

Transglycosylated starch accelerated intestinal transit and enhanced bacterial fermentation in the large intestine using a pig model

Short title: Transglycosylated starch and nutrient flow

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Supplemental Table 1. Dry matter content, pH, short-chain fatty acids (SCFA) and lactate in ileal of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 7 CON diet; *n* 6 TGS diet)

Item	CON	TGS	SEM	<i>P</i> -value
Dry matter (%)	11.8	16.5	0.66	0.002**
pH	8.2	7.8	0.11	0.036*
Concentrations (μmol/g)				
Total lactate	1.56	2.32	0.360	0.218
D-lactate	0.10	0.28	0.047	0.016*
L-lactate	1.45	2.03	0.346	0.328
Total SCFA	29.00	63.14	7.704	0.016*
Acetate	24.04	53.05	7.319	0.033*
Propionate	1.92	4.51	1.818	0.297
Butyrate	0.77	0.34	0.674	0.664
Iso-butyrate	1.62	3.92	0.620	0.038*
Valerate	0.22	0.18	0.062	0.524
Iso-valerate	0.24	0.10	0.039	0.013*
Caproate	0.20	0.01	0.092	0.222
Molar proportions (% of total SCFA)				
Acetate	83.16	86.29	2.385	0.332
Propionate	6.34	6.39	2.580	0.988
Butyrate	2.51	0.51	0.848	0.121
Iso-butyrate	5.75	6.14	0.819	0.729
Valerate	0.74	0.26	0.116	0.005**
Iso-valerate	0.80	0.16	0.078	<0.001***
Caproate	0.69	0.28	0.044	<0.001***

Significance level: **P*<0.05, ***P*<0.01, ****P*<0.001.

Supplemental Table 2. Dry matter content, pH, short-chain fatty acids (SCFA) and lactate in faeces of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 7 CON diet; *n* 6 TGS diet)

Item	CON	TGS	SEM	<i>P</i> -value
Dry matter (%)	42.0	34.9	1.69	0.041*
pH	8.3	6.1	0.12	<0.001***
Concentration (μmol/g)				
Total lactate	0.031	0.133	0.036	0.087
D-lactate	0.013	0.051	0.024	0.184
L-lactate	0.017	0.084	0.022	0.097
Total SCFA	37.08	118.37	17.829	<0.001***
Acetate	22.30	81.53	2.000	<0.001***
Propionate	6.66	14.46	3.218	0.119
Butyrate	1.71	9.21	1.739	0.010*
Iso-butyrate	3.12	5.96	0.460	<0.001***
Valerate	0.90	3.55	0.516	0.003**
Iso-valerate	2.19	2.95	0.507	0.281
Caproate	0.19	0.15	0.952	<0.001***
Molar proportion (% of total SCFA)				
Acetate	60.53	69.61	2.113	0.005**
Propionate	17.59	11.91	1.786	0.036*
Butyrate	4.50	7.53	0.737	0.005**
Iso-butyrate	8.47	4.97	0.306	<0.001***
Valerate	2.44	2.92	0.243	0.156
Iso-valerate	5.93	2.37	0.380	<0.001***
Caproate	0.54	1.49	0.139	<0.001***

Significance level: **P*<0.05, ***P*<0.01, ****P*<0.001.

Supplemental Table 3. Bacterial genera (hit counts; relative abundance > 0.01% of all reads) in ileal digesta of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 7 CON diet; *n* 6 TGS diet)

