

Early life antibiotic exposure affects pancreatic islet development and metabolic regulation

Jiaying Li ^{1,†}, Kaiyuan Yang ^{1,†}, Tingting Ju ¹, Tracy Ho ¹, Catharine A. McKay ¹, Yanhua Gao ¹, Shay K. Forget ¹, Stephanie R. Gartner ¹, Catherine J. Field ¹, Catherine B. Chan ^{1,2} and Benjamin P. Willing ^{1,*}

¹ Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada

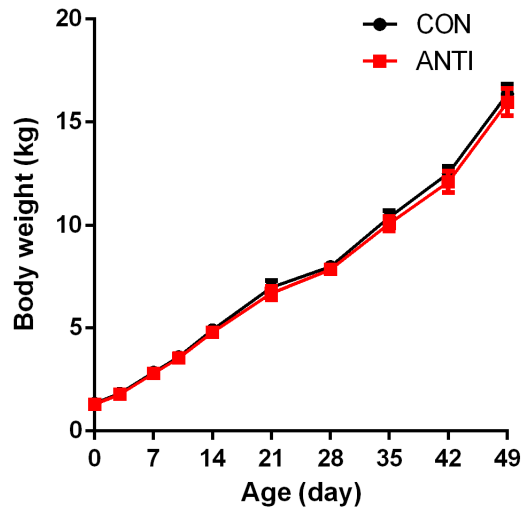
² Department of Physiology, University of Alberta, Edmonton, AB, T6G 2H7, Canada

