Effect of oat or rice flour on pulse-induced gastrointestinal symptoms and breath hydrogen in subjects sensitive to pulses and controls – a randomized cross-over trial with two parallel groups

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**Supplementary Figure S1.** Flow chart of the study.



**Supplementary Figure S2.** Flow chart of the study enrollment.

Discontinued intervention after the first leg (n= 0)

Excluded (n=11)

  Not meeting inclusion criteria (n=9)

  Declined to participate (n=2)



Analysed (n=21)
 Excluded from analysis (n= 1), outlying breath gas results

Allocated to intervention, parallel group 1 = sensitives (n=28)

 Received allocated intervention (n=22)

 Did not receive allocated intervention (give reasons) (n= 6), discontinued before the 1st intervention due to personal reasons

Allocated to 2 parallel groups (n=50)

Discontinued intervention after the first leg (n=0)

Allocated to intervention, parallel group 2 = controls (n= 22)

 Received allocated intervention (n=0)

 Did not receive allocated intervention (give reasons) (n= 0)

## Follow-Up

Analysed (n= 21)
 Excluded from analysis (n= 1), food diary indicated: diet instructions not followed

## Analysis

## Enrollment

## Allocation

Assessed for eligibility (n=61)

**Supplementary Table S1.** Food Neophobia, General Health Interest and Environmental Interest scales used in the online questionnaire (n=229 participants).

|  |  |  |
| --- | --- | --- |
| **Scale** | **Statements (shown to participants as Finnish translations)** | **Cronbach's alpha** |
| Food Neophobia a | I am constantly sampling new and different foods. (R) | 0.843 |
| I don't trust new foods. | 0.833 |
| (FNS) | If I don’t know what is in a food, I won’t try it. | 0.850 |
|  | I like foods from different countries. (R) | 0.841 |
|  | Ethnic food looks too weird to eat. | 0.836 |
|  | At dinner parties, I will try a new food. (R) | 0.846 |
|  | I am afraid to eat things I have never had before. | 0.838 |
|  | I am very particular about the foods I will eat. | 0.856 |
|  | I will eat almost anything. (R) | 0.845 |
|  | I like to try new ethnic restaurants. (R) | 0.832 |
|   |   | **0.855** |
| General Health Interest a | I am very particular about the healthiness of food I eat. (R) | 0.856 |
| I always follow a healthy and balanced diet. (R) | 0.864 |
| (GHI) | It is important for me that my daily diet contains a lot of vitamins and minerals. (R) | 0.862 |
|  | I try to avoid hard fats. (R) \* | 0.872 |
|  | I eat what I like and I do not worry much about the healthiness of food | 0.853 |
|  | The healthiness of food has little impact on my food choices | 0.851 |
|  | The healthiness of snacks makes no difference to me | 0.864 |
|  | I do not avoid foods, even if they may raise my cholesterol | 0.871 |
|   |   | **0.877** |
| Environmental Interest a | I believe my choices have impact on controlling the climate change. (R) | 0.818 |
| Climate change is not my problem. | 0.831 |
| (EIS) | I prefer seasonal foods produced near me instead of foreign products. (R) | 0.854 |
|  | Animal welfare and its promotion affects my purchase decisions. (R) | 0.818 |
|  | Mixed diet gives me all the nutrients I need. | 0.818 |
|  | Animal foods belong to a diverse diet.  | 0.816 |
|  | It is important for me that the food has been produced sustainably. (R) | 0.821 |
|  | Flavor and price are more important factor for me than sustainability. | 0.819 |
|  | I try to make ecological choices in my everyday life. (R) | 0.819 |
|  | Emphasis on ecological aspects annoys me.  | 0.843 |
|   |   | **0.841** |

a Answers to the statements given on 7-portal scale (1 = fully disagree; 4 = not agree or disagree; 7 = fully agree). FNS is from Pliner & Hobden (1992); GHI from Roininen et al. (1999) with minor modifications (\*). R= reversed statement in the calculation of the sum variable.

