**SUPPLEMENTARY METHODS**

*Production of Dairy Products*

Both dairy products were prepared at Agroscope, Federal Research Station for Agriculture (Bern) to industrial standards in accordance with Swiss food legislation. The yogurt culture was added with LGG to milk (preheated to 39oC) and stirred for five minutes before incubation at 39oC until pH reached 4.6. The acidified milk was prepared to mimic the appearance and texture of the yogurt by the addition of 2% D-(+)-glucono-delta-lactone powder (GDL, ≥ 99.0%, Jungbunzlauer AG, Switzerland) dissolved at room temperature by stirring for 5 min followed by incubation until the pH reached 4.6. Both products were stored immediately at 4oC upon reaching the target pH. The products were prepared in batches of 12-36 litres. Quality controls were carried out by the Quality Assurance Laboratory (Agroscope).

Fat was assessed by Roese-Gottlieb gravimetric method and dry matter assessed by gravimetric method (2h at 87°C). Nitrogen was assessed the Nitrogen Kjeldahl method with potentiometric titration. Protein content was defined as the nitrogen content multiplied by the Jones conversion factor for milk (6.38) (1). Enzymatic spectrophotometric analysis of the products were completed to quantify L-lactate, D-lactate and total lactate (adapted protocol from Boehringer Manheim, using an automated spectrophotometric analyzer, Thermo ScientificTM Gallery Automated Photometric Analyzer, Switzerland), acetate (protocol according to manufacturers, Boehringer Manheim), lactose (glucose moiety assessment by lactose hydrolysis with b-galactosidase followed by oxidation and redox reaction using ABTS), galactose (assessment of produced NADH at 340 nm, Thermo ScientificTM Gallery Automated Photometric Analyzer), and glucose (Enzymatic spectrophotometric determination, NADPH measurement at 340 nm). Free amino acids were assessed with Gallery spectrophotometric analyzer (Thermo, Switzerland) after derivatization with o-phthaldialdehyde in the presence of 2-Mercapto-ethansulfonic acid. All biochemical analyses of the products complied with ISO / IEC 17025:2005 requirements.

Carbohydrate and total sugar content of the products were calculated from the sum of the analysed sugars**.** Total energy content was calculated using Atwater conversion factors to assess total energy content (2). **Nutrient composition data is presented in Supplementary Table S1.**

**References**

1. Jones DB (1941. ) Factors for converting percentages of nitrogen in foods and feeds into percentages of protein pp. 22 [UDo Agriculture, editor]. Washington, DC: U.S. Government Printing Office.

2. Southgate DAT, Institute ARCFR (1981) THE RELATIONSHIP BETWEEN FOOD COMPOSITION AND AVAILABLE ENERGY [WHO Food and Agriculture Organization of the United Nations, The United Nations University, editor]. Provisional Agenda Item 4.1.3, Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements, Rome, 5 to 17 October 1981 .

**Online Supporting Material - Tables**

|  |  |  |
| --- | --- | --- |
|  | **Nutritional content of test products** **(per 100 g )** | **Nutritional content of normal milk (per 100ml)** |
| **Acidified milk** | **SEM** | **Probiotic yogurt** | **SEM** | ***UHT milk*** |
| **Energy (kJ)**  | 294 | 0.80 | 265 | 2.10 | *270*  |
| **Carbohydrate (g)**  | 4.9 | 0.01 | 2.7 | 0.05 | *4.9*  |
| **Protein (g) \*\***  | 3.3 | 0.02 | 3.4 | 0.03 | *3.2*  |
| **Fat (g) \*\***  | 3.4 | 0.01 | 3.5 | 0.01 | *3.6*  |
| **Total sugars (g)**  | 4.9 | 0.01 | 2.7 | 0.05 | *4.9*  |
| **Lactose monohydrate (g) \***  | 4.9 | 0.01 | 2.5 | 0.04 | *NA*  |
| **Galactose (g) \***  | 0.0 | 0.00 | 0.2 | 0.01 | *NA*  |
| **Glucose (g) \***  | 0.0 | 0.00 | 0.0 | 0.00 | *NA*  |
| **Total lactate (g) \***  | 0.0 | 0.00 | 1.1 | 0.03 | *NA*  |