* Corresponding author willing@ualberta.ca

† These authors contributed equally to this work

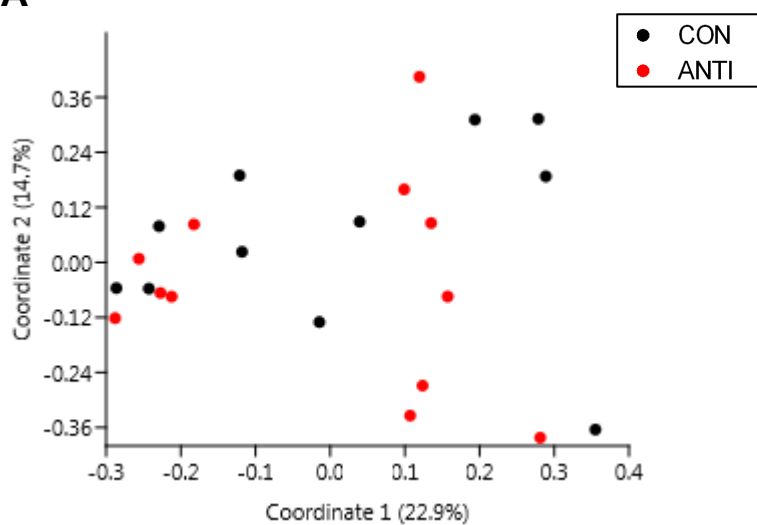
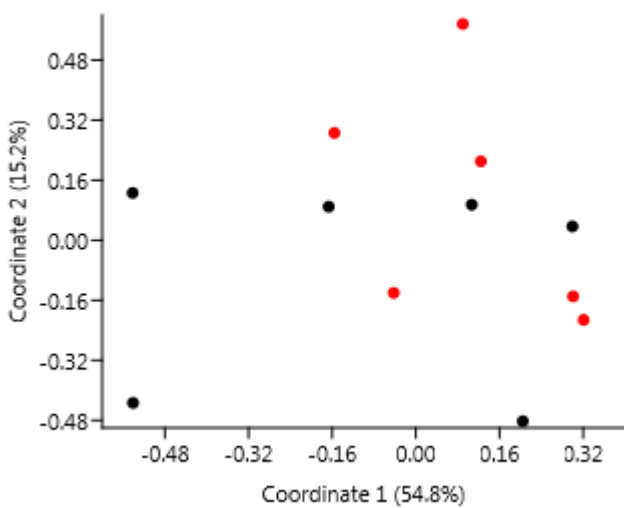
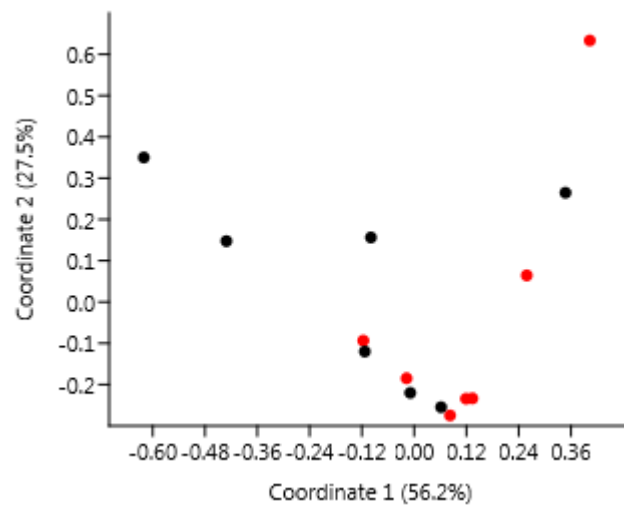
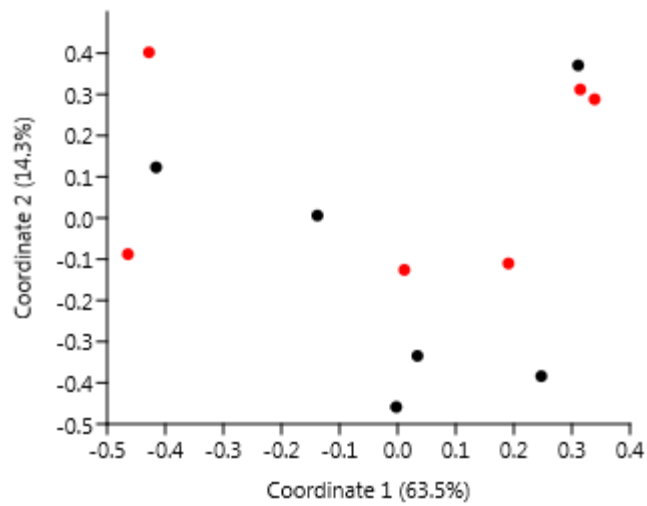
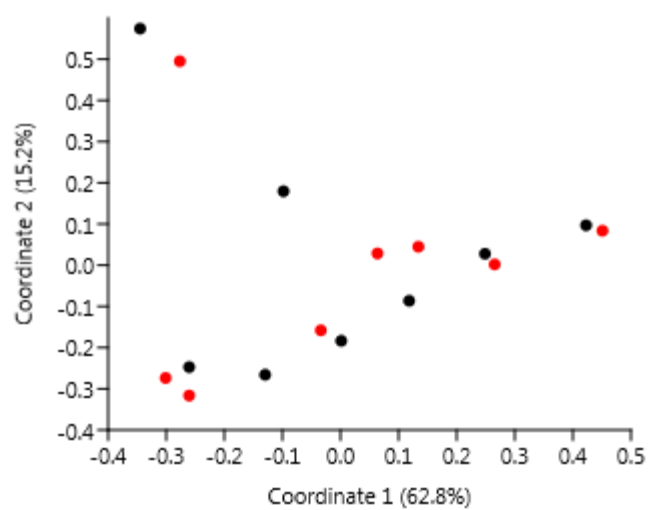
Supplemental information

Supplemental Figures



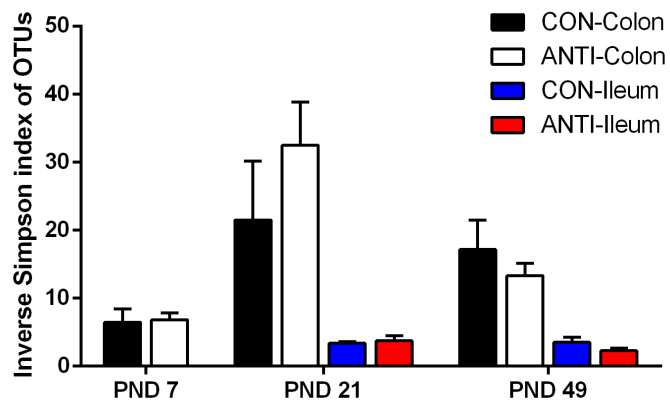
Supplemental Figure S1. Effect of postnatal antibiotics on body weight.

