Synbiotic (*Lactiplantibacillus pentosus* GSSK2 and isomalto-oligosaccharides) supplementation modulates pathophysiology and gut dysbiosis in experimental metabolic syndrome

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Supplementary Information

Supplementary Table S1: List of bacterial primers for q-PCR analysis of gut bacterial abundance

Bacterial primers	**Forward 5'-3'**	**Reverse 5'- 3'**
Total Bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Bacteroidetes	ACGCTAGCTACAGGCTTAACA	ACGCTACTTGGCTGGTTCA
Firmicutes	GCGTGAGTGAAGAAGT	CTACGCTCCCTTTACAC
Lactobacillus	CACCGCTACACATGGAG	AGCAGTAGGGAATCTTCCA
Bifidobacterium	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC
Akkermensia	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT
Faecalibacterium	GAGGAAGATAATGACGGTAC	ACCTCTGCACTACTCAAGA
Roseburia	GCGGTRCGGCAAGTCTGA	CCTCCGACACTCTAGTMCGA
Ruminococcus	CCCTGCGCTGTGCGAAAAAG	CCGCTGCGTACCTTTGGGAT
Prevotella	GATGGGGATGCGTCTGATTAG	TCCTGCACGCTACTTGGCT
Enterobacteriaceae	CCCTTATTGTTAGTTGCCAT	ACTCGTTGTACTTCCCATTG

Primer sequences were obtained from literature[13] and were procured from Eurofin Genomics India Pvt. Ltd.

PCR conditions for gut bacteria abundance : q-PCR was performed in 10µl reaction volume containing 1 µl template DNA (40 ng), 1 µl forward primer, 1 µl reverse primer, 2µl RNase free water and 5 µl iTaq Universal SYBR Green Supermix (2X) and was carried out using following conditions: 95°C for 2 minutes followed by 40 cycles of 95°C for 5 s, 60°C for 30 s with melt curve at 65-95°C for 5 s.

Supplementary Table S2: List of primers for q-PCR analysis of gene expression in tissues.

Gene	Forward (5'-3')	Reverse (5'-3')	Source
GAPDH	ACGGGAAACCCATCACCATC	CTCGTGGTTCACACCCATCA	*
Adiponectin	GGAAACTTGTGCAGGTTGGATG	GGGTCACCCTTAGGACCAAGAA	[1]
Leptin	TTCAAGCTGTGCCTATCCACAAAG	TGAAGCCCGGGAATGAAGTC	[1]
TLR-4	TGGCAGTTTCTGAGTAGCCG	GCTTTTCCATCCAACAGGGC	[2]
Claudin	TGTCCACCATTGGCATGAAG	GCCACTAATGTCGCCAGACC	[3]
CDX-2	CGCATCATCACCCTCACCAT	CGTCCTGGTTTTCACTTGGC	*
Muc-2	GCCAGATCCCGAAACCATGT	AGGACGGACTCTATGCTGGA	[2]
GLUT-4	CAACTGGACCTGTAACTTCATCGT	ACGGCAAATAGAAGGAAGACGTA	[4]
Glucokinase	CAGTGGAGCGTGAAGACAAA	CTTGGTCCAATTG AGGAGGA	*
CEBP-α	TTACAACAGGCCAGGTTTCC	GGCTGGCGACATACAGTACA	[5]
PPAR-γ	GACCACTCCCATTCCTTTGA	CGCACTTTGGTATTCTTGGAG	[6]
IL-6	TCCTACCCCAACTTCCAATGCTC	TTGGATGGTCTTGGTCCTTAGCC	[7]
TNF-α	GTCGTAGCAAACCACCAAGC	TGTGGGTGAGGAGCACATAG	[8]
FASN	GCAGCAGCATGATGTAGCAC	AGTTGCACACCACAAGGTCA	*
HSL	CGCCTTACGGAGTCTATGC	TCTGATGGCTCTGAGTTGC	[5]

*Primer sequences were designed using Primer BLAST software and were procured from Eurofin Genomics India Pvt. Ltd.