**Supplementary Table S1.** Nutritional composition of the milk products[[1]](#footnote-1).

|  |  |  |
| --- | --- | --- |
| **DAY 1** | **DAY 2** | **DAY 3** |
| **Food/Drink** | **Quantity** | **Food/Drink** | **Quantity** | **Food/Drink** | **Quantity** |
| *Breakfast* |  | *Breakfast* |  | *Breakfast* |  |
| Muesli | 55g | Muesli | 55g | Muesli | 55g |
| **Assigned dairy product\*** | **200ml/200g** | **Assigned dairy product\*** | **200ml/200g** | **Assigned dairy product\*** | **200ml/200g** |
| Sweet bread rolls | 70g | Sweet bread rolls | 70g | Sweet bread rolls | 70g |
| Margarine | 20g | Margarine | 20g | Margarine | 20g |
| *Snack* |  | *Snack* |  | *Snack* |  |
| Cereal bar  | 20g | Dried fruit bar | 40g | Apple | 140g |
|  |  |  |  | Cereal bar | 20g |
| *Lunch* |  | *Lunch* |  | *Lunch* |  |
| Tomato | 120g | Cucumber | 120g | Basmati rice | 95g |
| Sliced roast ham | 60g | Tomato | 120g | Tinned tuna in vegetable oil | 40g |
| Margarine | 20g | Couscous  | 90g | Tomato | 120g |
| Precooked baguette | 125g | Cooked chicken with herbs | 65g | Egg | 1 |
| Fruit and nuts mix | 35g | Olive oil | 15ml | Olive oil | 15ml |
| Apple juice | 250ml | Kitkat® | 30g | Precooked bread roll | 40g |
|  |  | Apple | 140g | Margarine | 10g |
| *Dinner* |  | *Dinner* |  | *Dinner* |  |
| Tinned ratatouille vegetables | 210g | Tricolour penne pasta | 110g | Gnocchi pasta | 110g |
| Basmati rice | 80g | Tomato pasta sauce | 200g | Tomato pasta sauce | 200g |
| Cooked chicken with herbs | 65g | Tinned tuna in vegetable oil | 40g | Leek | 100g |
| Margarine  | 10g | Tinned green beans | 120g | Sliced roast ham | 60g |
| Precooked bread roll | 40g | Precooked bread roll | 40g | Apple-strawberry puree | 90g |
| Madeline cake | 37g | Margarine  | 10g |  |  |
| *Snack* |  | *Snack* |  | *Snack* |  |
| Pear | 140g | Apple-strawberry puree | 90g | Banana | 110g |
| **Assigned dairy product\*** | **200ml/200g** | **Assigned dairy product\*** | **200ml/200g** | **Assigned dairy product\*** | **200ml/200g** |

**Supplementary Table S2.** Example menu of the 3-day controlled diet (2500Kcal menu). \*Assigned dairy product corresponds to 200ml full fat normal milk, 200g probiotic yogurt or 200g acidified milk according to the test phase.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline** **(*n*=10)** | **Run-in** **(*n*=12)** | **Dairy test phase probiotic yogurt (*n*=12)** | **Wash-out post- probiotic yogurt** **(*n*=12)** | **Dairy test phase acidified milk** **(*n*=12)** | **Wash-out post- acidified milk** **(*n*=12)** |  |
| **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | ***P*** |
| **Energy (MJ/d)** | 10.0 | 8.8 - 11.1 | 9.6 | 9.1 - 11.0 | 9.3 | 8.3 - 10.8 | 10.3 | 9.7 - 10.7 | 10.2 | 9.4 - 11.5 | 9.7 | 9.0 - 11.1 | 0.92 |
| **Protein (g/d)** | 86.7 | 75.7 - 92.0 | 90.0 | 82.7 - 95.7 | 93.3 | 88.7 - 105.1 | 94.4 | 91.4 - 97.2 | 83.2 | 80.6 - 92.6 | 90.2 | 82.8 - 105.9 | 0.27 |
| **Carbohydrate (g/d)** | 296.8 | 276.8 - 341.2 | 308.2 | 241.5 - 342.3 | 278.2 | 216.5 - 330.4 | 308.0 | 260.2 - 336.9 | 321.7 | 272.8 - 341.1 | 280.3 | 234.3 - 357.9 | 0.95 |
| **Sugar (g/d)** | 118.4 | 96.1 - 134.0 | 112.7 | 88.2 - 153.5 | 95.2 | 75.8 - 144.5 | 121.7 | 83.3 - 133.3 | 104.5 | 82.5 - 148.3 | 90.8 | 82.2 - 120.9 | 0.93 |
| **Fat (g/d)** | 79.8 | 64.3 - 93.2 | 80.8 | 67.5 - 89.7 | 75.6 | 68.9 - 83.4 | 76.4 | 69.9 - 83.4 | 84.5 | 75.9 - 88.5 | 76.5 | 72.6 - 83.2 | 0.76 |
| **Fibre (g/d)** | 26.8 | 21.2 - 28.6 | 21.4 | 17.4 - 26.5 | 25.0 | 18 - 28.8 | 21.4 | 16.4 - 29.2 | 23.0 | 19.1 - 25.8 | 21.7 | 18.9 - 30.9 | 0.90 |
| **Alcohol (units/d)** | 0.5 | 0.0 - 1.1 | 0.0 | 0.0 - 1.0 | 0.0 | 0.0 - 0.8 | 0.2 | 0.0 - 1.0 | 0.0 | 0.0 - 1.0 | 0.1 | 0.0 - 1.3 | 0.94 |
| **Fruit and vegetables (portions/d)** | 3.4 | 2.0 - 4.6 | 3.7 | 2 - 4.7 | 4.0 | 2.3 - 5.3 | 4.1 | 3.5 - 4.5 | 3.4 | 2.3 - 4.6 | 3.6 | 1.5 - 4.7 | 0.90 |
| **Sugary drinks (250ml/d)** | 1.6 | 1.2 - 2.5 | 1.5 | 0.9 - 2.8 | 1.6 | 0.7 - 2.9 | 1.8 | 0.6 - 2.5 | 1.2 | 0.5 - 2.8 | 1.3 | 0.6 - 3.3 | 0.99 |
| **Dairy products (portions/d)** | 2.0 | 1.8 - 2.6 | 2.0 | 2.0 - 2.1 | 2.0 | 2.0 - 2.0 | 2.0 | 2.0 - 2.0 | 2.0 | 2.0 - 2.0 | 2.0 | 2.0 - 2.0 | 0.70 |