ANTI, N = 16; CON, N=14.

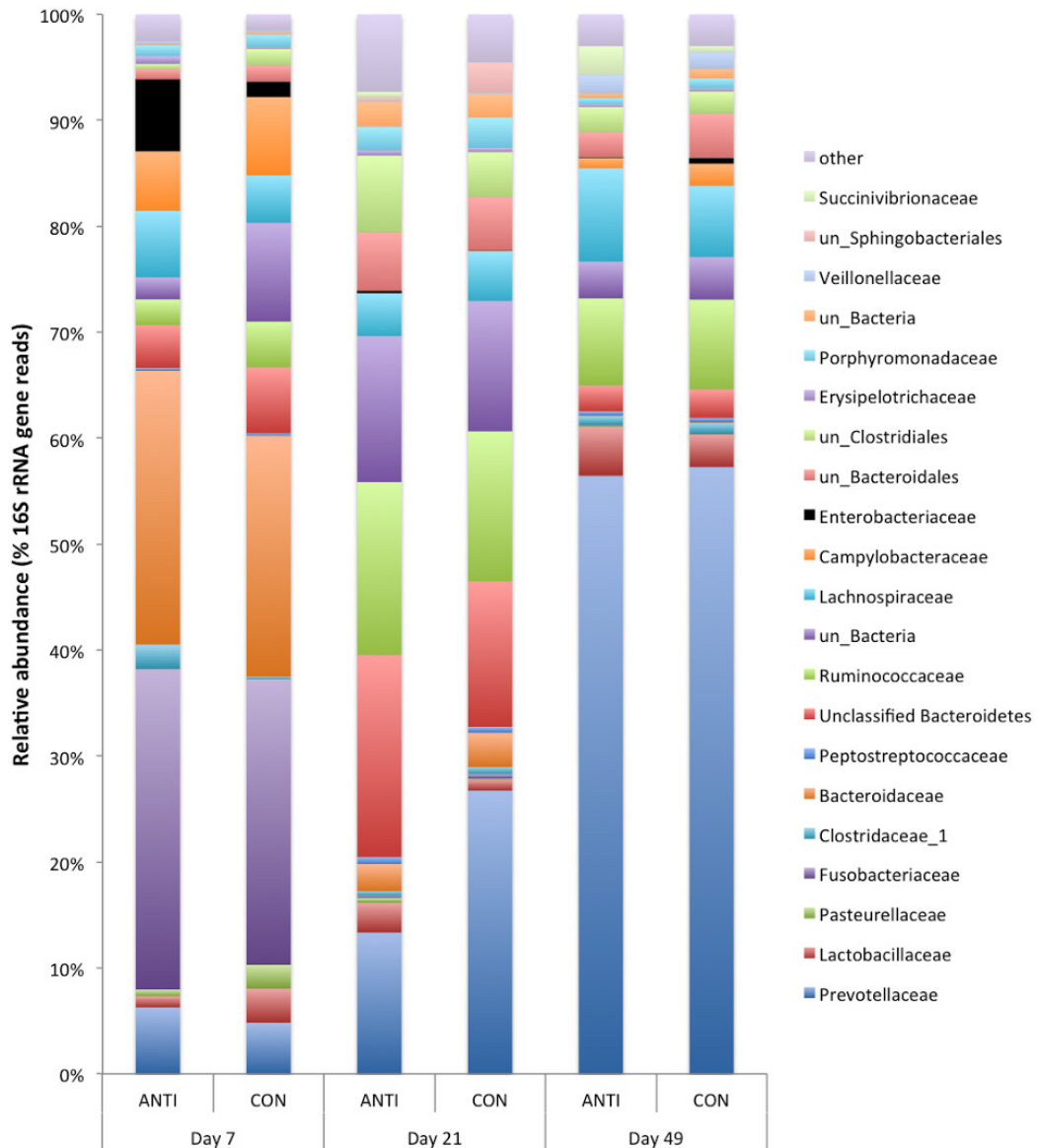
A**B****C****D****E**

Supplemental Figure S2. PCoA plots of bacterial community composition.

