Transcriptomic analysis of the stress response to weaning in the piglets gut

Lorenzo Bomba*[1], Andrea Minuti[1], Sonia J. Moisa[2], Erminio Trevisi[1], Elisa Eufemi[1], Michela Lizier[3], Franco Lucchini[4], Marcin Rzepus[5], Aldo Prandini[5], Filippo Rossi[5], Raffaele Mazza[6], Giuseppe Bertoni[1], Juan J. Loor[2], Paolo Ajmone Marsan[1].

1Istituto di Zootecnica, UCSC, via Emilia Parmense 84, 29122 Piacenza, Italy; 2Mammalian NutriPhysioGenomics, University of Illinois, Urbana, USA,3Istituto di Tecnologie Biomediche, CNR, via Cervi 93, 20090 Segrate, Milano, Italy; Istituto Clinico Humanitas IRCSS, via Manzoni 56, Rozzano, Milano, Italy; 4CRB, Istituto di Microbiologia, UCSC, via Milano 24, 26100 Cremona, Italy; 5Istituto di Scienze degli alimenti e della nutrizione, UCSC, via Emilia Parmense 84, 29122 Piacenza, Italy; 6Associazione Italiana Allevatori, Rome, Italy

*Corresponding author: lorenzo.bomba@unicatt.it

Introduction

Weaning is a very critical period for piglets, typically accompanied by low feed intake, weight loss after weaning and increased mortality. Weanling pigs usually experience a malabsorption syndrome characterized by digestive enzyme disorder and pathogenic bacterial over-growth, which often evolve in gut disorders, explaining part of the reduction in villous height and increased crypt depth. In the past, antibiotics were used to overcome weaning problems. Nowadays, the development of resistant pathogenic strain has promoted the use of organic acids.

Objective: We assessed the effect of weaning and a dietary acidification with sorbic acid on the transcriptional state of pig (*Sus Scrofa*) ileum, applying microarray based transcriptome analysis.

Materials & Methods

Experimental Design

28 pigs		Time at the sacrifice	
		T0(28 d. old)	T5(T0+5d)
Diets	Control	4	6
	Sorbic Acid		6

Diets

% on dry matter	Control	Fermentable
Protein	19.71	19.76
Lipid	4.92	4.93
Starch	39.27	39.49
Sorbic acid	0.50	-

Gene expression analyses were carried out in blood and Ileum samples respectively. CustomArray™ 90K (CombiMatrix, Irvine, CA, USA) were used to evaluate gene expression levels. Linear models of the limma package were used to identify genes differentially expressed (DEG) between diet and time points. To examine the functional relationships among the DEG we used the Ingenuity Pathway Analysis tool version 8.0 (IPA) and Dynamic Impact Approach (DIA). Microarray results were validated by real-time PCR.

Results and Discussion

Whole transcriptome microarray analysis was performed comparing diets and times. We found no significant differences in gene expression between diets. Comparing piglet post weaning (T5) with pre weaning (T0) gene expression profiles, a total of 205 transcripts were found to be differentially expressed at a FDR of 0.05 (Figure1). Among these 27 downregulated and 178 upregulated in post weaning (T5). The Log fold changes of significantly differentially expressed genes spanned between 4.32 and -2.03. .

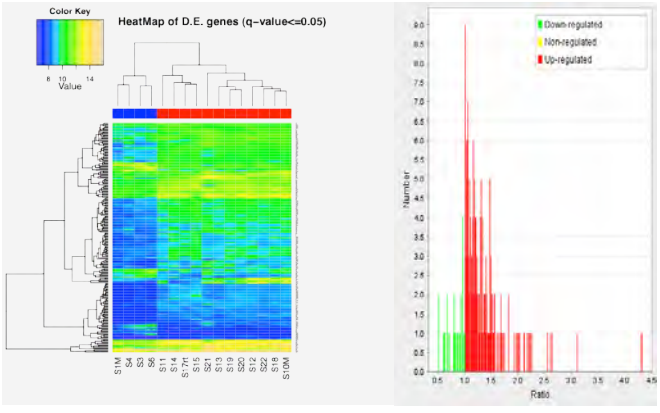

Figure 1: Left: Hierarchical clustering of all the samples based on genes differentially expressed (q-value ≤0.05). Relative levels of gene expression are depicted with a colour scale where yellow represents the highest level of expression and blue represents the lowest one. Unsupervised clustering identified 2 subsets of samples, one pre-weaning (blue) and the other post-weaning (red). Right: Number of DEG used for DIA analysis. Green colour depicted downregulated genes and red colour depicted upregulated genes (P value < 0.008 and FDR < 0.05).

