

Supplements

Gut microbiota-directed intervention with high-amylose maize ameliorates metabolic dysfunction in diet-induced obese mice

Received 00th January 20xx,
Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

Wei-Chung Chiou ^a, Wei-Han Lai ^a, Yu-Lin Cai ^a, Meng-Lun Du ^b, Hsi-Mei Lai ^b, Jui-Chieh Chen ^c, Hsiu-Chen Huang ^{d,e}, Hui-Kang Liu ^{f,g}, Cheng Huang ^{a,*}

Supplementary Tables

Table S1. Proximate compositions of HAM.

Ingredients (g/100g)	HAM
Ash	2.03 ± 0.03
Crude protein	12.14 ± 0.45
Crude fat	6.50 ± 0.67
Digestible starch	42.9 ± 0.52
Dietary fibers	34.83 ± 2.86

Data are shown as the mean ± SD.

Dietary fibers = Resistant starch + Crude fibers

Table S2. Compositions of the experimental diets.

Diets	ND	HFD	HAM
Carbohydrates (%)	76.2	40.7	40.6
Protein (%)	14.3	14.1	14.2
Fat (%)	9.5	45.2	45.2
Total energy (kcal/g)	3.7	4.6	4.6
Ingredients (g/kg diet)			
Cornstarch	465.7	289.3	190.6
Dextrinized cornstarch	155.0	96.3	96.3
Sucrose	100.0	100.0	100.0
Casein ($\geq 95\%$ protein)	140.0	175.0	147.9
L-Cystine	1.8	1.8	1.8
Lard	0.0	200.0	200.0
Soybean oil (no additives)	40.0	40.0	25.0
Cellulose	80.0	80.0	0.0
Mineral mix (AIN-93G-MX)	35.0	35.0	35.0
Vitamin mix (AIN-93-VX)	10.0	10.0	10.0
Choline bitartrate (41.1% choline)	2.5	2.5	2.5
Digestible starch from HAM	–	–	98.7
Protein from HAM	–	–	27.9
Fat from HAM	–	–	15.0
Dietary fibers from HAM	–	–	80.1

Table S3. Primers.

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')
MCP-1	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
TNF- α	TGGGAGTAGACAAGGTACAACCC	CATCTTCTCAAAATTCGAGTGACAA
IL-6	CCAGTTGCCTTCTTGGGACT	GGTCTGTTGGGAGTGGTATCC
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
LBP	GTGGCTGCTGAATCTCTTCC	GAGCGGTGATTCCGATTAAA
Leptin	TCTCCGAGACCTCCTCCATCT	TTCCAGGACGCCATCCAG
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT

Supplementary Figures

HAM dietary intervention modulated 40 genera in HFD-fed mice

HAM-driven changes were investigated at the genus level in the 37 families identified. Of the 40 genera (Fig. S1A), 20 genera, *Sporosarcina*, *Parabacteroides*, *Melissococcus*, *Lactococcus*, *Streptococcus*, *Ruminococcus*, *Mucispirillum*, *Marvinbryantia*, *Shuttleworthia*, *Johnsonella*, *Desulforudis*, *Sporobacter*, *Allobeggiatoa*, *Bifidobacterium*, *Anaerostipes*, *Pediococcus*, *Lactobacillus*, *Barnesiella*, *Robinsoniella*, and *Paraeggerthella*, were changed in the HFD group but were maintained at a level comparable to the ND group in the HAM group. Meanwhile, the HAM diet changed 20 other genera that showed no statistical significance between the ND and HFD groups, *Staphylococcus*, *Jeotgalicoccus*, *Anaerorhabdus*, *Corynebacterium*, *Hespellia*, *Desulfotomaculum*, *Proteocatella*, *Abiotrophia*, *Anaerotruncus*, *Butyricicoccus*, *Butyrivibrio*, *Rhodovulum*, *Bacteroides*, *Lebetimonas*, *Lachnospira*, *Trigonala*, *Allobaculum*, *Marina*, *Aerococcus*, and *Alistipes*. In parallel, correlations between these 40 genera are shown in Fig. S1B. Two key opposing groups of 31 genera are distinguished. One of the two groups comprises *Barnesiella*, *Robinsoniella*, *Allobeggiatoa*, *Bifidobacterium*, *Anaerostipes*, *Pediococcus*, *Anaerotruncus*, *Hespellia*, *Abiotrophia*, *Butyrivibrio*, *Lachnospira*, *Lebetimonas*, *Rhodovulum*, and *Proteocatella*, and the other is composed of *Parabacteroides*, *Sporosarcina*, *Marvinbryantia*, *Mucispirillum*,

Streptococcus, *Ruminococcus*, *Melissococcus*, *Lactococcus*, *Johnsonella*, *Aerococcus*, *Corynebacterium*, *Marina*, *Staphylococcus*, *Jeotgalicoccus*, *Anaerorhabdus*, *Allobaculum*, and *Alistipes*.

For the 20 genera that have a comparable profile in the ND and HAM groups, positive and negative correlations identified are shown in Fig. S1C. Likewise, there are two major opposing groups of 16 genera, where members of one group correlate positively amongst themselves but negatively with those of the other group. One of these groups comprises *Allobeggiatoa*, *Anaerostipes*, *Barnesiella*, *Bifidobacterium*, *Pediococcus*, and *Robinsoniella*, and the other is composed of *Johnsonella*, *Lactococcus*, *Marvinbryantia*, *Melissococcus*, *Mucispirillum*, *Parabacteroides*, *Shuttleworthia*, *Ruminococcus*, *Sporosarcina*, and *Streptococcus*. As for the 20 genera that were changed by the HAM intervention but showed no statistical difference between the ND and HFD groups, positive and negative correlations identified between them are shown in Fig. S1D. Similarly, two main opposing groups of 14 genera are discerned. One of these groups includes *Abiotrophia*, *Butyrivibrio*, *Lachnospira*, *Lebetimonas*, *Proteocatella*, and *Rhodovulum*, and the other comprises *Aerococcus*, *Alistipes*, *Allobaculum*, *Anaerorhabdus*, *Corynebacterium*, *Jeotgalicoccus*, *Marina*, and *Staphylococcus*. Meanwhile, the former group had inconsistent positive correlations with *Anaerotruncus* and *Hespellia*.