**Supplementary Table S2.**

A. Study diet for the first two days of the study period.

|  |  |  |
| --- | --- | --- |
|  | ALLOWED | AVOIDED |
| Drinks | Water and unsweetened sparkling waterCoffee and tea | Soft drinks and energy drinksBerry and fruit juicesAll alcohol beverages |
| Milk and other dairy products | All lactose-free dairy products (milk, sour milk, yoghurt, qvark, ice cream, cheeses, cream, curdled milk, vanilla sauces) | All dairy products containing lactoseProducts containing probiotics, (bifido/acidophilus) such as Gefilus, Rela |
| Probiotics | - | All fermented foods (f.e. sauerkraut) and products/supplements containing probiotic or lactic acid bacteria (e.g. Gefilus, Rela) |
| Fruits and berries | Banana, grapes, honeydew melon, cantaloupe melon, strawberry, blueberry, lingonberry, rasberry, orange, mandarin, satsuma, clementine | apple, pear, plum, peach, nectarine, mango, watermelon, cherry, apricot, persimmon, figs, date, black currant, tamarillo, all canned and dried fruits (eg raisins) |
| Vegetables | Potato, chives, herbs, broccoli, rose cabbage, cabbage, kale, cucumber, tomato, lettuce, carrot, pepper | artichoke, cauliflower, asparagus, mushrooms, garlic, onion, leek |
| Desserts and sweets | Products sweetened with sugar | All products containing sugar alcohols (xylitol, sorbitol, mannitol, maltitol, lactitol and isomalt), eg. chewing gums, pastilles, many sweets, sugar-free chocolate and protein bars |
| Cereal products | Oats, spelt, rice, corn, amaranth, quinoa, millet, buckwheat | Rye (eg. rye bread, crisp bread, porridge, Karelian pasty), wheat (bread, bun, pasta, couscous, pizza, hamburgers, bulgur, wheat bran, semolina porridge/pudding, bakery products), barley (groats, flakes, pastries, bakery products, pasta etc.) |
| Meat, poultry, fish and eggs | All products | Breaded products, sausages, frankfurters, meatballs, meat loaf, and ready-made meat products |
| Seeds, nuts, lentils, beans and legumes | - | All (eg. peas, beans, chickpeas, fava beans, lentils, soybeans and other soy products such as tofu), food ingredients containing pulses, (eg. Pulled Oats), spoonable pulse-containing products |
| Sweeteners and spices | SugarSalt, herbs, pepper | Artificial sweeteners, honeySoy sauce, onion and garlic salts and spice mixes |
| Fiber supplements | - | Products with added inulin, FOS- or GOS-fiberVi-Siblin and similar fiber supplements |
| Fats | Lactose-free butterOils and lactose-free margarines | All products containing lactose |

B. Study diet for third and fourth study days.

|  |  |  |
| --- | --- | --- |
|  | ALLOWABLE | AVOIDABLE |
| Drinks | Water and sparkling waterCoffee and tea | Soft drinks and energy drinksBerry and fruit juicesAll alcohol beverages |
| Milk and other dairy products | - | ALL DAIRY PRODUCTS ARE PROHIBITED! |
| Fruits and berriesVegetables | 1 banana | All (incl. tomato sauce) |
| Cereal products | White rice | All other cereal products (bread, porridge, breakfast cereals, muesli, cakes, biscuits, muffins and other bakery products)  |
| Meat, poultry and fishEggs | All (non-marinated)All eggs | Breaded products, sausages, frankfurters, meatballs, meat loaf, and ready-made meat products |
| Seeds, nuts, lentils, beans and legumes | - | All (eg. peas, beans, chickpeas, fava beans, lentils, soybeans and other soy products such as tofu), food ingredients containing pulses, (eg. Pulled Oats), spoonable pulse-containing products |
| Sweeteners and spices | SugarSalt and black pepper | Artificial sweeteners, honeySoy sauce, onion and garlic salts and spice mixes |
| Fiber supplements | - | Products with added inulin, FOS- or GOS-fiberVi-Siblin and similar fiber supplements |
| Probiotics | - | All fermented foods and products/supplements containing probiotic or lactic acid bacteria (e.g. Gefilus, Rela) |
| Fats | Oils and lactose-free margarines | Butter |