Genus [†]	CON	TGS	log ₂ fold change	SE [‡]	<i>p</i> value	<i>q</i> value [§]
Unclassified <i>Enterobacteriaceae</i>	81411	170875	1.070	0.817	0.191	0.391
<i>Turcibacter</i>	4344	4603	0.084	0.918	0.927	0.961
<i>Anaerovibrio</i>	68	7399	8.024	1.321	<0.001***	<0.001
Unclassified <i>Clostridiaceae</i>	3312	402	-3.043	0.832	<0.001***	0.003
<i>Actinobacillus</i>	815	2157	1.404	0.929	0.131	0.282
<i>Ruminococcus</i>	2840	85	-2.170	0.601	<0.001***	0.003
<i>Succinivibrio</i>	2706	27	-0.440	0.689	<0.001***	<0.001
Unclassified <i>Veillonellaceae</i>	13	1452	6.852	1.110	<0.001***	<0.001
<i>Megasphaera</i>	51	1116	5.240	1.153	<0.001***	<0.001
<i>Streptococcus</i>	555	439	-0.337	0.983	0.732	0.869
<i>Klebsiella</i>	37	661	4.165	1.464	0.004**	0.021
<i>Anaerobiospirillum</i>	75	476	2.661	1.355	<0.001***	<0.001
Unclassified <i>Clostridiales</i>	317	61	-2.375	0.657	<0.001***	0.003
<i>Clostridium</i>	211	167	-0.339	1.163	<0.001***	<0.001
<i>Helicobacter</i>	204	162	-0.334	1.360	0.806	0.912
<i>Campylobacter</i>	196	168	-0.219	0.646	0.734	0.869
<i>Desulfovibrio</i>	251	108	-1.209	0.641	0.059	0.151
Unclassified <i>Ruminococcaceae</i>	235	46	-2.359	0.655	<0.001***	0.003
<i>Lactobacillus</i>	25	235	3.220	0.857	<0.001***	0.003
<i>Selenomonas</i>	61	196	1.702	0.743	0.022*	0.077
Unclassified <i>Christensenellaceae</i>	193	49	-1.964	0.581	0.001**	0.004
<i>Mitsuokella</i>	6	192	5.082	0.968	<0.001***	<0.001
<i>Aggregatibacter</i>	67	99	0.557	0.786	0.479	0.732
<i>Phascolarctobacterium</i>	47	116	2.615	1.134	<0.001***	<0.001
<i>TG5</i>	96	63	-0.607	0.619	0.326	0.575
<i>Sutterella</i>	132	19	-2.799	1.262	0.027*	0.083
Unclassified <i>Coriobacteriaceae</i>	10	127	3.714	0.881	<0.001***	0.001
<i>Veillonella</i>	61	71	0.213	0.626	0.734	0.869
Unclassified <i>Micrococcaceae</i>	36	53	0.567	0.972	0.559	0.825
Unclassified <i>Lachnospiraceae</i>	53	31	-0.771	0.780	0.322	0.575
<i>Epulopiscium</i>	79	3	1.627	1.699	0.338	0.580
<i>Haemophilus</i>	59	22	-1.438	1.241	0.247	0.471
<i>Actinomyces</i>	34	47	0.493	0.862	0.567	0.825
<i>Acinetobacter</i>	62	12	-2.310	1.219	0.058	0.151
<i>Mannheimia</i>	33	38	0.234	1.061	0.825	0.912
<i>Enterococcus</i>	46	9	-2.293	1.190	0.054	0.151
<i>Bilophila</i>	34	8	-2.028	1.281	0.113	0.258
Unclassified <i>Desulfovibrionaceae</i>	38	1	-5.006	1.020	<0.001***	<0.001
<i>Oscillospira</i>	37	2	-2.493	0.735	0.001**	0.004

