

## Supplementary Information

### S1. Materials and Methods

#### S1.1. Nutritional characteristics of gluten-free diets

The dry matter, proteins, ash, and total starch (Table S1) of experimental GFDs were determined according to the methods 935.36, 950.36, 930.22, and polarization method 945.37, respectively [1]. The fat content was calculated by subtracting the sum of total solids (100 – proteins – ash - total starch - inulin; expressed in g/100g). Based on literature data, it was assumed that the calorific value of inulin is 1.5 kcal/g [2]. Mineral concentrations in diets (Table S1) was measured by flame (air – acetylene burner) atomic absorption spectrometry method (AAS) using an atomic absorption spectrophotometer (iCE 3000 SERIES - THERMO - England) equipped with an autosampler and the appropriate cathode lamp operating at the resonance line of the analysed bioelements (Ca: 422.7 nm; Mg: 285.2 nm). Before analysis, the samples were wet-digested with a mixture (9 : 1; v/v) of concentrated nitric acid (65 % HNO<sub>3</sub>; Merck, Darmstadt, Germany) and hydrochloric acid (30 % HCl; Merck) using a microwave system (Multiwave, Anton Paar GmbH, Graz, Austria). Calcium concentration was validated by adding a solution of lanthanum (III) chloride hydrate (LaCl<sub>3</sub> x 7H<sub>2</sub>O; Merck, Germany) to all samples in sufficient amounts to obtain 0.5% concentration of La<sup>3+</sup>. Phosphorus content was determined by the colorimetric molybdate method with hydroquinone (POCH S. A. Gliwice, Poland) and sodium (IV) sulphate (POCH S. A. Gliwice, Poland). Absorbance was measured using the VIS 6000 Spectrophotometer (KRÜSS- OPTRONIC, Germany) at  $\lambda = 610$  nm. The concentrations of Ca, Mg and P were automatically read from a calibration curve (Ca: range 0.5 – 4.0  $\mu\text{g/mL}$ ; Mg: range 0.05 – 0.8  $\mu\text{g/mL}$ ; P: range 0.4 – 2.0  $\mu\text{g/mL}$ ) prepared with the AAS standard solution of Ca, Mg and P, respectively (J.T.Baker®, Holland). The analyses were repeated (N = 8) for analytical quality control.

**Table S1.** Nutritional composition of experimental gluten-free diets

	O	R	OI	RI
Content of macronutrients [g/100g]				
Dry matter	9.44 ± 0.05	9.32 ± 0.04	9.26 ± 0.14	9.01 ± 0.07
Proteins*	13.97 ± 0.62	13.94 ± 0.71	13.68 ± 0.39	14.09 ± 0.44
Starch	65.06 ± 0.39	66.36 ± 0.43	57.80 ± 1.20	59.09 ± 0.68
Ash	2.10 ± 0.11	1.57 ± 0.08	1.94 ± 0.10	1.51 ± 0.04
Content of selected minerals [mg/g]				
Calcium	6.44 ± 0.03	2.71 ± 0.02	5.58 ± 0.01	2.56 ± 0.01
Magnesium	0.46 ± 0.003	0.48 ± 0.001	0.47 ± 0.001	0.46 ± 0.001
Phosphorus	2.54 ± 0.03	2.64 ± 0.02	2.51 ± 0.01	2.47 ± 0.00

\* N x factor 6.25

### S1.2. Microbiota characteristics with PCR-DGGE

**Table S2.** Primers and amplification conditions used for qualitative (PCR-DGGE) analysis of caecal microbiota.

Name	Sequence (5' 3')	PCR product (bp)	MgCl <sub>2</sub> (mM)	T <sub>a</sub> (°C)	Target	Reference
1401-r 968-f-GC	cgggtgtgtacaagaccc GC-aacgcgaagaacctta	486	5.0	58	Eubacteria	[3]
Bfrag-F Bfrag-R-GC	aacgctagctacaggctt GC-caatcggagttcttcgtg	388	1.5	56	<i>Bacteroides</i>	[4]
CLept-F CLept-R-GC	gcacaagcagtggagt GC-cttctccggtttgtcaa	239	2.0	53	<i>Clostridium leptum</i> group	[5]
Lac1F Lac2 GC R	agcagtagggaatctcca GC-attycaccgctacacatg	347	5.0	57	<i>Lactobacillus</i>	[6]
GC clamp	cgccccggggcgcgccggcgccggcgccggggggcaccggggg	-	-	-	-	[7]

**Table S3.** Denaturing gradient applied for separation of PCR products in DGGE technique.

Bacterial group	Gradient (%)	Electrophoretal conditions
Eubacteria	25-65	200V, 10 min.; 85V, 18 h
<i>Bacteroides</i>	25-55	200, 10 min.; 85V, 16 h
<i>Clostridium leptum</i> group	25-37.5	85V, 10 min; 200V, 4 h
<i>Lactobacillus</i>	40-55	200V, 10 min.; 85V, 20 h

### S1.3. Quantification of caecal microbiota by real-time PCR

**Table S4.** Primers used for real-time PCR analysis.

Target	Primer	Sequence (5' 3')	T <sub>a</sub> (°C)	Reference
<i>Bacteroides-Prevotella-Porphyromonas</i> group	BPP-F BPP-R	ggtgtcggcttaagtgccat cgga(c/t)gtaaggccgtgc	56	[8]
<i>Bifidobacterium</i>	BIF-F BIF-R	tcg cgtc(c/t)ggtgtgaaag ccacatccagc(a/g)tccac	58	[8]
<i>Clostridium leptum</i> group	sg-Clept-F sg-Clept-R3	gcacaagcagtggagt cttctccggtttgtcaa	53	[5]
<i>Enterococcus</i>	ECC-F ECC-R	cccttattgttagttgccatcatt actcgttgacttccattgt	61	[8]
<i>Lactobacillus</i>	Lac1F Lab667	agcagtagggaatctcca caccgctacacatggag	58	[7]
Total bacteria	UniF UniR	gtgstgcaygyygtcgta acgtrtccmnccttctc	60	[9]

## References

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