A transcriptomic analysis of gut response to stress induced by weaning in piglets

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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.

Aim of the research: 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	Fermentescible
Protein	19.71	19.76
Lipid	4.92	4.93
Starch	39.27	39.49
Sorbic acid	0.50	-

Hematological, histological and gene expression analyses were carried out in blood and Ileum samples respectively. CustomArrayTM 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), a Canonical pathway and regulatory network analyses software. Microarray results were validated by real-time PCR.

Results

Whole transcriptome microarray analysis was performed comparing diets and times. We found no significant difference 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).

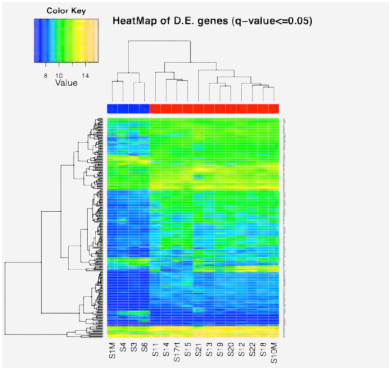

FIG.1: Hierarchical clustering of all the samples based on genes differentially expressed (q-value ≤0.05). Relative levels of gene expression are depicted with a colour scale where yellow represents the highest level of expression and blue represents the lowest one. Unsupervised clustering identified 2 subsets of samples, one pre-weaning (blue) and the other post-weaning (red).

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.

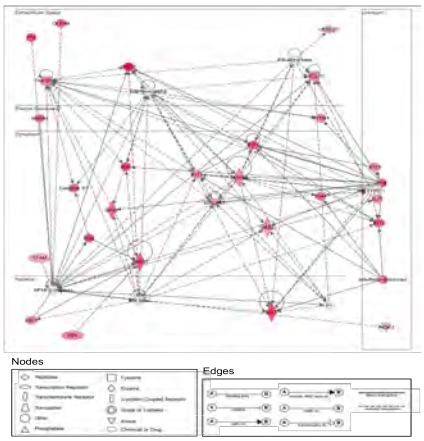

FIG.2:The second IPA network that depict genes related to Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry (score 36). The network displayed genes as nodes having different shapes that represent the functional class of the gene and the relationships between the nodes as edges (lines). 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 colour indicates the degree of modulation.

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. Ten statistically significant Canonical Pathways (FDR ≤ 0.05) were identified using IPA. The most significant Canonical Pathways was interferon signaling (FDR = 9.8E-4), in which IFIT3, IFIT1 and MX1 interferon inducing genes play a central role in initiating the immune response. To confirm microarray data, mRNA expression of 8 differentially expressed were successfully validated by real-time PCR.

Discussion

In conclusion, our results disentagled 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.

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