

1 **Consumption of transglycosylated starch down-regulates expression of mucosal innate**  
2 **immune response genes in the large intestine using a pig model**

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4 **Short title: Transglycosylated starch and gut mucosa**

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**Supplemental Table 1.** Oligonucleotide primer sequences used for qPCR

Gene	Accession number <sup>1</sup>	Primer sequence (5' to 3') <sup>2</sup>	Amplicon size (bp)	PCR efficiency (%)	R <sup>2</sup>	Reference
Target genes						
<i>ZOI</i>	XM_003353439.2	F AAGCCCTAAGTTCAATCACAATCT R ATCAAACCTCAGGAGGCGGC	130	107.2	0.99	1
<i>OCN</i>	NM_001163647.1	F TTGTGGGACAAGGAACGTATTTA R TGCCTGCCGACACGTTT	76	97.1	0.99	1
<i>MUC2</i>	XM_013989745.1	F TGCCATCTACACCAAGGTCTATTC R TCTGCAGGCCGTTGTAGTCTC	137	90.1	1.00	1
<i>MUC4</i>	XM_001926442.1	F GATGCCCTGGCCACAGAA R TGATTCAAGGTAGCATTCATTTGC	89	100.7	0.98	2
<i>IAP</i>	XM_003133729.2	F AGGAACCCAGAGGGACCATTC R CACAGTGGCTGAGGGACTTAGG	83	97.1	1.00	3
<i>TLR2</i>	NM_213761.1	F AATAAGTTGAAGACGCTCCCAGAT R GTTGCTCCTTAGAGAAAGTATTGATCGT	97	94.5	0.99	1
<i>TLR4</i>	AB188301.2	F TGTGGCCATCGCTGCTAAC R GGTCTGGGCAATCTCATACTCA	124	105.8	0.98	1
<i>SRBEF1</i>	NM_214157.1	F TCCATCAATGACAAGATCATCGA R CTGGTTGCTCTGCTGAAGGAA	123	100.7	1.00	4
<i>PPARG</i>	NM_214379.1	F CATTCCCGAGAGCTGATCC R GGAAGGCTCTTCGTGAGGTT	149	110.4	0.99	4
<i>FASN</i>	NM_001099930.1	F CGGTCTTGCTGACCAAGAAG R TTGGAACCGTCTGTGTTTCGT	82	97.5	0.99	4
<i>AMPK</i>	NM_214266.1	F GCTCCTTGACCGGAAGCA R CAGACTGGCACACATTTTCGAA	86	105.1	0.99	4
<i>IL1B</i>	NM_001005149.1	F AACTGGTACATCAGCACCTCTCAA R TCTTGGCGGCCTTTGGA	71	107.2	1.00	1
<i>IL6</i>	M86722.1	F CGCAGCCTTGAGGATTTCC R TCAGGTGCCCCAGCTACATT	67	109.5	0.99	1
<i>IL8</i>	NM_213867.1	F GCACTTACTCTTGCCAGAAGCTG	82	97.3	0.98	2

		R	CAAACCTGGCTGTTGCCTTCTT					
<i>IL10</i>	NM_214041.1	F	GCCTACATGACGATGAAGATGA	112	94.3	0.99	1	
		R	TGAAAGTCTCCAATTTGTATCCTAGAGT					
<i>INFG</i>	NM_213948.1	F	TTTAATGCAAGTACCTCAGATGTACCTAA	79	99.2	0.98	1	
		R	CACTCTCCTCTTTCCAATTCTTCAA					
<i>TNFA</i>	EU682384.1	F	CAGCTGGAGAAGGATGATCGA	71	92.0	0.99	1	
		R	CCAGATTCAGCAAAGTCCAGATAG					
<i>TGFB1</i>	NM_214015.1	F	ATCTCGCCCATCTCGGTTT	100	85.6	0.99	1	
		R	AAGTTGAGGCTCTCAGGGAGAA					
<i>SOCS1</i>	EW101597	F	CGCCCTCAGTGTGAAGATGG	110	98.2	0.99	2	
		R	GCTCGAAGAGGCAGTCTGAAG					
<i>SOCS2</i>	NM_001097461	F	CTGCGCATCGAATACCAAG	190	108	0.98	2	
		R	TGTAGAGCGGTTTGGTCAG					
<i>SOCS3</i>	NM_001123196	F	CACTCTCCAGCATCTCTGTC	105	105.0	0.99	2	
		R	TCGTAAGTGGTCCAGGAACTC					
<i>SOCS4</i>	ES445034	F	TCCTGGGACAGGCTCTATG	170	108.4	0.98	2	
		R	GGTACTTGGGAGGTGTTTC					
<i>SOCS5</i>	DB784235	F	ACGCTGTGTTTGCAGTCTC	89	98.1	0.96	2	
		R	ACTTCCAAGCTCCCTGTC					
<i>SOCS6</i>	XM_001926570	F	ATCTCTAGCCGGTGACTTCG	178	109.2	0.99	2	
		R	GCCCTTCTGCTTCTGTTTCG					
House-keeping genes								
<i>ACTB</i>	XM_003357928.1	F	GGGCATCCTGACCCTCAAG	89	97.3	0.99	3	
		R	TGTAGAAGGTGTGATGCCAGATCT					
<i>GAPDH</i>	NM_001206359.1	F	GGCGTGAACCATGAGAAGTATG	60	96.5	0.99	3	
		R	GGTGCAGGAGGCATTGCT					
<i>B2M</i>	NM_213978.1	F	CCCCGAAGGTTTCAGGTT	66	102.2	0.99	3	
		R	GCAGTTCAGGTAATTTGGCTTTC					
<i>HPRT</i>	NM_001032376.2	F	AGAAAAGTAAGCAGTCAGTTTCATATCAGT	131	92.1	0.99	3	
		R	ATCTGAACAAGAGAGAAAATACAGTCAATAG					
<i>OAZ1</i>	NM_001122994.1	F	TCGGCTGAATGTAACAGAGGAA	70	99.2	0.99	3	
		R	GAGCCTGGATTGGACGTTTAAA					

