridium difficile, Yersinia, Vibrio parahaemolyticus, Enteroinvasive E. coli, Klebsiella oxytoca (rare)</td>
</tr>
<tr>
<td>Virus</td>
<td>Rotavirus, Norovirus</td>
<td>Cytomegalovirus, Adenovirus, Herpes simplex virus</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Cryptosporidium, Microsporidium, Isospora, Cyclospora, Giardia lamblia</td>
<td>Entamoeba histolytica</td>
</tr>
</tbody>
</table>
**Overarching Hypothesis:**

*Rice bran* contains bioactive phytochemicals that when converted during digestion by gastrointestinal microflora, modulate gut mucosal immunity for protection against gut pathogens.

*Rice Bran* phytochemical contents differ in health properties and efficacy varies across genetically diverse cultivars.
Experimental Design to Assess Dietary Rice Bran Effects on Gut Mucosal Immunity and Salmonella Fecal Shedding in mice

Day -14
All 129SvEv mice fed AIN-93 W control diet.

Day -7
10% rice bran (Wells, Red Wells or IAC 600 variety) or left as AIN-93 W control diet.

Day 0
Infect mice orally with $2 \times 10^7$ CFU Salmonella enterica 14028s.

Day 7
Euthanize mice for analysis of immune response to S. enterica infection.

Measure diet changes and induction of immune response.
Effect of dietary rice bran varieties on *Salmonella* fecal shedding

Rice bran consumption maintains intestinal immune populations despite S. enterica infection.

LTH rice bran inhibits salmonella entry and intracellular replication in intestinal epithelial cells better than SHZ rice bran

<table>
<thead>
<tr>
<th>Metabolite Type</th>
<th>LTH</th>
<th>SHZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid amide</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fatty acid conjugate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fatty acid ester</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fatty acid, Amino</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fatty acid, Dicarboxylate</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>*Galactolipids</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Glycerolipids</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Oxylipins</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>*Phospholipids</td>
<td>57</td>
<td>47</td>
</tr>
<tr>
<td>Sphingolipid</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Sterols</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>163</td>
<td>148</td>
</tr>
</tbody>
</table>

*Galactolipids and Phospholipids highlighted in red to indicate significance.
Correlations between rice bran metabolite profiles and *Salmonella* fecal shedding

- An approach directed towards understanding the ratios and distinct stochiometry of components required for protection against *Salmonella*

- A process that may be useful to inform rice crop improvement for diarrheal disease protective/health traits

<table>
<thead>
<tr>
<th>Fiber</th>
<th>Component</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Organic Matter</td>
<td>-0.94</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>Total Fiber</td>
<td>0.31</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Soluble Fiber</td>
<td>-0.8</td>
<td>0.03 *</td>
</tr>
<tr>
<td></td>
<td>Insoluble Fiber</td>
<td>0.54</td>
<td>0.3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenolics</th>
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<tbody>
<tr>
<td></td>
<td>GAE</td>
<td>-0.83</td>
<td>0.06 §</td>
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</table>

