

Suppl. table 1: Composition of isocaloric diet used in hyperphagic food intake and food induced hormone release

Ingredients	Diet(g/kg)
Sucrose	200.0
Dextrin	298.6
Glucose	131.1
Casein	189.6
Cellulose	47.4
Maltodextrin	33.2
Soybean oil	42.7
Calcium phosphate	12.3
Vitamin and mineral mix	19.0
Potassium citrate	15.6
Calcium carbonate	0.52
L-cysteine	2.8
Choline bitartrate	1.9

Suppl. Information for RNA isolation and gene expression analysis

The qRT-PCR assay was performed according to MIQE guidelines¹. Animal tissues were collected during the animal sacrifice and immediately stored in RNA later (Sigma-Aldrich, USA) at -80 °C. Total RNA was extracted from stomach (fundus), ileum and colon using Trizol RNA extraction agent-based method. 10-20 mg of frozen tissue sample was initially homogenized in Trizol (MP Biomedicals, USA), followed by chloroform (Sigma-Aldrich,

USA) addition to separate nucleic acid layers from protein and sub-cellular contents. Once the layer has been separated, RNA is precipitated using chilled iso-propanol (Sigma-Aldrich, USA) and washed with freshly prepared 75% ethanol. Dried RNA pellet was reconstituted in nuclease-free water (Thermo Fischer Scientific, USA). Once extracted, RNA quality was checked on 1.2% agarose gel and quantified using spectrophotometer (Nanodrop, Thermo Scientific, USA).

RNA samples having $A_{260/280}$ of 2.0 ± 0.09 were used for further experiments. DNase treatment was given prior to reverse transcription using commercially available kit from Thermo Fischer Scientific. DNase-treated RNA (1 μ g) was then reverse transcribed to cDNA using commercially available kit (Thermo Fisher scientific, RevertAid cDNA synthesis kit). The reaction conditions for reverse transcription were 37 °C for 30 min for DNase treatment followed by inactivation of DNase enzyme at 65 °C for 10 min, then primer annealing at 65 °C for 5 min and finally reverse transcription at 25 °C for 5 min, then 42 °C for 60 min and finally 70 °C for 5 min.

Primers Designing: - The gene sequence for primer designing was procured from NCBI's nucleotide database. The CDS (coding sequence) region was used for primer designing using NCBI's Primer designing tool. Primer pair was evaluated for T_m (melting temperature), GC content and presence of secondary structure using OligoCalc software (Oligonucleotide properties calculator) and finally nucleotide BLAST was performed to assess the local alignment of selected primers and to omit the primer sequences having matching similarity with non-target genes.

The cDNA (1 μ g) synthesized was stored at -20 °C till qRT-PCR analysis. For gene expression analysis, 10 μ l reaction volume was used (Composition of reaction volume: cDNA- 25ng; Primer- 1 μ l (1pmol/ μ l) each amplification primer; SYBR- 5 μ l and Nuclease-free water-2.5

μl) and qPCR amplifications were carried out on CFX Maestro software from Biorad. The thermal cycling comprised an initial denaturation at 95 °C for 2 min, followed by 40 cycles of annealing and elongation at 60 °C for 30 s, and denaturation at 95 °C for 5 s. Melting curves were analysed by increasing the temperature from 60 °C to 95 °C with a plate reading every 0.5 °C. The complete experiment was performed using a minimum of four biological replicates. Analysis of relative gene expression was done using $2^{-\Delta\Delta ct}$ method² and values were expressed in terms of fold change with reference to control gene (β -actin Forward primer 5' TGGTGGGAATGGGTCAGAAG 3' and reverse primer 5' ACGGTTGGCCTTAGGGTTCA 3'). TRPA1 primers sequence was as Forward primer 5' TGCCACTCTGGTACTTACGC 3' and Reverse primer 5' GGAAGTGAGGTCCTTCAGCC 3'.

- 1 S. A. Bustin, V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele and C. T. Wittwer, *Clin. Chem.*, 2009, **55**, 611–622.
- 2 K. J. Livak and T. D. Schmittgen, *Methods*, , DOI:10.1006/meth.2001.1262.