**Supplementary Table S3.** Nutritional analysis by test phase, assessed by Kruskal Wallis test (all results NS; *P* > 0.05). [[2]](#footnote-2)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Run in** **(n=8)** | **Dairy test phase** **probiotic yogurt** **(*n*=10)** | **Wash-out post-** **probiotic yogurt** **(n=10)** | **Dairy test phase** **acidified milk** **(n=8)** | **Wash-out post-** **acidified milk** **(n=9)** | ***P*** |
| **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** |
| **Activity energy expenditure (AEE) (kJ/d)** | 1994 | 1522 - 2384 | 1420 | 1248 - 1805 | 1392 | 913 - 2149 | 1762 | 1511 - 2087 | 1609 | 1183 - 2525 | 0.49 |
| **Average Hourly AEE (kJ/h)** | 85 | 64 - 99 | 62 | 54 - 78 | 60 | 39 - 90 | 73 | 67 - 90 | 70 | 52 - 107 | 0.63 |
| **MET** | 1.3 | 1.2 - 1.3 | 1.2 | 1.2 - 1.2 | 1.2 | 1.1 - 1.3 | 1.2 | 1.2 - 1.3 | 1.2 | 1.2 - 1.4 | 0.43 |
| **Number of Bouts** | 1.3 | 1.2 - 1.6 | 0.3 | 0.1 - 1.0 | 0.6 | 0.2 - 1.2 | 1.1 | 0.5 - 1.4 | 1.4 | 0.5 - 2.0 | 0.19 |
| **Total Time in Bouts (min)** | 19.9 | 14.8 - 27.6 | 4.5 | 1.5 - 14.0 | 8.1 | 2.4 - 19.9 | 16.6 | 9.0 - 19.3 | 22.3 | 6.6 - 32.7 | 0.27 |
| **Total Counts in Bouts** | 78877 | 59465 - 117103 | 14349 | 4801 - 58694 | 32216 | 11969 - 77043 | 63293 | 37584 - 81630 | 75655 | 21826 - 116284 | 0.30 |
| **Number of Sedentary Bouts** | 15.2 | 14.3 - 17.3 | 14.3 | 10.6 - 15.6 | 12.6 | 11.4 - 14.2 | 14.3 | 11.0 -15.0 | 11.5 | 10.8 - 16.5 | 0.80 |
| **Total Time in Sedentary Bouts (min)** | 415.0 | 324.5 - 480.3 | 431.7 | 304.3 - 486.0 | 371.3 | 327.6 - 401.8 | 377.4 | 351.8 - 428.6 | 364.6 | 306.3 - 428.3 | 0.76 |
| **Light PA (counts)** | 801.7 | 716.7 - 861.7 | 703.8 | 654.3 - 763.8 | 679.2 | 608.8 - 765.0 | 687.4 | 620.7 - 842.0 | 675.5 | 640.8 - 757.3 | 0.58 |
| **Moderate PA (counts)** | 56.3 | 48.1 - 63.0 | 39.2 | 33.0 - 41.8 | 37.4 | 29.2 - 49.7 | 41.8 | 39.2 - 46.8 | 46.3 | 37.2 - 65.4 | 0.06 |
| **Vigorous PA (counts)** | 5.2 | 1.0 - 11.9 | 1.6 | 0.7 - 4.4 | 1.9 | 0.7 - 4.2 | 3.6 | 1.8 - 6.8 | 2.0 | 1.1 - 4.6 | 0.71 |
| **Very Vigorous PA (counts)** | 0.0 | 0.0 - 0.2 | 0.0 | 0.0 - 0.1 | 0.0 | 0.0 - 0.3 | 0.0 | 0.0 - 0.0 | 0.0 | 0.0 - 0.2 | 0.69 |
| **Total MVPA (counts)** | 62.9 | 52.0 - 71.0 | 40.0 | 38.3 - 44.5 | 38.4 | 30.5 - 52.9 | 46.0 | 42.4 - 54.4 | 47.1 | 38.7 - 69.4 | 0.05 |
| **Vector Magnitude Counts** | 478191 | 428628 - 606017 | 352223 | 316167 - 436269 | 390330 | 284251 - 519971 | 454976 | 412480 - 522034 | 440930 | 348085 - 589976 | 0.57 |
| **Vector Magnitude (CPM)** | 625.0 | 548.4 - 679.2 | 444.8 | 420.9 - 699.2 | 594.1 | 431.4 - 688.3 | 606.0 | 500.4 - 731.4 | 603.7 | 476.7 - 763.0 | 0.49 |
| **Time (min)** | 872.7 | 766.3 - 932.3 | 743.8 | 691.8 - 806.5 | 707.4 | 648.8 - 826.6 | 742.0 | 675.1 -875.5 | 731.4 | 700.8 - 826.4 | 0.46 |