Bacterial community composition of (A) PND 7 rectal swabs, (B) PND 21 distal colon, (C) PND 21 ileum, (D) PND 49 distal colon, and (E) PND 49 ileum were shown in PCoA plots using Bray-Curtis dissimilarity metrics in amoxicillin treated (ANTI, red) and control (CON, black) pigs. All OTUs were retained in the analysis.

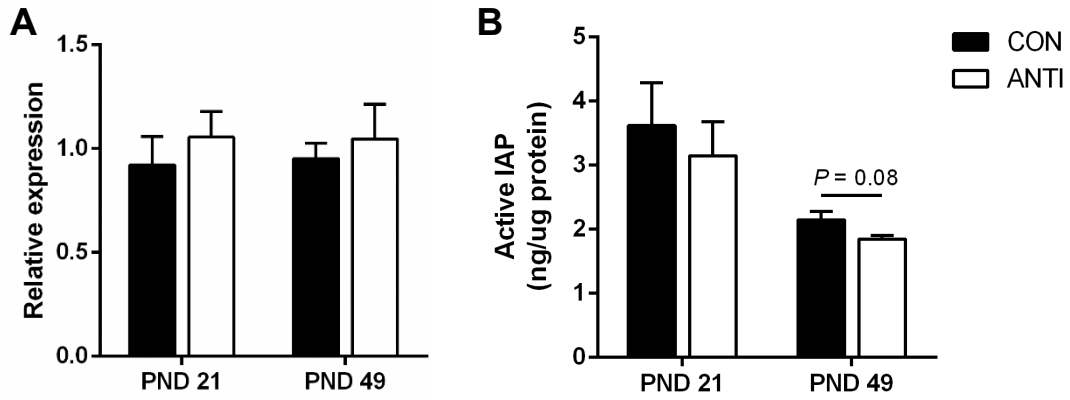


Supplemental Figure S3. Effect of antibiotic treatment on alpha diversity of gut microbiota.