**Supplementary Table S3.** Comparison of subgroups formed from participants to the online questionnaire in selected questions concerning the usage of pulses and receiving gut symptoms from pulses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | All | Symptoms | Food neophobia (FNS) | Environmental Interest Scale (EIS) | General Health Interest (GHI) |
|  | n=229 | yes (n=96) | no (n=133) | high (n=46) | mid (n=89) | low (n=94) | high (n=84) | mid (n=95) | low (n=50) | high (n=163) | low (n=63) |
| *How often do you consume following pulses as part of your diet? \** |
| Peas | 2(4)2.18 ±0.96 | 2(4)2.00 ±0.95 | 2(4)2.21 ±0.94 | 2(3) b2.02 ±0.95 | 2(4) a2.27 ±0.95 | 2(4) a 2.17 ±0.98 | 2(4)2.39 ±1.03 | 2(3)2.13 ±0.91 | 2(3)1.92 ±0.88 | 2(4)2.20 ±0.98 | 2(4)2.12 ±0.92 |
| Beans | 2(4)2.33 ±1.13 | 1(3) b1.84 ±1.01 | 3(4) a2.59 ±1.09 | 1(3) b1.78 ±1.03 | 2(4) a2.39 ±1.13 | 3(4) a2.53 ±1.10 | 3(4) a2.87 ±1.11 | 2(4) b2.09 ±1.03 | 1.5(3) b 1.86 ±1.01 | 2(4)2.37 ±1.13 | 2(4)2.21 ±1.14 |
| Chickpeas | 2(4)2.04 ±1.08 | 1(3) b1.76 ±0.96 | 2(4) a2.16 ±1.13 | 1(3) b1.48 ±0.89 | 2(4) a2.12 ±1.09 | 2(4) a2.24 ±1.08 | 3(4) a2.69 ±1.04 | 1(4) b1.79 ±0.96 | 1(3) b1.44 ±0.81 | 2(4)2.10 ±1.09 | 1.5(3)1.91 ±1.06 |
| Favabeans | 1(3)1.62 ±0.93 | 1(3) b1.42 ±0.78 | 2(3) a1.77 ±1.00 | 1(3) b1.24 ±0.67 | 1(3) a1.63 ±1.02 | 2(3) a1.81 ±0.91 | 2(3) a2.10 ±1.07 | 1(3) b1.39 ±0.75 | 1(2) b1.28 ±0.64 | 1(3)1.70 ±0.97 | 1(3)1.44 ±0.81 |
| Lentils | 2(3)1.95 ±1.02 | 1(3) b1.63 ±0.86 | 2(3) a2.05 ±1.06 | 1(3) b1.52 ±0.86 | 2(3) a1.96 ±1.05 | 2(3) a2.16 ±1.00 | 3(3) a2.57 ±0.97 | 1(3) b1.72 ±0.91 | 1(3) b1.36 ±0.72 | 2(3)2.01 ±1.04 | 1(3)1.80 ±0.95 |
| Soybeans | 2(4)2.39 ±1.31 | 2(4)2.18 ±1.21 | 3(4)2.38 ±1.28 | 1(3) b1.65 ±0.92 | 2(4) a2.47 ±1.38 | 3(4) a2.67 ±1.27 | 3.5(4) a3.30 ±1.11 | 2(4) b2.01 ±1.20 | 1(3) b1.58 ±0.86 | 2(4)2.40 ±1.31 | 2(4)2.35 ±1.30 |
| Meal with pulses | 2(3)2.06 ±1.12 | 1(3)1.86 ±1.08 | 2(3)2.17 ±1.11 | 1(3) b1.48 ±0.81 | 2(3) a2.18 ±1.18 | 2(3) a2.23 ±1.11 | 3(3) a2.79 ±1.09 | 1(3) b1.75 ±0.92 | 1(3) b1.44 ±0.86 | 2(3)2.18 ±1.13 | 1(3)1.77 ±1.06 |