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National Center for Biotechnology Information (NCBI) Entrez Gene (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>).

F, forward primer; R, reverse primer.

## References

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**Supplemental Table 2.** Relative expression of genes within the innate immune signalling cascade in the jejunum and ileum of pigs fed either the control (CON) or transglycosylated starch (TGS) diet (Mean values with their standard errors (SEM); *n* 8 per diet)

Gene of interest*	Control diet	TGS diet	SEM	<i>P</i> -value
<b>Jejunum</b>				
<i>NFKB</i>	0.163	0.124	0.0176	0.140
<i>IL1B</i>	0.554	0.459	0.0893	0.462
<i>IL6</i>	0.052	0.031	0.0126	0.266
<i>IL8</i>	0.344	0.372	0.0935	0.318
<i>IL10</i>	0.155	0.124	0.0207	0.301
<i>TGFBI</i>	0.220	0.221	0.0332	0.981
<i>TNFA</i>	0.128	0.102	0.0191	0.353
<i>INFG</i>	0.305	0.230	0.0441	0.251
<i>SOC1</i>	0.231	0.270	0.0501	0.596
<i>SOC2</i>	0.225	0.243	0.0367	0.741
<i>SOC3</i>	0.121	0.098	0.0215	0.463
<i>SOC4</i>	0.223	0.207	0.0289	0.706
<i>SOC5</i>	0.152	0.131	0.0190	0.454
<i>SOC6</i>	0.248	0.244	0.0499	0.954
<i>AMPK</i>	0.156	0.160	0.0288	0.926
<i>FASN</i>	0.149	0.141	0.0336	0.864
<i>PPARG</i>	0.020	0.028	0.0055	0.366
<b>Ileum</b>				
<i>NFKB</i>	0.238	0.185	0.0394	0.360
<i>IL1B</i>	0.086	0.132	0.0234	0.185
<i>IL6</i>	0.196	0.385	0.0942	0.177
<i>IL8</i>	0.596	0.469	0.0699	0.219
<i>IL10</i>	0.264	0.329	0.0689	0.517
<i>TGFBI</i>	0.397	0.586	0.9663	0.188
<i>TNFA</i>	0.230	0.364	0.0682	0.184
<i>INFG</i>	0.447	0.406	0.0839	0.735
<i>SOCS1</i>	0.508	0.466	0.0793	0.708
<i>SOCS2</i>	0.299	0.396	0.0639	0.300
<i>SOCS3</i>	0.201	0.254	0.0421	0.408
<i>SOCS4</i>	0.297	0.349	0.0779	0.647
<i>SOCS5</i>	0.185	0.184	0.0333	0.996
<i>SOCS6</i>	0.233	0.183	0.0493	0.485
<i>AMPK</i>	0.186	0.301	0.0765	0.307
<i>FASN</i>	0.178	0.259	0.0428	0.202
<i>PPARG</i>	0.012	0.010	0.0020	0.486

\*Gene names: *AMPK*, AMP-activated protein kinase; *FASN*, fatty acid synthase; *IL*, interleukin; *INFG*, interferon-  $\gamma$ ; *NFKB*, nuclear transcription factor kappa-light-chain-enhancer of activated B cells; *PPARG*, peroxisome-proliferator-activated receptor- $\gamma$ ; *SOCS*; suppressor of cytokine signaling; *TGFB1*, transforming growth factor beta-1; *TNFA*, tumor necrosis factor- $\alpha$ .