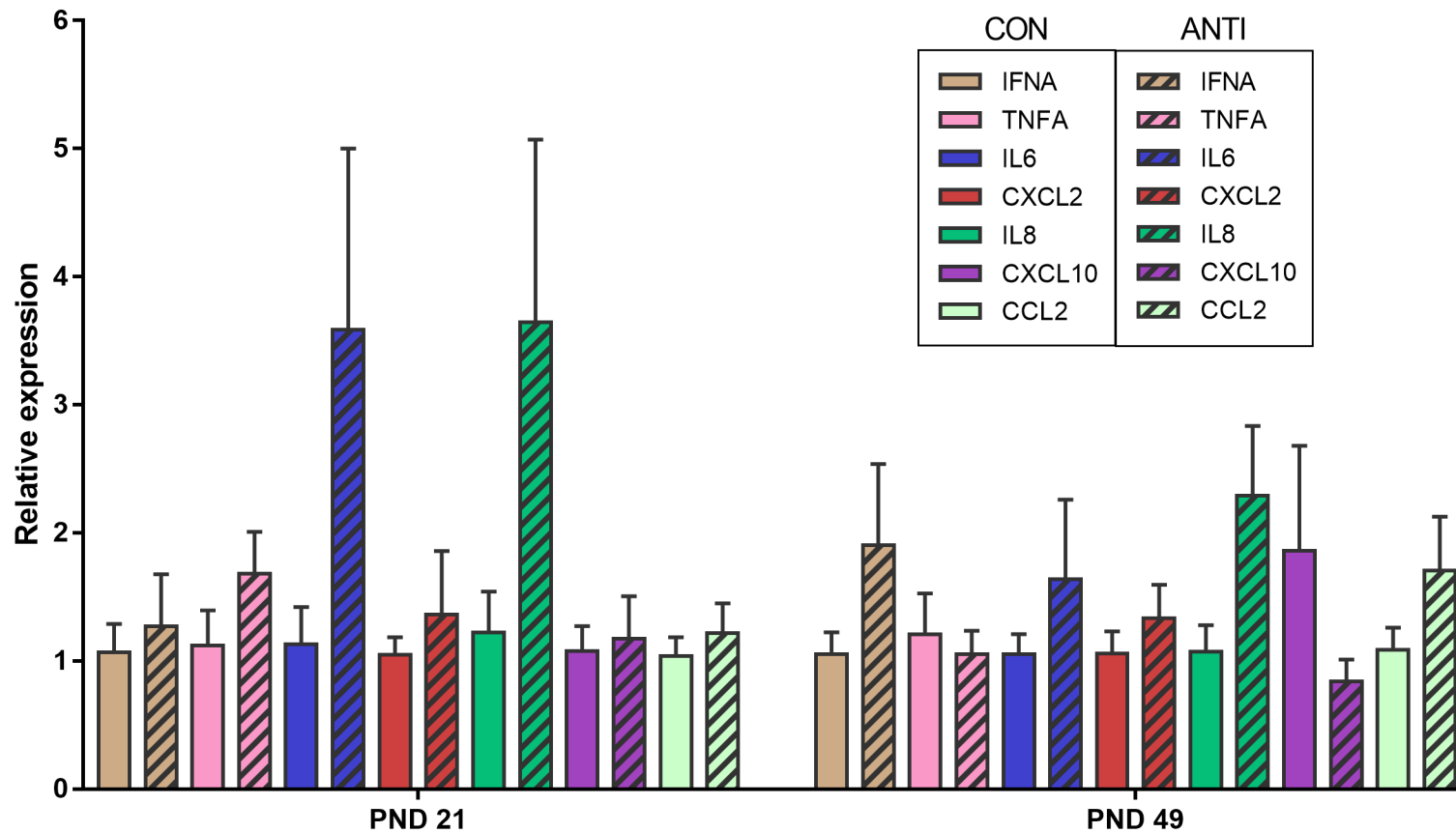
Supplemental Figure S4 Proportion of bacterial families in distal colon samples.

Mean relative proportion of bacterial families in distal colon samples of piglets treated with amoxicillin (ANTI) or placebo (CON) for first two weeks of life at postnatal day 7, 21 and 49. Enrichment of Enterobacteriaceae in ANTI pigs is only observed during antibiotic treatment at day 7 ($P < 0.05$).



Supplemental Figure S5. Intestinal alkaline phosphatase (IAP) gene expression levels and activity.

The relative gene expression (A) and activity (B) of IAP at PND 21 and PND 49 were measured in ileum.



Supplemental Figure S6. Pancreatic gene expression of cytokines and chemokines.

The relative gene expression of IFN α , TNF α , IL6, CXCL2, IL8, CXCL10, and CCL2 at PND 21 and PND 49 were measured in whole pancreas.