**Supplementary Table S4.** Analysis of changes in physical activity parameters, evaluated by test phase. Kruskal Wallis test (all results NS, *P*>0.05).[[3]](#footnote-3)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Group 1: acidified milk – probiotic yogurt (*n*=6) | Group 2: probiotic yogurt - acidified milk (*n*=6) | Statistical assessments |
| **Parameter** | **Delta change acidified milk** | **Delta change probiotic yogurt** | **Delta change acidified milk** | **Delta change probiotic yogurt** | **Carry-over effect** | **Intervention effect** |
| **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | **Median** | **IQR** | ***P*** | ***P*** |
| Glycemia (mmol/l) | 0.3 | 0.1 - 0.4 | 0.0 | -0.1 - 0.1 | 0.1 | -0.1 - 0.1 | 0.0 | -0.3 - 0.2 | 0.30 | 0.23 |
| Insulin (mU/l) | 0.6 | -0.4 - 1.6 | 1.4 | 0.9 - 1.6 | -0.4 | -1.5 - 0.2 | -0.7 | -0.8 - 0.2 |  0.02\* | 0.70 |
| Total cholesterol (mmol/l) | -0.3 | -0.3 - -0.2 | -0.2 | -0.3 - 0.0 | -0.2 | -0.4 - 0.2 | -0.1 | -0.3 - 0.1 | 0.75 | 0.75 |
| HDL Cholesterol (mmol/l) | -0.1 | -0.1 - 0.0 | 0.0 | -0.2 - 0.2 | 0.0 | 0.0 - 0.1 | 0.0 | -0.1 - 0.1 | 0.94 | 1.00 |
| LDL Cholesterol (mmol/l) | -0.2 | -0.2 - 0.0 | -0.2 | -0.3 - 0.0 | -0.1 | -0.4 - 0.3 | -0.1 | -0.2 - 0.0 | 0.57 | 1.00 |
| TAG (mmol/l) | 0.1 | 0.1 - 0.1 | 0.0 | -0.2 - 0.2 | -0.1 | -0.2 - 0.1 | 0.1 | -0.2 - 0.1 | 0.30 | 1.00 |
| NEFA (µmol/l) | -76.0 | -161.5 - -10 | -18.0 | -184.3 - 209.8 | -193.0 | -269 - -166.5 | -39.5 | -45.8 - -16 | 0.24 | 0.39 |
| hsCRP (mg/l) | 0.0 | -0.1 - 0.1 | 0.2 | 0.0 - 1.9 | -0.1 | -0.5 - 0.1 | -0.1 | -0.4 - 0.1 | 0.25 | 0.85 |
| LPS (EU/ml) | 0.0 | -0.3 - 0.3 | -0.3 | -0.5 - 0.1 | -0.1 | -0.5 - 0.5 | -0.3 | -0.5 - 0.2 | 0.59 | 0.24 |
| IL6 (pg/ml) | -0.3 | -1.1 - 0.5 | 0.4 | -0.3 - 1.9 | 1.6 | -0.9 – 5.0 | -1.3 | -2.4 - -0.6 | 0.82 | 0.82 |
| TNFα (pg/ml) | 0.3 | -0.3 - 0.8 | 1.2 | -0.1 - 2.6 | 1.6 | -1.4 - 2.6 | 0.3 | -0.1 - 1.5 | 0.82 | 0.48 |
| CCL2 (pg/ml) | 12.3 | 6.2 - 15.7 | 0.7 | 0.0 - 6.1 | 12.8 | 8.9 - 21.1 | 2.9 | 1.6 - 7.0 | 0.70 |  0.01\* |
| CCL5 (pg/ml) | 19.5 | -107.8 - 33.5 | -36.0 | -128.5 - 73 | -34.7 | -61.1 - 84.3 | 10.5 | -51 - 59.3 | 0.94 | 0.48 |