<i>Leptotrichia</i>	13	21	0.632	1.524	0.679	0.869
Unclassified <i>Helicobacteraceae</i>	24	8	1.758	1.332	0.187	0.391
<i>Erwinia</i>	5	27	2.477	1.314	0.059	0.151
Unclassified <i>Bacteroidales</i>	28	1	-4.644	1.371	0.001**	0.004
Unclassified <i>Alcaligenaceae</i>	24	2	-3.442	1.518	0.023*	0.079
<i>Enterobacter</i>	19	4	0.349	1.132	0.758	0.884
<i>Comamonas</i>	10	11	0.169	0.905	0.852	0.918
<i>Schwartzia</i>	6	14	1.127	1.375	0.413	0.667
Unclassified <i>Neisseriaceae</i>	8	12	0.469	0.914	0.608	0.852
<i>rc4-4</i>	20	0	-5.552	1.507	<0.001***	0.003
<i>Pyramidobacter</i>	19	0	-5.058	1.843	0.006**	0.027
<i>Pseudomonas</i>	17	1	-4.443	1.361	0.001**	0.006
Unclassified <i>Synergistaceae</i>	15	1	-3.902	1.256	0.002**	0.010
<i>Moraxella</i>	10	5	-0.989	0.842	0.240	0.469
Unclassified <i>Pasteurellaceae</i>	9	6	-0.881	0.941	0.349	0.580
<i>Blautia</i>	1	14	4.005	1.572	<0.001***	<0.001
<i>Desulfomicrobium</i>	9	5	-0.791	2.304	0.731	0.869
<i>Salmonella</i>	12	2	-2.610	1.497	0.081	0.195
<i>Collinsella</i>	7	6	-0.060	1.051	0.954	0.975
<i>Catenibacterium</i>	0	13	6.395	2.091	0.002**	0.011
Unclassified <i>p-2534-18B5</i>	12	1	-3.807	1.477	0.010*	0.038
Unclassified <i>Gemellaceae</i>	4	8	0.874	0.895	0.329	0.575
Unclassified <i>Elusimicrobiaceae</i>	12	0	1.055	3.108	0.734	<0.001
Unclassified <i>Erysipelotrichaceae</i>	1	11	3.273	1.365	0.017*	0.060

Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[†]Relative abundance >0.004% of all reads.

[‡]Standard error of the log₂fold change.

[§]False discovery rate (Benjamini-Hochberg) corrected p value.

Supplemental Table 4. Bacterial genera (hit counts; relative abundance > 0.01% of all reads) in faeces of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 7 CON diet; *n* 6 TGS diet)

Genus [†]	CON	TGS	log ₂ fold change	SE [‡]	<i>p</i> value	<i>q</i> value [§]
Unclassified <i>Christensenellaceae</i>	22669	2362	-3.263	0.824	<0.001***	<0.001
Unclassified <i>Ruminococcaceae</i>	13944	2361	-2.562	0.854	0.003**	0.007
Unclassified <i>Clostridiales</i>	11644	1122	-3.375	0.652	<0.001***	<0.001
Unclassified <i>Coriobacteriaceae</i>	70	10269	7.192	1.025	<0.001***	<0.001
<i>Ruminococcus</i>	2440	5927	1.281	0.577	0.026*	0.053
<i>Megasphaera</i>	28	7728	8.085	0.696	<0.001***	<0.001
Unclassified <i>Enterobacteriaceae</i>	5429	1022	-2.409	0.587	<0.001***	<0.001
Unclassified <i>Veillonellaceae</i>	57	1089	4.257	1.025	<0.001***	<0.001
<i>Desulfovibrio</i>	289	820	1.504	0.576	0.009**	0.023
<i>Methanobrevibacter</i>	387	705	0.867	0.733	0.237	0.327
Unclassified <i>p-2534-18B5</i>	904	0	-10.542	1.191	<0.001***	<0.001
Unclassified <i>Lachnospiraceae</i>	414	439	0.081	0.642	0.899	0.899
<i>Butyrivibrio</i>	1	576	9.786	1.639	<0.001***	<0.001
Unclassified <i>Bacteroidales</i>	383	189	-1.016	0.899	0.258	0.339
Unclassified <i>Clostridiaceae</i>	502	12	-5.322	0.738	<0.001***	<0.001
<i>Turcibacter</i>	338	130	-1.383	0.830	0.096	0.163
<i>Collinsella</i>	36	417	3.554	0.799	<0.001***	<0.001
<i>Oscillospira</i>	406	38	-3.429	0.841	<0.001***	<0.001
<i>Mitsuokella</i>	4	437	6.733	1.120	<0.001***	<0.001
Unclassified <i>RF39</i>	406	20	-4.344	1.339	0.001**	0.003
<i>RFN20</i>	49	374	2.935	0.688	<0.001***	<0.001
<i>Succinivibrio</i>	364	5	-6.083	1.140	<0.001***	<0.001
Unclassified <i>Synergistaceae</i>	248	10	-2.924	1.190	<0.001***	<0.001
Unclassified <i>BS11</i>	255	1	-3.489	2.631	0.185	0.284
Unclassified <i>Desulfovibrionaceae</i>	166	82	-1.013	0.788	0.199	0.294
Unclassified <i>ML615J-28</i>	63	134	1.092	1.505	0.468	0.543
<i>Streptococcus</i>	114	67	-0.776	1.308	0.553	0.606