PCR conditions for gene expression analysis in tissues: q-PCR was performed in 10µl reaction volume containing 1 µl template cDNA (20 ng), 1 µl forward primer, 1 µl reverse primer,1 µl RNase free water and 5 µl iTaq Universal SYBR Green Supermix (2X). qPCR was carried out under the following conditions: 95°C for 2 minutes followed by 40 cycles of 95°C for 5 s, 60°C for 30 s with melt curve at 65-95°C for 5 s.

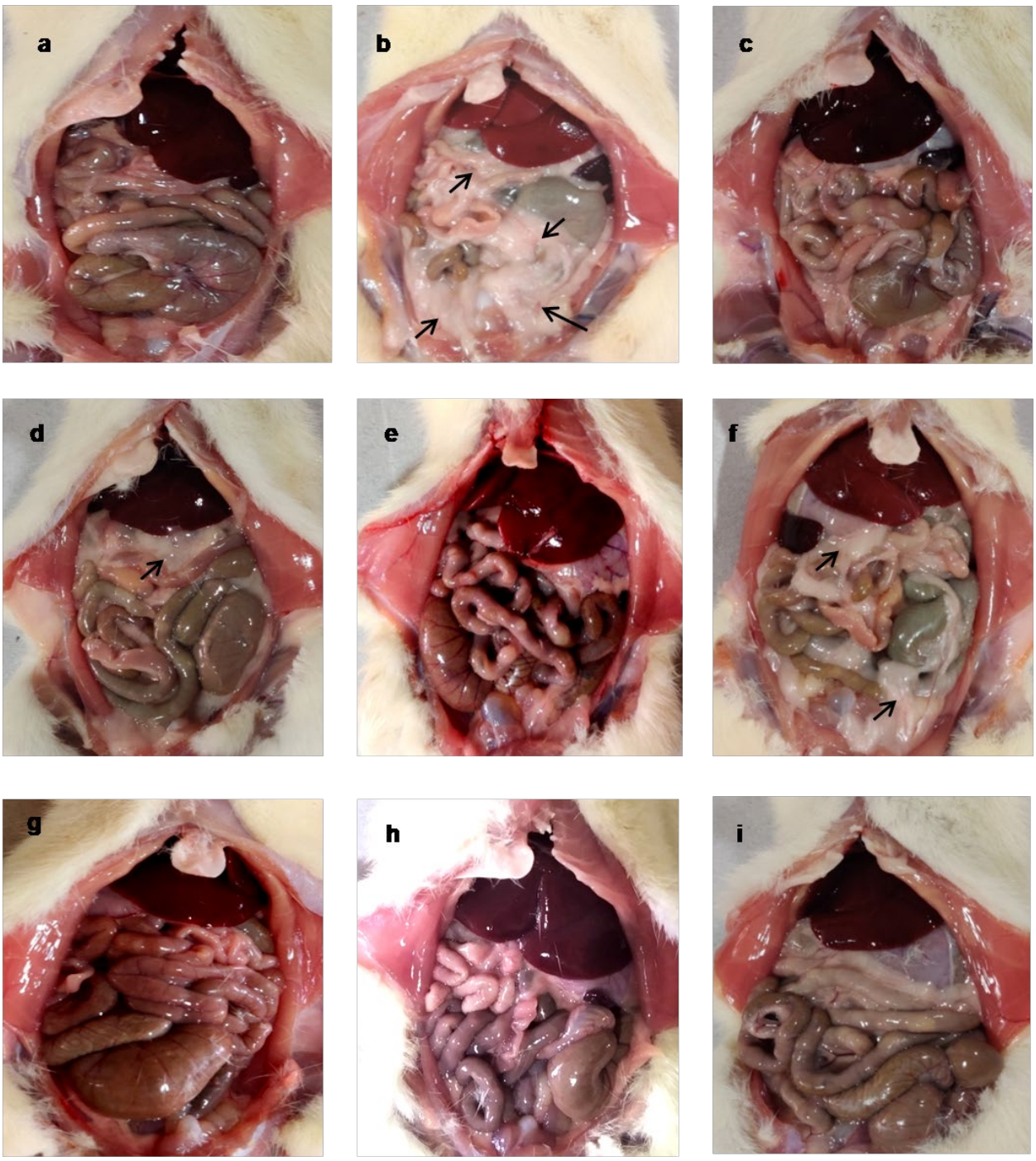

Figure S1: Gross macroscopic observation of adipose tissue of animals belonging to different groups: (a) Control, (b) HFD, (c) *L. pentosus*, (d) *L. pentosus* + HFD, (e) IMOs, (f) IMOs + HFD, (g) Synbiotic, (h) Synbiotic + HFD, (i) Orlistat + HFD. Arrows indicate adipose tissue deposits.