**Supplementary Table S5.** Change in fasting parameters after two-week intake of acidified milk compared to probiotic yogurt intake. [[4]](#footnote-4)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **OTU[[5]](#footnote-5) (species assigned) *n*=294** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| OTU\_729 (*Streptococcus salivarius thermophilus*) | 291.8 | 7.8 | 12.4 | .< 0.01 |
| OTU\_344 (*Lactobacillus delbrueckii* spp*. Bulgaricus*) | 149.8 | 3.7 | 5.0 | .< 0.01 |
| OTU\_135 (*Intestinibacter bartlettii*) | 346.0 | 2.2 | 3.8 | 0.01 |
| OTU\_280 (*Bifidobacterium kashiwanohense/**Bifidobacterium pseudocatenulatum*) | 1451.1 | -1.7 | -3.6 | 0.03 |
| **Species *n*=92** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Streptococcus salivarius thermophilus* | 313.9 | 7.6 | 12.6 | < 0.01 |
| *Lactobacillus delbrueckii* spp. *bulgaricus* | 153.7 | 3.6 | 5.2 | < 0.01 |
| *Bifidobacterium kashiwanohense/**Bifidobacterium pseudocatenulatum* | 1519.3 | -1.7 | -3.9 | < 0.01 |
| *Intestinibacter bartlettii* | 335.8 | 2.1 | 3.8 | < 0.01 |
| **Genus *n*=107** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Streptococcus* | 1985.0 | 1.8 | 4.3 | < 0.01 |
| *Intestinibacter* | 317.2 | 2.1 | 3.7 | 0.01 |
| *Bifidobacterium* | 1658.3 | -1.5 | -3.4 | 0.03 |
| *Megasphaera* | 1217.2 | -1.7 | -3.3 | 0.03 |
| **Family *n*=36** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P*** |
| *Streptococcus* | 1985.0 | 1.8 | 4.3 | < 0.01 |
| *Intestinibacter* | 317.2 | 2.1 | 3.7 | 0.01 |
| *Bifidobacterium* | 1658.3 | -1.5 | -3.4 | 0.03 |
| *Megasphaera* | 1217.2 | -1.7 | -3.3 | 0.03 |
| **Order *n*=24** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Bifidobacteriales* | 2214.2 | -1.6 | -3.8 | < 0.01 |
| *Lactobacillales* | 1794.0 | 1.2 | 3.2 | 0.02 |
| **Class *n*=21** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Actinobacteria* | 2123.1 | -1.4 | -3.4 | < 0.01 |
| *Bacilli* | 1842.5 | 1.4 | 3.5 | < 0.01 |
| **Phylum *n*=12** |  |  |  |  |
| No significant taxa |  |  |  |  |

**Supplementary Table S6.** Bacterial groups differentially present after probiotic intake compared to acidified milk intake (*P*adj.≤0.05). Statistic = value of the Wald test statistic.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **OTU[[6]](#footnote-6) (Assigned species) *n*=292** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| OTU\_344 (*Lactobacillus delbrueckii*spp. *bulgaricus*) | 45.4 | 7.4 | 14.1 | < 0.01 |
| OTU\_729 (*Streptococcus**salivarius thermophilus*) | 107.8 | 6.4 | 11.0 | < 0.01 |
| **Species *n*=99** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Lactobacillus delbrueckii* spp*.bulgaricus* | 39.8 | 7.0 | 14.3 | < 0.01 |
| *Streptococcus salivarius thermophilus* | 108.6 | 6.0 | 11.4 | <0.01 |
| *Bilophila wadsworthia* | 610.8 | -1.3 | -3.3 | 0.03 |
| *Haemophilus parainfluenzae* | 960.4 | -1.5 | -3.3 | 0.03 |
| **Genus *n*=106** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Lactobacillus* | 1231.2 | 3.3 | 5.7 | <0.01 |
| *Streptococcus* | 1719.2 | 1.4 | 4.1 | < 0.01 |
| *Ruminococcaceae UCG-004* | 104.3 | -1.8 | -3.9 | < 0.01 |
| *Bilophila* | 646.8 | -1.4 | -3.5 | 0.01 |
| *Haemophilus* | 862.1 | -1.6 | -3.6 | 0.01 |
| *Veillonella* | 3243.0 | -1.2 | -3.2 | 0.02 |
| *Flavonifractor* | 451.2 | -1.0 | -3.1 | 0.03 |
| *Oscillospira* | 83.6 | -1.0 | -3.0 | 0.03 |
| *Erysipelotrichaceae UCG-003* | 133.7 | 1.0 | 2.9 | 0.05 |
| *Escherichia-Shigella* | 7810.8 | -1.4 | -2.8 | 0.05 |
| **Family *n*=33**  | **Base mean**  | **FC** | **Statistic**  | **Adjusted *P***  |
| *Lactobacillus*  | 1231.2  | 3.3  | 5.7  | < 0.01  |
| *Streptococcus*  | 1719.2  | 1.4  | 4.1  | < 0.01  |
| *Ruminococcaceae UCG-004*  | 104.3  | -1.8  | -3.9  | < 0.01  |
| *Bilophila*  | 646.8  | -1.4  | -3.5  | 0.01  |
| *Haemophilus*  | 862.1  | -1.6  | -3.6  | 0.01  |