| Spoonable pulse foods | 2(4)2.31 ±1.46 | 1(4)2.16 ±1.47 | 2(4)2.37 ±1.40 | 1(4) b1.59 ±1.05 | 2(4) a2.56 ±1.52 | 2(4) a2.43 ±1.46 | 3(4) a3.14 ±1.42 | 1(4) b1.95 ±1.33 | 1(4) b1.60 ±1.03 | 2(4)2.40 ±1.46 | 1(4)2.08 ±1.43 |
| *Do you get gut symptoms after eating pulses? \*\** |
| Peas | 2(4)2.31 ±1.31 | 3(4) a3.31 ±1.24 | 1(3) b1.59 ±0.79 | 3(4) a3.02 ±1.41 | 2(4) b2.17 ±1.27 | 2(4) b2.11 ±1.20 | 2(4) b2.02 ±1.27 | 2(4) ab2.35 ±1.3 | 3(4) a2.74 ±1.32 | 2(4)2.39 ±1.32 | 2(4)2.12 ±1.30 |
| Beans | 2(4)2.36 ±1.36 | 4(4) a3.49 ±1.20 | 1(3) b1.55 ±0.74 | 3(4) a3.17 ±1.36 | 2(4) b2.29 ±1.38 | 2(4) b2.03 ±1.17 | 2(4)2.25 ±1.27 | 2(4)2.34 ±1.37 | 2.5(4)2.60 ±1.46 | 2(4)2.40 ±1.36 | 2(4)2.26 ±1.36 |
| Chickpeas | 2(4)2.15 ±1.35 | 3(4) a3.23 ±1.31 | 1(3) b1.37 ±0.67 | 3(4) a2.85 ±1.53 | 1(4) b2.00 ±1.26 | 1(4) b1.95 ±1.23 | 1.5(4)1.93 ±1.17 | 2(4)2.24 ±1.45 | 2(4)2.34 ±1.41 | 2(4)2.22 ±1.37 | 1(4)1.97 ±1.28 |
| Favabeans | 2(4)1.91 ±1.31 | 3(4) a2.73 ±1.49 | 1(3) b1.32 ±0.71 | 2(4) a2.35 ±1.45 | 1(4) b1.87 ±1.34 | 1(4) b1.73 ±1.16 | 1(4)1.85 ±1.29 | 1(4)1.89 ±1.33 | 1(4)2.04 ±1.31 | 1(4)1.94 ±1.32 | 1(4)1.82 ±1.28 |
| Lentils | 1(4)1.92 ±1.30 | 3(4) a2.93 ±1.41 | 1(2) b1.19 ±0.46 | 2(4) a 2.48 ±1.52 | 1(4) b1.94 ±1.32 | 1(4) b1.62 ±1.07 | 1(4)1.83 ±1.17 | 1(4)1.89 ±1.36 | 1(4)2.10 ±1.39 | 1(4)1.99 ±1.32 | 1(4)1.74 ±1.24 |
| Soybeans | 1(4)1.67 ±1.11 | 2(4) a2.33 ±1.31 | 1(4) b1.20 ±0.58 | 1(4) a2.17 ±1.42 | 1(4) ab1.66 ±1.07 | 1(4) b1.44 ±0.89 | 1(4)1.54 ±0.90 | 1(4)1.63 ±1.20 | 1(4)1.98 ±1.20 | 1(4)1.67 ±1.10 | 1(4)1.67 ±1.14 |
| Meal with pulses | 1(4)1.64 ±1.09 | 2(4) a2.31 ±1.32 | 1(3) b1.15 ±0.49 | 1(4) a2.07 ±1.27 | 1(4) ab1.64 ±1.07 | 1(4) b1.43 ±0.96 | 1(4)1.63 ±1.04 | 1(4)1.62 ±1.14 | 1(4)1.68 ±1.10 | 1(4)1.68 ±1.11 | 1(4)1.53 ±1.04 |
| Spoonable pulse foods | 1(4)1.34 ±0.86 | 1(4) a1.76 ±1.18 | 1(2) b1.05 ±0.24 | 1(4) a1.74 ±1.20 | 1(3) b1.37 ±0.87 | 1(4) c1.13 ±0.51 | 1(4) b1.19 ±0.69 | 1(4) b1.28 ±0.81 | 1(3) a1.72 ±1.09 | 1(4)1.36 ±0.90 | 1(3)1.32 ±0.75 |