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Component</th>
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<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>γ-oryzanol</td>
<td>0.09</td>
<td>0.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamin E</th>
<th>Component</th>
<th>r</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>α-tocopherols</td>
<td>-0.89</td>
<td>0.03 *</td>
</tr>
<tr>
<td></td>
<td>γ-tocopherols</td>
<td>0.6</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>δ-tocopherols</td>
<td>0.4</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>α-tocotrienol</td>
<td>-0.54</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>γ-tocotrienol</td>
<td>0.94</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>δ-tocotrienol</td>
<td>-0.54</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Total Vitamin E</td>
<td>0.14</td>
<td>0.8</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Component</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myristic acid (14:0)</td>
<td>-0.41</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Palmitic acid (16:0)</td>
<td>0.49</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Palmitoleic acid (16:1)</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Stearic acid (18:0)</td>
<td>-0.94</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>Oleic acid (18:1n9)</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Vaccenic acid (18:1 n7)</td>
<td>0.37</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid (18:2 n6)</td>
<td>-0.14</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>α-linolenic acid (18:3n3)</td>
<td>0.84</td>
<td>0.06 §</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid (20:0)</td>
<td>-0.03</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gadoleic acid (20:1)</td>
<td>0.37</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Behenic acid (22:0)</td>
<td>-0.81</td>
<td>0.06 §</td>
</tr>
<tr>
<td></td>
<td>Lignoceric acid (24:0)</td>
<td>-0.94</td>
<td>0.02 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Elements</th>
<th>Component</th>
<th>r</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Boron</td>
<td>-0.89</td>
<td>0.03 *</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>-0.6</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Cobalt</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>-0.03</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>-0.09</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>0.2</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Molybdenum</td>
<td>-0.26</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Nickel</td>
<td>-0.77</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
<td>-0.09</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Sulfur</td>
<td>-0.43</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>-0.54</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Rice Bran protects gnotobiotic neonatal pigs against human rotavirus induced diarrhea

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>% with diarrhea</th>
<th>Mean days to onset</th>
<th>Mean duration days</th>
<th>Mean cumulative scores</th>
<th>Fecal virus shedding (ELISA)</th>
<th>(CCIF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB+LGG+EcN</td>
<td>6</td>
<td>0°</td>
<td>8 (0)°</td>
<td>0 (0)°</td>
<td>6.2 (0.5)°</td>
<td>100°</td>
<td>2.8 (0.3)°</td>
</tr>
<tr>
<td>LGG+EcN</td>
<td>6</td>
<td>50°</td>
<td>5.2 (1.3)°</td>
<td>0.7 (0.3)°</td>
<td>8.9 (0.6)°</td>
<td>100°</td>
<td>1.2 (0.2)°</td>
</tr>
<tr>
<td>RB only</td>
<td>5</td>
<td>20°</td>
<td>7.2 (0.8)°</td>
<td>0.2 (0.2)°</td>
<td>4.4 (1.6)°</td>
<td>100°</td>
<td>1.6 (0.2)°</td>
</tr>
<tr>
<td>Mock</td>
<td>9</td>
<td>100°</td>
<td>1.4 (0.2)°</td>
<td>5.6 (0.3)°</td>
<td>14.4 (1.0)°</td>
<td>100°</td>
<td>2.0 (0.3)°</td>
</tr>
</tbody>
</table>

- Rice bran or Lactobacillus (LGG)+ Ecoli Nissle (EcN) alone reduced HRV diarrhea
- Addition of RB to LGG+EcN colonized pigs completely protected against HRV diarrhea and can prevent the increase of HRV shedding in the LGG+EcN fed pigs

Yang et. al. Clinical and Vaccine Immunology (2014) and Scientific Reports (2015)
Locally grown

Multidisciplinary Efforts to reduce diarrheal disease, malnutrition, and growth stunting

Provide nutritionally dense foods to at-risk infants

Community-based dietary interventions & clinical trials
Nicaragua/Mali RB Project Overview

- Objective: To assess feasibility of rice bran consumption in healthy weaning children for enteric/diarrheal disease prevention.
  - Is it realistic for mothers to feed 6 - 12 month old children heat-stabilized dietary rice bran daily in their diet?
  - Does heat-stabilized dietary rice bran consumption modulate the stool microbiome/metabolome?
  - Can daily consumption of heat-stabilized rice bran prevent diarrheal disease episodes and indicators of malnutrition?
Rice bran for health, nutrition and disease prevention

- Phytochemicals across varieties
- Environmental exposure (e.g. Salmonella, Rotavirus, Arsenic, Pesticides)
- Growth failure, Stunting
- Cognitive impairment
- Overweight / Obesity
- Gut microbiome

Infectious (e.g. enteric pathogen, HIV, malaria)
Chronic (e.g. diabetes, cardiovascular disease, cancer)