**Supplementary Table S7.** Bacterial groups differentially present after probiotic yogurt relative to baseline milk phases (*P*adj*.*≤0.05). Statistic = value of the Wald test statistic. No significant differences at Pylum level (*n*=9) (Table continued on next page).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Order *n*=24** | **Base mean** | **FC**  | **Statistic** | **Adjusted *P***  |
| *Lactobacillales* | 1329.5 | 1.5 | 4.2 | < 0.01 |
| *Desulfovibrionales* | 1232.5 | -1.2 | -3.1 | 0.01 |
| *Pasteurellales* | 671.7 | -1.4 | -3.1 | 0.01 |
| *Selenomonadales* | 16040.9 | -0.8 | -2.9 | 0.02 |
| **Class *n*=21** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Bacilli* | 1047.5 | 1.6 | 4.3 | < 0.01 |
| *Deltaproteobacteria* | 1219.2 | -1.1 | -2.9 | 0.03 |
| *Gammaproteobacteria* | 8111.0 | -1.2 | -2.9 | 0.03 |
| *Negativicutes* | 14341.9 | -0.7 | -2.7 | 0.03 |

**Supplementary Table S7.** (continued)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***OTU[[7]](#footnote-7) (species assigned)******n*=277** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P*** |
| OTU\_314 (unassigned) | 10.8 | -1.7 | -4.0 | 0.02 |
| OTU\_21 (*Bilophila wadsworthia)* | 572.8 | -1.6 | -3.6 | 0.03 |
| OTU\_109 (unassigned) | 89.7 | -1.9 | -3.7 | 0.03 |
| OTU\_45 (unassigned) | 129.7 | -1.3 | -3.6 | 0.03 |
| OTU\_104 (unassigned) | 12.0 | -2.2 | -3.4 | 0.04 |
| OTU\_133 (unassigned) | 454.5 | 2.3 | 3.2 | 0.04 |
| OTU\_145 (unassigned) | 441.0 | 1.0 | 3.3 | 0.04 |
| OTU\_280 (*Bifidobacterium kashiwanohense/ Bifidobacterium pseudocatenulatum* | 597.2 | 1.4 | 3.3 | 0.04 |
| OTU\_135(*Intestinibacter bartlettii*) | 215.7 | -1.7 | -3.2 | 0.04 |
| **Species *n*=89** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P*** |
| *Bifidobacterium kashiwanohense/ Bifidobacterium pseudocatenulatum* | 602.9 | 1.4 | 3.5 | 0.04 |
| *Bilophila wadsworthia* | 632.0 | -1.5 | -3.3 | 0.05 |
| **Genus *n*=105** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P*** |
| *Bilophila*  | 651.5  | -1.6 | -3.5  | 0.01  |
| *Clostridiaceae Family XIII AD3011 group*  | 168.1  | -1.2 | -3.6  | 0.01  |
| *Intestinibacter*  | 204.5  | -1.8 | -3.5  | 0.01  |
| *Ruminococcaceae UCG-004*  | 104.2  | -1.7 | -3.5  | 0.01  |
| *Desulfovibrio*  | 490.7  | -1.9 | -3.4  | 0.02  |
| *Lachnospiraceae UCG-004*  | 425.5  | 1.0 | 3.3  | 0.02  |
| *Ruminococcaceae UCG-014*  | 763.3  | 2.0 | 3.2  | 0.02  |
| *Bifidobacterium*  | 711.9  | 1.3 | 3.1  | 0.03  |
| *Clostridiaceae Family XIII UCG-001*  | 35.1  | -1.5 | -2.9  | 0.05  |