IPA software identify 11 networks. In the network having the highest likelihood (Figure 2), IFNα and NFκB complexes represent central hubs and connect genes involved in antimicrobial and inflammatory response. The second network includes transcripts related to Lipid Metabolism, Molecular Transport and Small Molecule Biochemistry. Among these, APOA1 and SST were downregulated and associated with PPARalfa/RXR activation and with APCS, involved in acute phase response signaling. Steroid biosynthesis (CYP51A1, MSMO1), RIG-I-like (DDX58, IL8, CXCL10, ISG15) and NOD-like (HSP90B1, IL8, BIRC2) receptor signaling pathways and apoptosis (CASP3, BIRC2, TNFSF10) were the most impacted KEGG Canonical Pathways (FDR ≤ 0.05) (Table 1). As part of those pathways, IFIT3, IFIT1 and MX1 interferon-inducing genes play a central role in initiating the immune response.

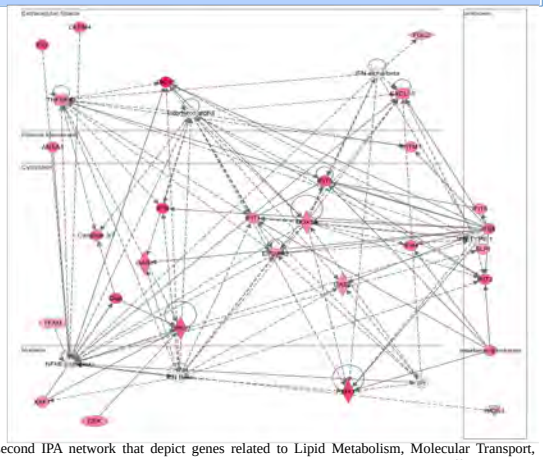

Figure.2:The second IPA network that depict genes related to Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry (score 36). The network displayed genes as nodes having different shapes that represent the functional class of the gene and the relationships between the nodes as edges (lines). The diagrams show direct (solid lines) and indirect (dashed lines) interactions between genes. Red and green shading denotes genes increased and decreased in expression, respectively, and the intensity of the color indicates the degree of modulation.

KEGG CATEGORY	KEGG SUB-CATEGORY	KEGG PATHWAY	FLUX	IMPACT
1. Metabolism	1.3 Lipid Metabolism	Steroid biosynthesis		
1. Metabolism	1.1 Carbohydrate Metabolism	Fructose and mannose metabolism		
1. Metabolism	1.1 Carbohydrate Metabolism	Glycolysis / Gluconeogenesis		
1. Metabolism	1.1 Carbohydrate Metabolism	Citrate cycle (TCA cycle)		
1. Metabolism	1.5 Amino Acid Metabolism	Valine, leucine and isoleucine degradati on		
1. Metabolism	1.6 Metabolism of Other Amino Acids	Glutathione metabolism		
2. Geneti cInformati on Processing	2.3 Folding, Sorti ng and Degradati on	Ubiquiti n mediated proteolysis		
2. Geneti cInformati on Processing	2.1 Transcripti on	Spliceosome		
3. Environmental Informati on Processing	3.3 Signaling Molecules and Interacti on	Cytokine-cytokine receptor interacti on		
3. Environmental Informati on Processing	3.2 Signal Transducti on	MAPK signaling pathway		
4. Cellular Processes	4.3 Cell Growth and Death	Apoptosis		
4. Cellular Processes	4.3 Cell Growth and Death	p53 signaling pathway		
4. Cellular Processes	4.4 Cell Communicati on	Focal adhesion		
4. Cellular Processes	4.4 Cell Communicati on	Tight juncti on		
4. Cellular Processes	4.1 Transport and Catabolism	Endocytosis		
4. Cellular Processes	4.2 Cell Moti lity	Regulati on of acti n cytoskeleton		
5. Organismal Systems	5.1 Immune System	RIG-I-like receptor signaling pathway		
5. Organismal Systems	5.1 Immune System	NOD-like receptor signaling pathway		
5. Organismal Systems	5.1 Immune System	Toll-like receptor signaling pathway		
5. Organismal Systems	5.1 Immune System	Cytosolic DNA-sensing pathway		
5. Organismal Systems	5.1 Immune System	Natural killer cell mediated cytotoxicity		
5. Organismal Systems	5.1 Immune System	Chemokine signaling pathway		
5. Organismal Systems	5.8 Development	Axon guidance		
5. Organismal Systems	5.2 Endocrine System	PPAR signaling pathway		

Table 1. Bioinformatics analysis of flux and impact analysis using differentially expressed genes (*P* value < 0.008; FDR < 0.05). Analysis was performed using the Dynamic Impact Approach (DIA) based on Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways database. Flux denotes the degree of activation or inhibition of each category and subcategory (green shades=inhibition, yellow shades=stable, red shades=activation). Blue horizontal bars denote the impact of each category and subcategories.

Conclusions

Our results disentangled some of the mechanisms of response to the stress of weaning occurring in the piglet gut. In this period several pathways related to immune and inflammatory response are activated and can induce small intestine atrophy. The dietary acidification that we administered to mitigate weaning stress had no detectable effect during the first 5 days after weaning. Further research is needed to identify compounds able to control the molecular mechanisms triggering inflammation. Improved knowledge will permit a better design of specialized feeds providing at the same time nutrients and the best combination of molecular signals to optimize animal stress response and welfare in critical periods.

Work supported by Romeo and Enrica Invernizzi Foundation