<i>Sutterella</i>	50	130	1.389	0.647	0.032*	0.060
<i>rc4-4</i>	113	0	-7.928	1.061	<0.001***	<0.001
<i>Catenibacterium</i>	0	89	7.727	1.245	<0.001***	<0.001
<i>Campylobacter</i>	45	34	-0.395	0.813	0.627	0.678
<i>Dorea</i>	73	0	-7.995	0.987	<0.001***	<0.001
<i>Blautia</i>	15	51	1.753	0.782	0.025*	0.053
Unclassified <i>GMD14H09</i>	57	0	-8.355	2.086	<0.001***	<0.001
<i>Phascolarctobacterium</i>	34	21	-0.708	0.801	0.377	0.468
Unclassified <i>Pirellulaceae</i>	48	2	-4.439	1.287	0.001**	0.002
<i>Bilophila</i>	33	14	-1.279	1.024	0.211	0.302
<i>Anaerovibrio</i>	14	29	1.050	1.202	0.383	0.468
<i>Helicobacter</i>	24	10	-1.283	0.853	0.133	0.208
Unclassified <i>R4-45B</i>	32	0	-8.017	1.392	<0.001***	<0.001
Unclassified <i>Rikenellaceae</i>	2	30	4.239	1.721	<0.001***	<0.001
Unclassified <i>Victivallaceae</i>	28	2	-3.494	0.969	<0.001***	0.001
<i>Dehalobacterium</i>	30	0	-7.892	1.169	<0.001***	<0.001
<i>Succiniclasticum</i>	15	12	-0.308	1.479	0.835	0.868
<i>Paludibacter</i>	24	1	-0.462	2.745	0.866	0.889
Unclassified <i>Mogibacteriaceae</i>	22	2	-3.817	0.881	<0.001***	<0.001
<i>Coprococcus</i>	15	4	-1.895	0.881	0.031*	0.060
<i>Klebsiella</i>	1	17	4.200	1.699	0.013*	0.032
<i>vadinCA11</i>	16	1	-3.849	1.045	<0.001***	0.001
<i>Bacteroides</i>	13	3	1.007	1.513	0.506	0.570
Unclassified <i>Dehalobacteriaceae</i>	15	0	-6.933	1.061	<0.001***	<0.001
<i>Clostridium</i>	12	3	-1.764	1.077	0.101	0.169
<i>Oxalobacter</i>	11	4	-1.598	0.758	0.035*	0.064
<i>Dialister</i>	0	14	5.840	1.211	<0.001***	<0.001
<i>Enterococcus</i>	12	1	-3.308	1.277	0.010*	0.024
<i>Lactobacillus</i>	7	6	-0.376	0.980	0.701	0.738
<i>Acidaminococcus</i>	0	11	5.522	1.143	<0.001***	<0.001

Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

†Relative abundance >0.01% of all reads.

‡Standard error of the \log_2 fold change.

§False discovery rate (Benjamini-Hochberg) corrected p value.