Disease

Impaired immune defense
Gastro-Intestinal damage & inflammation

Malnutrition

Nutrient malabsorption
Lack of food security

Bioactive components

Damaged Gastro-Intestinal integrity

Legumes

Soybeans
Peanuts

Fresh Peas & Beans

Pulses
Peas, lentils, chickpeas, and dry beans.
Current Recommendations for Cancer Prevention

1. Be as lean as possible without becoming underweight.
2. Be physically active for at least 30 minutes every day.
3. Avoid sugary drinks. Limit consumption of energy-dense foods.
4. Eat more of a variety of vegetables, fruits, whole grains and legumes such as beans.
5. Limit consumption of red meats and avoid processed meats.
6. If consumed at all, limit alcoholic drinks to 2 for men and 1 for women per day.
7. Limit consumption of salty foods and foods processed with salt (sodium).
8. Don’t use supplements to protect against cancer.
9. *It is best for mother breastfeed exclusively for up to 6 months and then add other liquids and foods.
10. *After treatment, cancer survivors should follow the recommendations for cancer prevention.
   *Special population recommendations.
11. do not smoke or chew tobacco.

Following these recommendations, one third of total cancers could be prevented. Combined with eliminating tobacco use, one half of total cancers could be prevented. (AICR, 2012)
Health Promoting Properties of Dry Beans (Phaseolus vulgaris L.)

1. Dry cooked beans have unique nutrient profiles compared to corn and cereal grains.
2. Consumption of common beans has been shown to alter chronic disease processes and risk factors.
   - Reduce inflammation
   - Promote weight loss (>50% of dogs are overweight)
   - Inhibit tumor growth (one in four dogs get cancer)
   - Alter tumor metabolism
   - Reduce serum cholesterol

3. Common dry beans are a promising staple food for chronic disease prevention.
### Whole grains and dry beans consumption across socioeconomic groups

#### Percentages of adults’ that meet minimum recommended consumption levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Dry beans &amp; peas</th>
<th>Total Grains</th>
<th>Whole grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Adults</td>
<td>3.5 ± 0.6</td>
<td>58.9 ± 1.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Lowest, poverty income ratio (≤1.30)</td>
<td>4.8 ± 1.0</td>
<td>53.9 ± 2.4</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Middle, poverty income ratio (1.31-1.85)</td>
<td>6.1 ± 1.3</td>
<td>55.4 ± 3.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Highest, poverty income ratio (≥1.86)</td>
<td>2.7 ± 0.7</td>
<td>61.0 ± 1.5</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>2.2 ± 0.7</td>
<td>59.9 ± 1.6</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>1.7 ± 0.9</td>
<td>45.0 ± 1.9</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Mexican American</td>
<td>20 ± 2.6</td>
<td>70.5 ± 2.5</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Kirkpatrick et al. J Acad Nutr Diet 2012
Community-Academic Partnerships in Northern Colorado

Healthy Hearts
- Improve limetabolism in children at risk for hypercholesteremia with dietary interventions

Beans/Bran Enriching Nutritional Eating For Intestinal health Trial
- Enhance gut & immune health in cancer survivors

Colorectal cancer control & chemoprevention
- Establish biomarkers for reduced gut inflammation and colorectal cancer prevention

Sooper Staples
- Educate & empower community on whole grains and legumes at point of purchase
Worldwide burden of cancer

By 2030

The number of new cancer cases will increase by 54%.

The number of cancer related deaths will increase by 59%.
Building Partnerships in Research, Training, Education and Outreach on Sustainable Foods with evidence for Gut Health
Complex Interactions to determine the health benefits of whole grains
Effects of Navy bean intake on the companion dog stool metabolome

PC1 (18% Variance)

PC2 (14% Variance)

Control Baseline
Control 4 Wk

Navy Bean Baseline
Navy Bean 4 Wk

Forster et. al. Current Metabolomics 2015
A Simple, Sustainable Goal
Achieve Health benefits from a general increase in whole grain and legume consumption across the lifespan