Effects of an Additional Iron Dextran Injection Administered to Piglets on Differential Gene Expression at Weaning

uniferon®
Iron(III) Dextran

Introduction

An administration of 200 mg Fe at 1-3 d of age has been demonstrated to meet the Fe requirement of nursing piglets until approximately 4 kg BW.

The combination of larger litters of faster growing pigs can contribute to a potential "iron gap" before weaning[1].

Objectives

The objective of this experiment was to evaluate the differential gene expression between pigs receiving one or two 200 mg Fe dextran injections at weaning.

Methods

- Six female littermate pairs were given their first 1 mL injections of iron dextran (200 mg Fe/mL, Uniferon®, Pharmacosmos Inc. Watchung, NJ)
- On d 7, six female pigs were paired by weight (1.72 ± 0.13 kg) and one pig from each pair was randomly selected as control (CON) and the other received a second injection (+Fe)
- At weaning on d 22 each piglet was anesthetized, and samples of liver and duodenum were taken from the anesthetized piglets and placed in DNA/RNA Shield (Zymo, Irvine, CA)
- RNA-Seq libraries were sequenced to a depth of at least 30 million read pairs (150 bp paired-end sequencing) per sample
- Differential Gene Expression data were analyzed with GeneSpring (Agilent, Santa Clara, CA) with a fold-change (FC) of |1.2| P < 0.05. Pathway analysis was conducted with Ingenuity Pathway Analysis (IPA QIAGEN, Redwood City, CA) software with Z-score cutoff of P < 0.05

Results

- In liver, 362 genes were expressed with a FC ≥|1.2| P < 0.05
- In the duodenum, 435 genes were significantly changed with a FC ≥|1.2| P < 0.05

Conclusions

Pigs that received two doses of Uniferon® had gene expression that helps explain the following:

- Growth rate
- Genes and pathway responsible for increased hematocrit
- Body Temperature Regulation associated with a 4.2 FC increase in TRPV1 expression in liver
- Gut health improvement due to the 40 FC in Claudin-1. Increased tight junctions
- Decrease in Gluconeogenesis in the duodenum. Saves 4 ATP per molecule
- Decrease in urea spares 4 ATP for each urea molecule not produced

~2.5% of genes expressed were affected by two iron dextran injections.

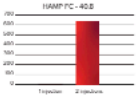
Liver: Individual Genes of Note

Biochemical Pathway Changes

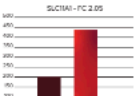
Liver Urea Synthesis

Z = -.0832
P < .05

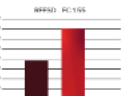
Spares 4 ATP Per Molecule



HAMP (Hepcidin Antimicrobial Peptide) Liver-produced hormone. It is the main circulating regulator of iron absorption and distribution.

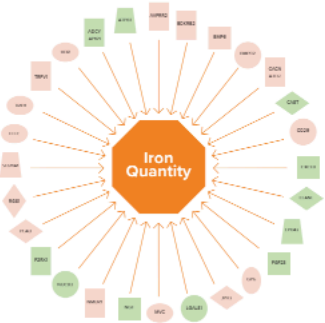


Solute carrier family 11 is a proton-coupled divalent metal ion transporter. Functions as a divalent transition metal (iron and manganese) transporter.



Binder 2 Fe-2S cluster binding. Predicted functions: oxidoreductase (oxidoreductase) activity

Liver: Gene Expression Related to Transition Metal Quantity



Body Weight, Hb, and tissue weight at birth, d 7, and weaning (d 22)

Treatment

Variables	1 injection	2 injections	SEM	TRT	Pair(sex)
BW, kg					
Birth	1.15	1.23	0.09	0.58	0.46
Weaning	4.84	5.00	0.14	0.52	0.02
Hb, g/dL					
Birth	10.4	10.3	0.85	0.96	0.37
Weaning	10.5	12.7	0.74	0.17	0.79

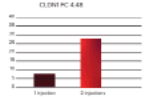
Duodenum: Individual Genes of Note

Biochemical Pathway Changes

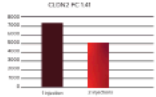
Duodenal Glucose Synthesis — **Z = -1.671**, **P < .05** — **Spares 4 ATP Per Molecule**

Duodenal Sterol Metabolism — **Z = -1.850**, **P < .05**

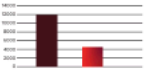
Duodenal Epithelial Cell Protection — **Z = 0.361**, **P < .05**



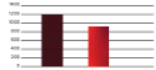
Claudin 1 – Gene responsible for tight junctions of cell-to-cell adhesion in epithelial or endothelial cell sheets.



Claudin 2 – Claudin-2 is expressed in colon-leaky epithelia.

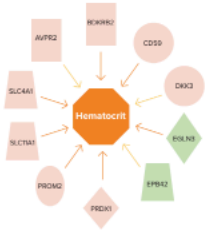


Transferrin Receptor Protein 1 - This gene encodes a cell surface receptor necessary for cellular iron uptake by the process of receptor-mediated endocytosis.



Hemostatic Iron Regulator - regulates iron absorption by regulating the interaction of the transferrin receptor with transferrin.

Liver: Genes and Pathways Related to Hematology



P < .05

Liver Hematocrit Synthesis — **Z = -1.36**, **P < .05**

Gene Symbol	FC	P value	Associated Biological Functions
AVPR2	3.24	0.041	Stimulates urine concentration to maintain water homeostasis
BDKRB2	3.14	0.013	Mediates inflammation by vasodilation and increased vascular permeability
SLC4A1	4.70	0.015	CO2 transport from tissues
SLC11A1	2.05	0.039	Divalent transition metal transporter (Fe and Mn)
PROM2	2.04	0.018	Pentaspan membrane glycoprotein
PRDX1	1.22	0.034	Reduce hydrogen peroxide an alkyl peroxides
CD59	1.25	0.028	Glycoprotein that regulates complement-mediated cell lysis. Also involved in coagulation
DKK3	1.78	0.011	Enhances insulin sensitivity and glucose tolerance
EGLN3	-1.56	0.024	Egl-9 family hypoxia-inducible factor 3
EPB42	-2.02	0.044	Erythrocyte membrane protein band. Expressed due to anemia
F2RL2	-7.3	0.004	coagulation factor II thrombin receptor like 2

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