Supplements

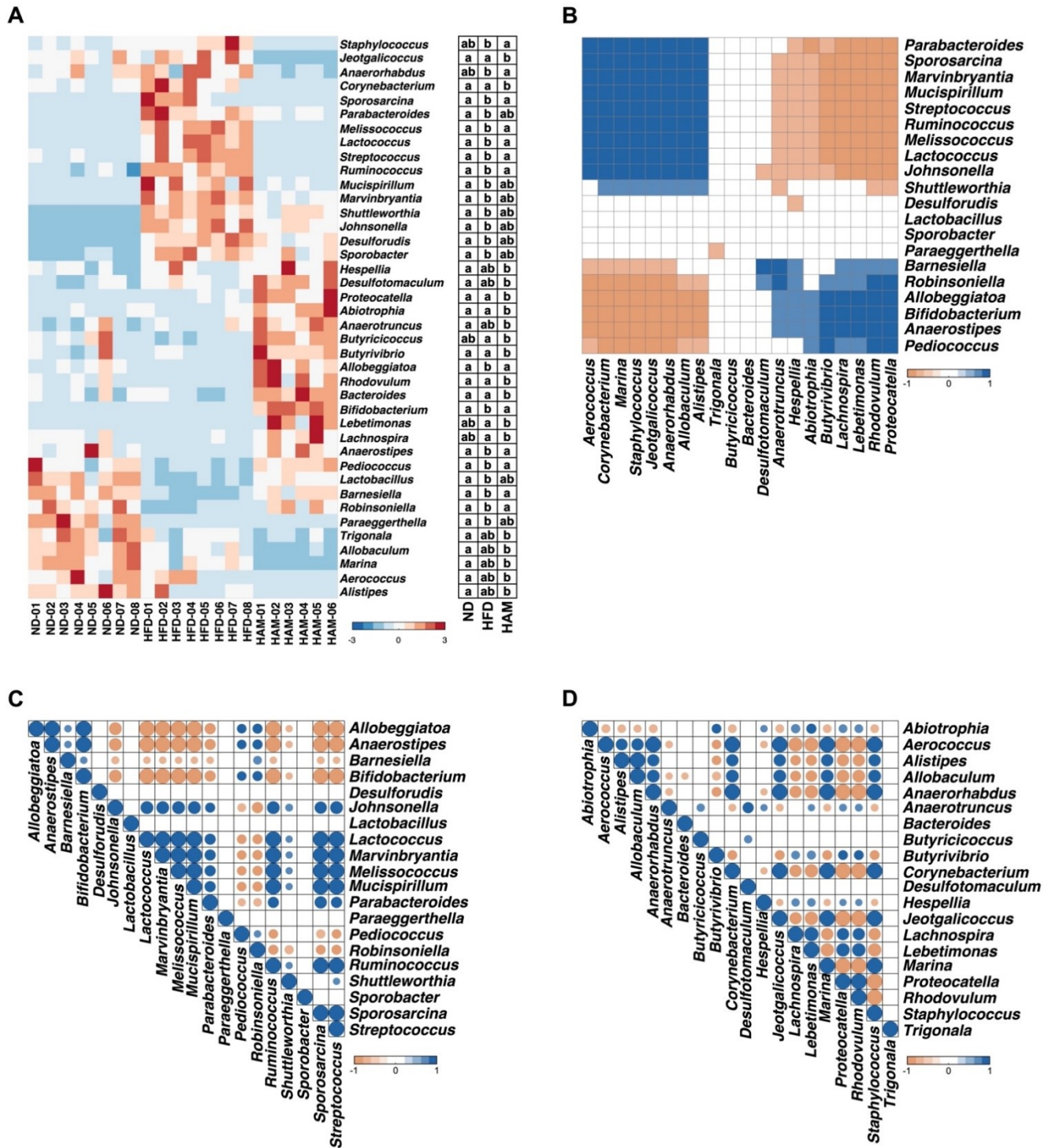


Fig. S1. Effects of the HAM diet on bacterial genera and correlations in HFD-fed mice. (A) Heatmap of the bacterial genera modulated by the HAM diet. The genera listed had a relative abundance of 0.01% or more in at least one group. Heatmaps are shown and were

analyzed using hierarchical clustering analysis. The color scale bar indicates high (red) and low (blue) abundance along the rows of the data. Statistical significance between groups, determined by Kruskal-Wallis with Dunn's post-hoc tests, is listed in the right panel, where different letters (a, b) indicate statistical significance ($p < 0.05$) between groups and letters shared between groups indicate no statistical significance ($p \geq 0.05$). **(B)** Correlations of the bacterial genera modulated by the HAM diet. **(C)** Correlations of the bacterial genera that were significantly changed by HFD feeding, compared to the ND diet, and were stabilized by the HAM dietary intervention. (Correlations of the changes in bacterial genera between the HFD and HAM groups.) **(D)** Correlations of the bacterial genera that were not significantly changed by HFD feeding but were modulated by the HAM dietary intervention. Positive and negative correlations of statistical significance ($p < 0.05$), with an absolute correlation coefficient of at least 0.6 in Kendall rank correlation analysis, are indicated by blue and orange shading (B) or circles (C, D), respectively.