Data shown as medians (ranges) and means ± standard deviations. Symptoms groups refer to statement in Figure 1. Statistically significant differences between 2-3 subgroups are based on Kruskall-Wallis test and Mann-Whitney (p<0.05) and shown with letters a-c. \* scale: 1 almost never; 2 about once a month; 3 a few times in a month; 4 a few time in a week; 5 daily; \*\* scale: 1 not at all (including answers: I don't know); 2 slightly; 3 some; 4 quite much; 5 very much.

**Table S4**. Median (Interquartile range) of A. Breath gas measurements B. Gut symptoms and C. Defecation pattern by the Bristol stool scale during the study period.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Sensitives, Rice Mix | Sensitives, Oat flour | Controls, Rice mix | Controls, Oat flour |
| **A. Breath gas measurements** |  |  |  |  |
| H2 AUC | 28.9 (27.6) | 75.2 (59.7) | 31.0 (16.2) | 93.3 (42.1) |
| H2 highest value | 30 (14) | 60 (31) | 26 (17) | 78 (40) |
| H2 mean of 5 highest values | 20.2 (12) | 51.2 (36.4) | 20 (12.6) | 65.6 (38.2) |
| H2 time of peak value (h) | 6.5 (2) | 5.8 (1.8) | 6 (2.5) | 5 (0.8) |
| CH4 AUC | 2.7 (64.8) | 2.9 (66.6) | 2.5 (5.7) | 2.4 (5.2) |
| CH4 highest value | 2 (28) | 2 (21) | 1 (1) | 1 (1) |
| CH4 mean of 5 highest values | 1.4 (24.6) | 1.4 (20.2) | 1 (0.2) | 1 (0.6) |
| CH4 time of peak value (h) | n.a. | n.a. | n.a. | n.a. |
| **B. Gut symptoms** |
| Sum of symptoms of 3 first days | 10 (20) | 11 (15) | 1 (4) | 0 (5) |
| Sum of symptoms of the 4th day | 3 (9) | 7 (8) | 0 (0) | 0 (2) |
| Very mild symptoms, 3 first days | 3 (5) | 3 (5) | 0 (2) | 0 (3) |
| Mild symptoms, 3 first days | 3 (4) | 4 (5) | 0 (1) | 0 (1) |
| Moderate symptoms, 3 first days | 2 (4) | 1 (6) | 0 (0) | 0 (0) |
| Severe symptoms, 3 first days | 0 (1) | 0 (0) | 0 (0) | 0 (0) |
| Very mild symptoms, 4th day | 1 (3) | 2 (4) | 0 (0) | 0 (1) |
| Mild symptoms, 4th day | 1 (3) | 2 (4) | 0 (0) | 0 (0) |
| Moderate symptoms, 4th day | 0 (2) | 0 (2) | 0 (0) | 0 (0) |
| Severe symptoms, 4th day | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Upper abdomen pain, 3 first days | 0 (1) | 0 (1) | 0 (0) | 0 (0) |
| Lower abdomen pain, 3 first days | 0 (2) | 0 (1) | 0 (0) | 0 (0) |
| Cramping, 3 first days | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Bloating, 3 first days | 3 (5) | 1 (6) | 0 (0) | 0 (0.5) |
| Flatulence, 3 first days | 3 (4) | 3 (6) | 0 (1) | 0 (2) |
| Rumbling, 3 first days | 2 (2) | 1 (3) | 0 (0) | 0 (0) |
| Other symptoms, 3 first days | 0 (0.3) | 0 (1) | 0 (0) | 0 (0) |
| Upper abdomen pain, 4th day | 0 (1) | 0 (0) | 0 (0) | 0 (0) |
| Lower abdomen pain, 4th day | 0 (1) | 0 (1) | 0 (0) | 0 (0) |
| Cramping, 4th day | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Bloating, 4th day | 0 (2) | 1 (4) | 0 (0) | 0 (0) |
| Flatulence, 4th day | 1 (2) | 2 (2) | 0 (0) | 0 (1) |
| Rumbling, 4th day | 0 (0) | 0 (1) | 0 (0) | 0 (0) |
| Other symptoms, 3 first days | 0 (0) | 0 (2) | 0 (0) | 0 (0) |
| **C. Defecation pattern by Bristol stool scale during the study period** |
| 1 | 0 (1) | 0 (1) | 0 (0) | 0 (0) |
| 2 | 0 (0) | 0 (1) | 0 (0) | 0 (0) |
| 3 | 0 (0) | 0 (1) | 0.5 (1) | 0.5 (1.3) |
| 4 | 1 (2) | 1 (1) | 2 (1.5) | 1.5 (2) |
| 5 | 2 (3) | 2 (2) | 3 (0.5) | 1 (1.3) |
| 6 | 0 (1) | 0 (1) | 0 (0) | 0 (0) |
| 7 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Total | 5 (5) | 6 (5) | 4 (3) | 4.5 (2.3) |

H2 = hydrogen; AUC = Area under curve; CH4 = methane; n.a. = not analyzed. No clear peaking point could be determined for methane values.

**Supplementary Figure S3. A.** The average hydrogen production rate during the fourth study day (n=42). Lunch was served at the time point of 4 hours. **B**. Area under curve values of hydrogen production (medians +/- IQR). a differs significantly from b (p<0.05), (Wilcoxon Rank Test).