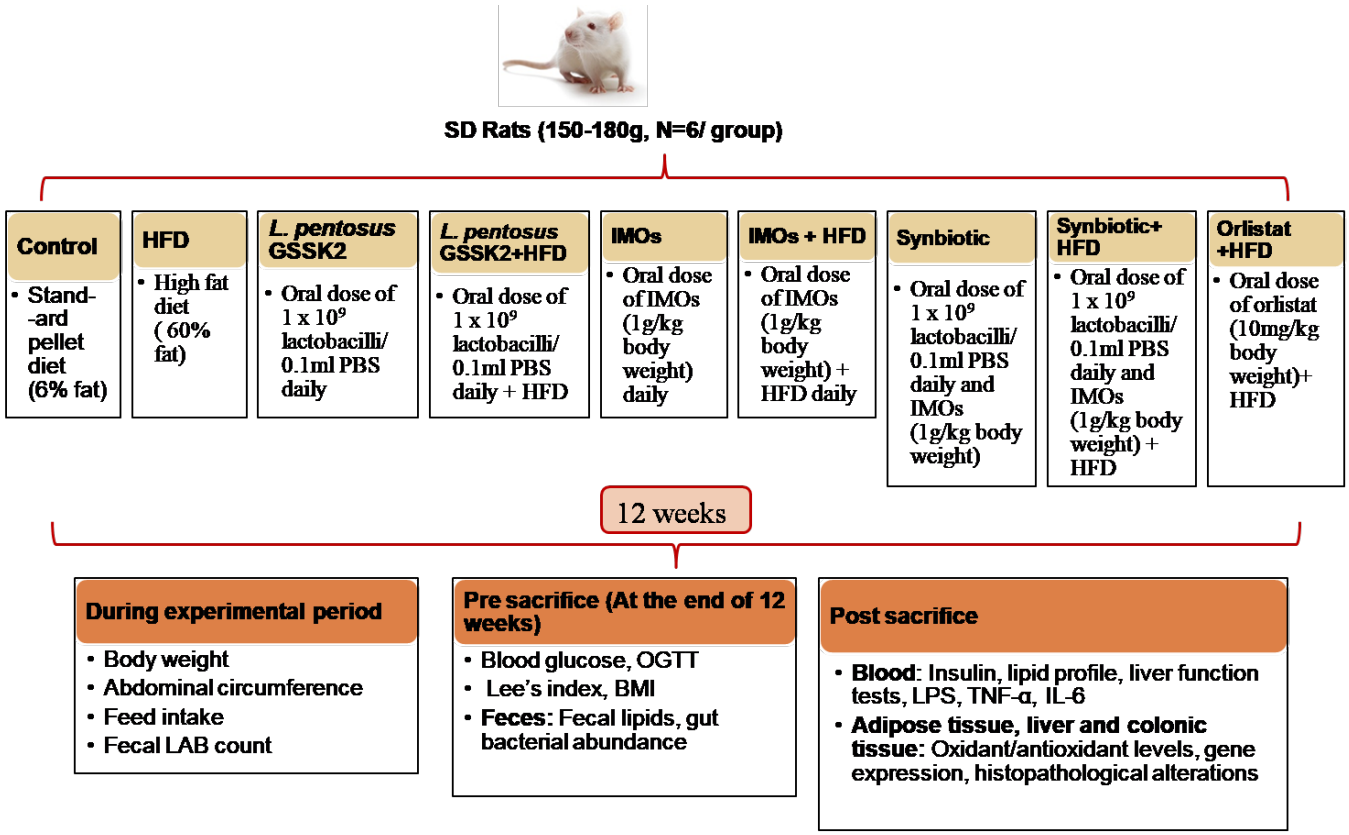

Figure S2: Flow diagram depicting different experimental groups and interventions.

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