**Supplementary Table S8.** Bacterial groups differentially present after acidified milk relative to baseline milk phases (*P*adj*.*≤0.05). Statistic = value of the Wald test statistic (Table continued on next page).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Family *n*=34** | **Base Mean** | **FC**  | **Statistic** | **Adjusted *P***  |
| *Bilophila* | 651.5 | -1.6 | -3.5 | 0.01 |
| *Clostridiaceae Family XIII**AD3011 group* | 168.1 | -1.2 | -3.6 | 0.01 |
| *Intestinibacter* | 204.5 | -1.8 | -3.5 | 0.01 |
| *Ruminococcaceae UCG-004* | 104.2 | -1.7 | -3.5 | 0.01 |
| *Desulfovibrio* | 490.7 | -1.9 | -3.4 | 0.02 |
| *Lachnospiraceae UCG-004* | 425.5 | 1.0 | 3.3 | 0.02 |
| *Ruminococcaceae UCG-014* | 763.3 | 2.0 | 3.2 | 0.02 |
| *Bifidobacterium* | 711.9 | 1.3 | 3.1 | 0.03 |
| *Clostridiaceae Family XIII UCG-001* | 35.1 | -1.5 | -2.9 | 0.05 |
| **Order *n*=22** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Bifidobacteriales* | 956.2 | 1.2 | 3.1 | 0.03 |
| *Desulfovibrionales* | 1228.3 | -1.3 | -3.3 | 0.03 |
| **Class *n*=19** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Actinobacteria* | 963.6 | 1.2 | 3.0 | 0.02 |
| *Deltaproteobacteria* | 1137.9 | -1.3 | -3.2 | 0.02 |
| **Phylum *n*=10** | **Base Mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Actinobacteria* | 1389.0 | 1.2 | 3.2 | 0.02 |

**Supplementary Table S8. (**continued).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Genus *n*= 105** | **Base mean** | **FC [[8]](#footnote-8)** | **Statistic** | **Adjusted *P***  |
| *Clostridiaceae Family XIII**AD3011 group* | 127.3 | -1.3 | -3.7 | 0.02 |
| **Family *n*= 34** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Clostridiaceae Family XIII**AD3011 group* | 127.3 | -1.3 | -3.7 | 0.02 |
| **Order *n*= 22** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Erysipelotrichales* | 289.5 | -1.3 | -4.4 | < 0.01 |
| **Class *n*= 19** | **Base mean** | **FC** | **Statistic** | **Adjusted *P*** |
| *Erysipelotrichia* | 295.3 | -1.3 | -3.9 | 0.00 |

**Supplementary Table S9.** Bacterial groups differentially present between baseline milk phases (*P*adj.≤0.05) to assess time-course changes: Wash-out 1 compared to Wash out 2. Statistic = value of the Wald test statistic.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **OTU[[9]](#footnote-9) (Assigned species)** ***n*= 277** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| OTU\_67 (unassigned) | 882.1 | -2.6 | -4.1 | 0.01 |
| OTU\_296 (unassigned) | 4226.9 | -2.7 | -3.8 | 0.02 |
| **Species *n*= 89** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Prevotella copri* | 130769.2 | -2.2 | -3.9 | 0.01 |
| *Clostridium celatum /**Clostridium disporicum* | 589.8 | -2.4 | -3.5 | 0.02 |
| *Parasutterella excrementihominis* | 1001.1 | -2.0 | -3.3 | 0.02 |
| *Phascolarctobacterium succinatutens* | 6315.7 | -2.3 | -3.4 | 0.02 |
| **Genus *n*= 105** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Prevotella 9* | 83322.1 | -1.7 | -3.3 | 0.05 |
| **Family *n*= 34** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Prevotella 9* | 83322.1 | -1.7 | -3.3 | 0.05 |
| **Order *n*= 22** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Lactobacillales* | 1859.6 | 1.5 | 4.0 | < 0.01 |
| **Class *n*= 19** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Bacilli* | 1732.3 | 1.5 | 4.0 | < 0.01 |

**Supplementary Table S10.** Bacterial groups differentially present between baseline milk phases (*P*adj.≤0.05) to assess time-course changes: Run in compared to wash-out 1. Statistic = value of the Wald test statistic.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **OTU[[10]](#footnote-10) (Assigned species) *n*= 277** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| OUT\_125 (unassigned) | 159.0 | 1.8 | 4.1 | 0.01 |
| OTU\_67 (unassigned) | 609.7 | -1.9 | -3.6 | 0.05 |
| **Species *n*= 89** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Bacteroides eggerthii* | 385.1 | -2.3 | -3.5 | 0.04 |
| **Genus *n*= 105** |  |  |  |  |
| No significant taxa |  |  |  |  |
| **Family *n*= 34** |  |  |  |  |
| No significant taxa |  |  |  |  |
| **Order *n*= 22** |  |  |  |  |
| No significant taxa |  |  |  |  |
| **Class *n*= 19** |  |  |  |  |
| No significant taxa |  |  |  |  |
| **Phylum *n*= 9** | **Base mean** | **FC** | **Statistic** | **Adjusted *P***  |
| *Synergistetes* | 18.0 | 2.4 | 3.5 | 0.01 |

**Supplementary Table S11.** Bacterial groups differentially present between baseline milk phases (*P*adj.≤0.05) to assess time-course changes: Run in compared to wash out 2. Statistic = value of the Wald test statistic.