Supplemental Tables

Supplemental Table S1. Ingredients and chemical composition of the piglet diets

Ingredients	Diet ^a (g/kg)			
	Phase 1	Phase 2	Phase 3	Pre-grower
Wheat, ground	270.3	463.8	599.7	508.4
Soybean meal, 460g CP/kg	138.3	168	123	124
Canola meal	0	0	0	55
Peas, ground	0	0	0	75
HI-Lactose whey	171.2	125	0	0
Distillers wheat husky	0	30	50	0
Corn	0	0	94	150
Oat	220	100	59	0
Fish meal, Menhaden 620g CP/kg	60	37.5	0	0
Fat	15	40	25	18
Canola oil	10	0	0	0
Limestone / glass rock	8.5	15	15	19.2
Mono/dicalcium phosphate	0	0	4.3	0
Salt	0	0.5	4.7	3.9
L-Lysine HCl 780g/kg	4.3	5.2	8.3	4.4
L-Threonine 990g/kg	0.8	2.2	3.3	1.5
DL-Methionine 990g/kg	1.9	2	2.7	1.2
L-Tryptophan 990g/kg	0	0.1	0.4	0
Animal plasma 920	35	0	0	0
Water-glycol blend	35	0	0	0
Proform pig starter premix	25	0	0	0
extraPRO™	0	0	0	35
Others	4.7	10.7	10.6	4.4
Dry matter (%)	87.6	89.7	88.8	89.5
Digestible energy (kcal/kg)	3577	3627	3533	3483
Metabolizable energy (kcal/kg)	3385	3403	3337	3268
Net energy (kcal/kg)	2463	2622	2500	2349
Crude protein (%)	22.8	20.2	18.1	21.5
Crude fat (%)	5.0	5.8	4.3	5.4
Lysine (%)	1.64	1.36	1.30	1.30
Threonine (%)	1.00	0.91	0.87	0.92
Methionine (%)	0.54	0.49	0.48	0.43
Tryptophan (%)	0.30	0.24	0.24	0.25

^a Phase 1, 2,3 and pre-grower diets are standard feed pallets produced by HI-PRO FEEDS, Sherwood Park, AB.

Supplemental Table S2 Porcine-specific primer sequences used in quantitative RT-PCR analysis

Gene	Accession No.	Primer	Primer sequence (5'→3')	Ta (°C)	Amplicon length
<i>IGF2</i>	NM_213883.2	Forward	AGGGCATCCAAACCACAAAC	60	96 bp
		Reverse	GGGTTCAATTTTGGTATGTAAGT		
<i>INS-2</i>	NM_001109772.1	Forward	CAGAAGCGTGGCATCGTG	60	76 bp
		Reverse	CCTAGTTGCAGTAGTTCTCCAG		
<i>PDX-1</i>	NM_001141984.1	Forward	AAGTCTACCAAGGCTCACGC	60	159 bp
		Reverse	GCGCGGCCTAGAGATGTATT		
<i>GPR41 (FFAR3)</i>	XM_005664489.1	Forward	TGGAGACCTTACGTGTTG	57	75 bp
		Reverse	CGAGGATGAGAAGTAGTAGAT		
<i>GPR43 (FFAR2)</i>	NM_001278758.1	Forward	CGTGTTTCATCGTTCAGTA	57	76 bp
		Reverse	GAAGTTCTCATAGCAGGTA		
<i>IAP</i>	XM_003133729.2	Forward	CTAAAGGGGCAGATGAATGG	60	105 bp
		Reverse	CACCTGTCTGTCCACGTTGT		
<i>TNFα</i>	X57321.1	Forward	CACCACGCTCTTCTGCCTACTGC	60	162 bp
		Reverse	TCGGCTTTGACATTGGCTACAA		
<i>IFNα</i>	M28623	Forward	GGACCTGGAAGCCTGTGTCA	60	326 bp
		Reverse	CACGGGCAGGATGATACAGAAA		
<i>IL6</i>	M86722.1	Forward	GCCCACCAGGAACGAAAG A	60	109 bp
		Reverse	AAGCAGCCCCAGGGAGAA		
<i>IL8</i>	NM_213867	Forward	CCGTGTCAACATGACTTCCAA	60	74 bp
		Reverse	GCCTCACAGAGAGCTGCAGAA		
<i>CCL2</i>	NM_214214 (USDA)	Forward	GCGGCTGATGAGCTACAGAAG	60	74 bp
		Reverse	CCGCGATGGTCTTGAAGATC		
<i>CXCL2</i>	NM_001001861	Forward	CCGTGCAAGGAATTCACCTC	60	125 bp
		Reverse	TGCGGGGTTGAGACAAACTT		
<i>CXCL10</i>	NM_001008691	Forward	CCCACATGTTGAGATCATTGC	60	168 bp
		Reverse	CATCCTTATCAGTAGTGCCG		
<i>GAPDH</i>	NM_001206359.1	Forward	CACCTGTCTGTCCACGTTGT	60	147 bp
		Reverse	ATGGACCGTGGTCATGAGT		

Ta: annealing temperature