**Supplemental Table 3.** Covariation scores for associations of bacterial genera and luminal microbial metabolites with the relative gene expression in the ileal, caecal and colonic mucosa of pigs using sparse partial least squares regression and relevance networking

Bacterial genus/microbial metabolite	Association score with*	Bacterial genus/microbial metabolite	Association score with*
Ileum		Caecum	
	<i>OCLN</i> expression		<i>SOCS2</i> expression
<i>Desulfovibrio</i>	-0.52	Unclassified <i>Ruminococcaceae</i>	0.67
<i>Megasphaera</i>	-0.66	Unclassified <i>RFP13</i>	0.67
<i>Turicibacter</i>	-0.53	Unclassified <i>Mogibacteriaceae</i>	0.79
<i>Helicobacter</i>	0.61	Unclassified <i>Dehalobacteriaceae</i>	0.71
<i>Actinobacillus</i>	-0.52	Propionate	-0.5
<i>Mannheimia</i>	0.51	Isobutyrate	-0.45
<i>Lactobacillus</i>	-0.51	Isovalerate	0.61
<i>Campylobacter</i>	-0.50	Caproate	0.49
Unclassified <i>Helicobacteraceae</i>	-0.60	Lactate	-0.43
Unclassified CAB.1	-0.59	pH	0.55
<i>Aggregatibacter</i>	-0.63		<i>SOCS4</i> expression
SMB53	0.56	<i>Succinivibrio</i>	0.76
Unclassified <i>Gemellaceae</i>	-0.51	<i>Oscillospira</i>	0.69
Unclassified <i>Lachnospiraceae</i>	-0.66	Unclassified <i>Succinivibrionaceae</i>	0.64
		Unclassified	
<i>Flexispira</i>	-0.63	<i>Dethiosulfovibrionaceae</i>	0.79
<i>Sphingomonas</i>	-0.67	<i>Bilophila</i>	0.77
		Isovalerate	0.48
Cecum		Lactate	-0.43
	<i>TLR4</i> expression		<i>ZO1</i> expression
Unclassified <i>Veillonellaceae</i>	-0.46	<i>Slackia</i>	0.50
<i>Megasphaera</i>	-0.47	<i>Anaerobiospirillum</i>	-0.70
Unclassified <i>Bacteroidales</i>	0.61	<i>Catenibacterium</i>	-0.72
Unclassified			
<i>Christensenellaceae</i>	0.51	<i>Anaerovibrio</i>	-0.70
Unclassified <i>Coriobacteriaceae</i>	-0.50	Unclassified <i>Microbacteriaceae</i>	-0.63
<i>Treponema</i>	0.46	<i>Selenomonas</i>	-0.57
Unclassified			
<i>Alphaproteobacteria</i>	0.50	Propionate	-0.41
<i>Pyramidobacter</i>	0.54	Isovalerate	0.52
Unclassified <i>GMD14H09</i>	0.64	Caproate	0.42
Unclassified <i>WPS2</i>	0.57	Lactate	-0.41
<i>Paludibacter</i>	0.54	pH	0.45
Unclassified <i>Pirellulaceae</i>	0.47		<i>MUC2</i> expression
<i>Dialister</i>	-0.46	Unclassified <i>Helicobacteraceae</i>	0.80
<i>Sphaerochata</i>	0.47	<i>Ruminobacter</i>	0.89

Unclassified <i>Mogibacteriaceae</i>	0.54	<i>Acinetobacter</i>	0.84
Unclassified <i>BS11</i>	0.55	Unclassified <i>Tremblayales</i>	0.86
Unclassified <i>Cerasicoccaceae</i>	0.51	<i>Akkermansia</i>	0.66
Total SCFA	-0.45	<i>Peptococcus</i>	0.88
Isobutyrate	-0.54	<i>Enterococcus</i>	0.83
Propionate	-0.56	Unclassified <i>Aeromonadaceae</i>	0.89
pH	0.66	Unclassified <i>Neisseriaceae</i>	0.81
	<i>NFKB</i> expression	<i>Roseburia</i>	0.71
Unclassified			
<i>Desulfovibrionaceae</i>	0.50		
<i>Anaerobiospirillum</i>	-0.54	Colon	
<i>Catenibacterium</i>	-0.59		<i>TLR4</i> expression
Unclassified RFP13	0.52	Unclassified <i>Helicobacteraceae</i>	-0.5
<i>Coprococcus</i>	0.54	<i>Sutterella</i>	-0.64
Unclassified <i>Mogibacteriaceae</i>	0.63	<i>Turicibacter</i>	-0.65
<i>Selenomonas</i>	-0.52	<i>Collinsella</i>	0.59
Unclassified			
<i>Dehalobacteriaceae</i>	0.63	<i>Blautia</i>	-0.61
Unclassified <i>Microbacteriaceae</i>	-0.60	<i>Megasphaera</i>	-0.57
Unclassified <i>Comamonadaceae</i>	-0.54	Unclassified <i>Lachnospiraceae</i>	-0.61
Propionate	-0.41	Unclassified <i>Erysipelotrichaceae</i>	-0.65
Isovalerate	0.52	<i>Dialister</i>	-0.64
Caproate	0.42	RFN20	-0.68
Lactate	-0.41	Unclassified <i>Ruminococcaceae</i>	0.53
pH	0.45	Isovalerate	0.6
		Lactate	-0.34
		pH	0.55

\*Only the strongest pair-wise associations were projected, with score threshold depending of the respective association. Gene names: *MUC2*, mucin 2; *NFKB*, nuclear factor- $\kappa$ B; *OCN*, occludin; *SOCS*, suppressor of cytokine signaling; *TLR4*, toll-like-receptor 4; *ZO1*, zonula occludens-1.