Supplemental Table 5. Alpha-diversity indices of bacterial communities in ileal digesta and faeces of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 7 CON diet; *n* 6 TGS diet)

Item	CON	TGS	SEM	<i>P</i> -value
Ileum				
OTU richness*	289	349	74.5	0.578
Shannon	1.63	1.44	0.288	0.658
Simpson	0.50	0.48	0.093	0.830
Faeces				
OTU richness	858	553	120.8	0.106
Shannon	4.14	2.93	0.117	0.001**
Simpson	0.94	0.86	0.010	0.002**

Significance level: ***P*<0.01.

†OTU, operational taxonomic unit.

Supplemental Table 6. Covariation scores for associations (Pearson's correlations) of ileal bacterial genera, microbial metabolites, dry matter and pH with ileal passage rate markers for liquid and solids in pigs fed either the control (CON) or transglycosylated starch (TGS) diet using sparse partial least squares regression and relevance networking

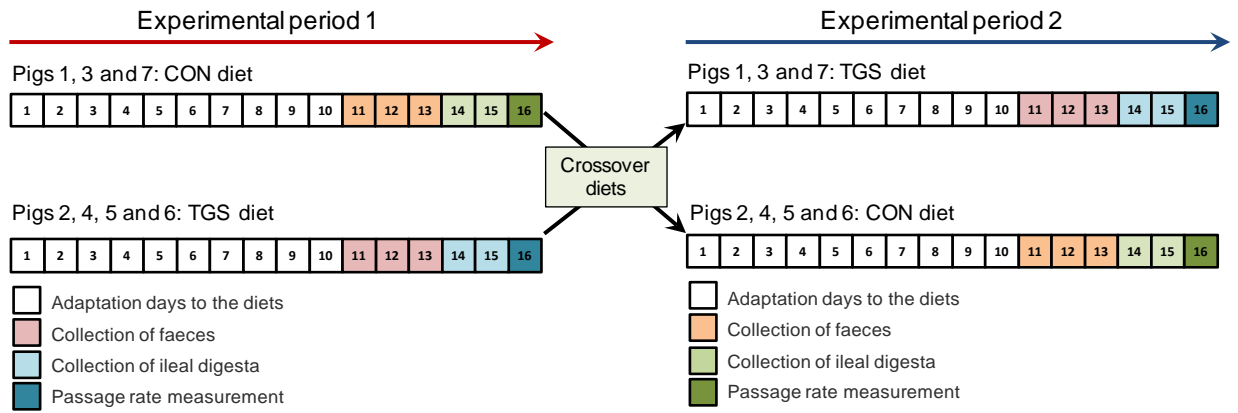
Bacterial genus/ microbial metabolite	Association score ($r =$)*					
	CrRT1Comp	CrRT2Comp	CrRTtotal	YbRT1Comp	YbRT2Comp	YbRTtotal
<i>Klebsiella</i>	-0.74	0	-0.77	-0.64	0	-0.62
<i>Erwinia</i>	-0.74	0	-0.77	-0.65	0	-0.64
Acetate	0	0	0	-0.76	0	-0.78
Isobutyrate	0	0	0	-0.73	0	-0.75
Total short-chain fatty acids	0	0	0	-0.74	0	-0.76
D-lactate	0	0	0	0	0	-0.63
Dry matter	0	0	0	-0.71	0	-0.73

*Only the strongest pairwise associations are presented: bacterial genera, $|r| > 0.8$; fermentation metabolites, $|r| > 0.6$. CrRT1Comp, liquid marker fractional passage rate for compartment 1 (stomach); CrRT2Comp, liquid marker fractional passage rate for compartment 2 (small intestine); CrRTtotal, liquid marker fractional passage rate for both compartments; DM, dry matter; SCFA, short-chain fatty acids; YbRT1Comp, solid marker fractional passage rate for compartment 1 (stomach); YbRT2Comp, solid marker fractional passage rate for compartment 2 (small intestine); YbRTtotal, solid marker fractional passage rate for both compartments.