**Online Supporting Material- Figures**

**Supplementary Fig S1. Stability of the three bacteria present in the probiotic yogurt on days 1, 7 and 14 following probiotic yogurt production.** Analyses completed for products sampled at day 1 post-production (dark grey), day 2 post-production (medium grey) and day 14 post-production (light grey). Participants received the products on between days 4 and 15 following production.

Assessed for eligibility (*n*= 393 )

Excluded (*n*= 379)

   Not meeting inclusion criteria

(*n*= 185)

  Declined to participate/no further contact after study protocol provided (*n*= 192)

  Other reasons (*n*= 2)

Randomised (*n*=14)

Allocated to group **probiotic yogurt-acidified milk**

 (*n*= 7)

   Received allocated intervention (*n*=7)

    Did not receive allocated intervention (*n*=1), acute illness during study test day

Allocated to group **acidified milk-probiotic yogurt**

(*n*=7)

   Received allocated intervention (*n*= 7)

    Did not receive allocated intervention

(*n*= 0)

Analysed (*n*=6)
    Excluded from analysis (*n*=1), (non-compliance with dietary protocol)

Analysed (*n*= 6)
    Excluded from analysis (*n*=1), (acute infection)

**Supplementary Fig S2. Flow chart of the study** (adapted from CONSORT 2010)**.**

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**Supplementary Fig S3.** Raw reads of yogurt and probiotic bacterial strains for study phases with dietary restrictions. Panels A-E show *Streptococcus salivarius* spp*. thermophilus* (purple) and *Lactobacillus delbrueckii* spp. *bulgaricus* (green); Panels F-J show *Lactobacillus rhamnosus* GG(blue) and *Lactobacillus casei/ Lactobacillus paracasei* (red). Samples coded by study day (RI=run-in, Y1/Y2=probiotic yogurt group 1/2, M1/M2=acidified milk group 1/2, WO1/WO2=wash-out 1/2), subject number (002-021), and Illumina run number (1-8). Repeated sample analysis completed for samples WO2\_018 and Y2\_018.

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**Supplementary Fig S4.** Postprandial kinetic response for inflammatory markers, (a) TNFα, (b) IL6, and (c) CCL5, to high fat test meal after two week intake of acidified milk or probiotic yogurt.Basal=white, post-acidified milk=light grey, post-yogurt=dark grey.



3e+06

0e+00

**Supplementary Fig S5.** Cluster analysis by Spearman’s distance using OTUs present at a mean abundance of 0.01% in a minimum of three volunteers (*n*=292). All samples included in the analysis are presented. Samples coded by study day (RI=run-in, Y1/Y2=probiotic yogurt group 1/2, M1/M2=acidified milk group 1/2, WO1/WO2=wash-out 1/2), subject number (002-021). Colour-coding by subject ID number.

1. All control UHT milk nutrients as indicated by manufacturer.\*Nutrients assayed by enzymatic spectrophotometric analysis; \*\*nutrients assayed by Roese-Gottlieb gravimetric method and Kjeldahl method with potentiometric titration. Total sugars calculated as the sum of assayed sugars. Carbohydrate calculated by sum of all assayed carbohydrates. Protein calculated by assessment of total nitrogen content using Jones conversion factor for milk (1). Atwater factors used to calculate total energy content (2). [↑](#footnote-ref-1)
2. IQR, interquartile range. Only data from volunteers that completed all test days analysed (*n*=12). [↑](#footnote-ref-2)
3. IQR, interquartile range; AEE, activity energy expenditure; MET, Metabolic equivalent of task; PA, Physical activity; MVPA, Moderate-vigorous PA; CPM, counts per min.

 Minimum wear time is 8 h/d for three days. [↑](#footnote-ref-3)
4. IQR, interquartile range; hsCRP, high sensitivity C-reactive protein; LPS, lipopolysaccharide; CCL2, chemokine ligand 2; CCL5, chemokine ligand 5. \* = significant at *P*≤ 0.05. Wilcoxon test used to assess significance of carry-over effects and to assess differences between the two interventions. [↑](#footnote-ref-4)
5. OTU, Operational taxonomic unit; FC, Fold change (log2). [↑](#footnote-ref-5)
6. OTU, Operational taxonomic unit; FC, Fold change (log2). [↑](#footnote-ref-6)
7. OTU, Operational taxonomic unit; FC, Fold change (log2). [↑](#footnote-ref-7)
8. FC, Fold change (log2). [↑](#footnote-ref-8)
9. OTU, Operational taxonomic unit; FC, Fold change (log2). [↑](#footnote-ref-9)
10. OTU, Operational taxonomic unit; FC, Fold change (log2). [↑](#footnote-ref-10)