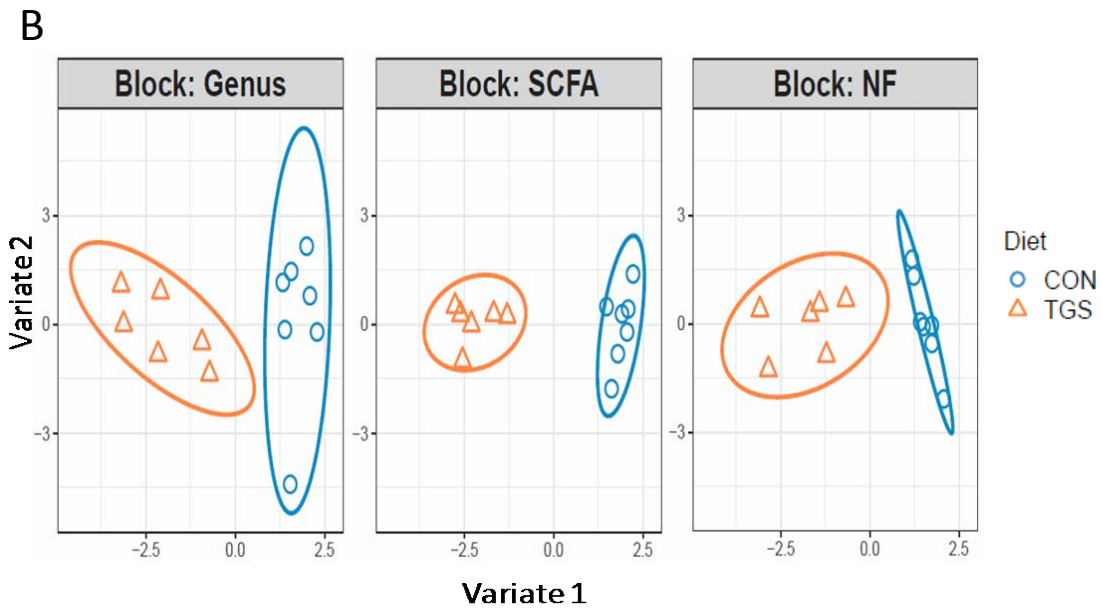
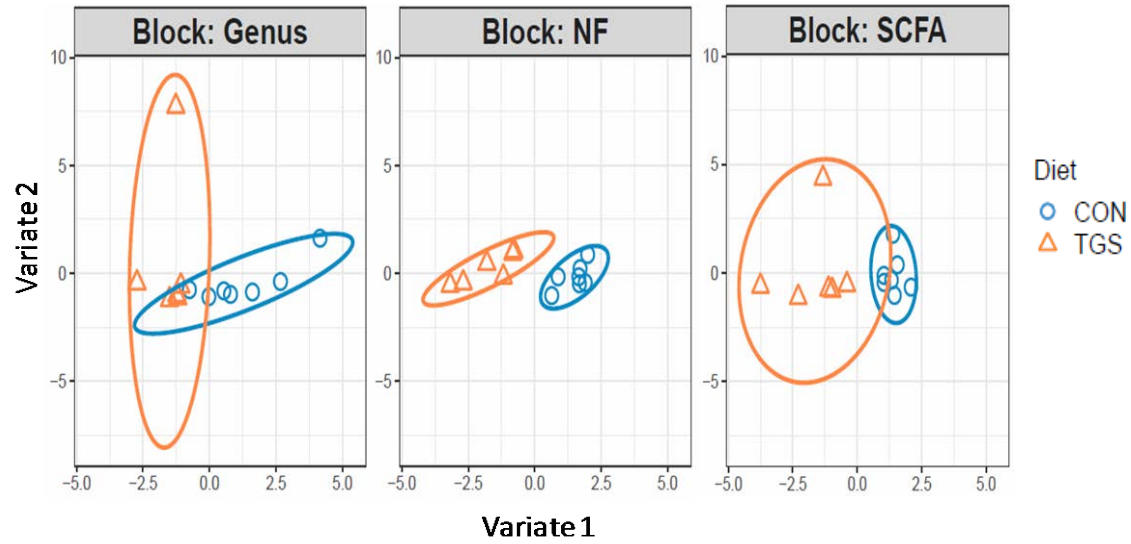
Supplemental Table 7. Covariation scores for associations (Pearson's correlations) of ileal and fecal bacterial genera, microbial metabolites, dry matter and pH with ileal nutrient flow and fecal nutrient excretion, respectively, in pigs fed either the control (CON) or transglycosylated starch (TGS) diet using sparse partial least squares regression and relevance networking

Bacterial genus/microbial metabolite	Association score ($r =$)*					
	Dry matter flow	Ash flow	Crude protein flow	Starch flow	Calcium flow	Phosphorous flow
Ileum						
Unclassified <i>Clostridiales</i>	-0.67	0	0	-0.65	0	0
Unclassified <i>Ruminococcaceae</i>	0	0	0	-0.61	0	0
<i>Enterococcus</i>	-0.6	0	0	0	0	0
<i>Oscillospira</i>	-0.64	0	0	-0.65	0	0
Unclassified <i>Desulfovibionaceae</i>	-0.65	0	0	-0.66	0	0
Unclassified <i>Bacteroidales</i>	-0.64	0	0	-0.64	0	0
<i>rc4.4</i>	0	0	0	-0.61	0	0
Acetate	0.74	0	0	0.78	0	0
Isobutyrate	0.7	0	0	0.73	0	0
Butyrate	0	0	0	-0.61	0	0
Isovalerate	0	0	0	-0.62	0	0
Total short-chain fatty acids	0.67	0	0	0.71	0	0
Dry matter	0.68	0	0	0.72	0	0
Feces						
Unclassified <i>Ruminococcaceae</i>	-0.86	0	-0.84	-0.84	0	0
<i>Megasphaera</i>	0.92	0	0.87	0.91	0	0
<i>Collinsella</i>	0.84	0	0.82	0.82	0	0
<i>Oscillospira</i>	-0.92	0	-0.90	-0.90	0	-0.82
<i>Catenibacterium</i>	0.83	0	0.82	0.80	0	0
Unclassified <i>Victivallaceae</i>	0	-0.80	0	0	0	0
<i>Dialister</i>	0.82	0	0.80	0.80	0	0
Acetate	0.94	0	0.94	0.93	0.82	0.85
Butyrate	0.93	0	0.91	0.92	0	0.83
Valerate	0	0	0.81	0	0	0
Caproate	0.91	0	0.88	0.92	0	0
Total short-chain fatty acids	0.93	0	0.93	0.91	0.83	0.85
pH	-0.93	0	-0.90	-0.93	0	-0.80

*Only the strongest pairwise associations presented: ileal genera, fermentation metabolites and dry matter, $|r| > 0.6$; fecal genera, fermentation metabolites and dry matter, $|r| > 0.8$.



Supplemental Fig. 1. Schematic of the study including the adaptation to the diets, and subsequent collections of ileal digesta and feces and passage rate measurements for experimental period 1 and 2. CON, control starch; TGS, transglycosylated starch.



Supplemental Fig. 2. Sample plots with confidence ellipses for each block displaying the dissimilarity (Bray-Curtis) between samples for the most discriminant genera ($n = 10$), short-chain fatty acids (SCFA, $n = 5$) and flow of nutrients (NF, $n = 4$) in ileal digesta (A) and faeces (B), which were identified using horizontal sparse partial least squares-discriminant analysis. Relative abundance of bacterial genera $> 0.01\%$ of all reads.



Supplemental Fig. 3. Loading plots of each feature selected on the first component in each block using horizontal sparse partial least squares-discriminant analysis. The best discriminant genera ($n = 10$), short-chain fatty acids (SCFA, $n = 5$) and flow of nutrients (NF, $n = 4$) for component 1 in ileal digesta (A) and faeces (B) are displayed. Relative abundance of bacterial genera $> 0.01\%$ of all reads.