



Differentially expressed genes in Piétrain sired pigs when feeding a high-fat high-fibre diet

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Introduction

- Pig production in Flanders focuses on producing high quality lean meat.
- This can be accomplished by using Piétrain sires and crossbreeding with a highly prolific hybrid dam
- In order to maintain high carcass leanness and conformation, the progenies of Piétrain have been from inception of selection fed on grain-based feeds rich in starch as major energy source
- In the future: shift from standard (Control) diet $\Leftrightarrow$ High-fat high-fibre diet
= maize and soy-bean = fibrous byproducts

It is not yet known how Piétrain-offspring will cope with a shift in diet from grain to high-fibre-high-fat feeds



Objectives

- To identify candidate genes that are involved in feed resilience mechanisms through transcriptomics in tissues involved in the regulation of digestion
- To investigate if there is an effect of the EBV for feed intake of the sire
- To investigate if there is an effect of the sex of the animal
- To identify the biological pathways of the differentially expressed genes

Materials and methods

- Experimental design

A 2x2x2 factorial trial with genetic background, diet and sex as factors

Sire type	Feed	Sex	Number of piglets
EBV high DFI	Control diet	Gilt	4
		Barrow	4
	High-fat-high-fibre diet	Gilt	4
		Barrow	4
EBV Low DFI	Control diet	Gilt	4
		Barrow	4
	High-fat-high-fibre diet	Gilt	4
		Barrow	4

- Euthanasia
 - At the end of 15 weeks = after 1 week diet change

■ Feeding regime

- Fed on the same feed after weaning at 4 week
- Reared up to 14 weeks, thereafter,
- Diet shift was from 14 to 15 weeks, in which 16 pigs were fed differently on control diet and high-fat-high-fibre diet respectively.

■ Feed composition

	phase1 (4-14 wks)	phase 2 (14-15 wks)	
		Control	High-fat high-fibre diet
Crude protein (g/kg)	160	160	160
Raw fat (g/kg)	49	46	75
Crude fibre (g/kg)	40	45	60
Carbohydrate (g/kg)	353	349	246
Energy (MJ/kg)	9.6	9.4	9.4

- Genetic background:

2 groups of sires: High or Low EBV for daily feed intake

Average EBVs	High feed intake boars	Low feed intake boars
Feed intake	149	82
Growth rate	188	84
Carcass quality	107	141
Feed conversion	123	127

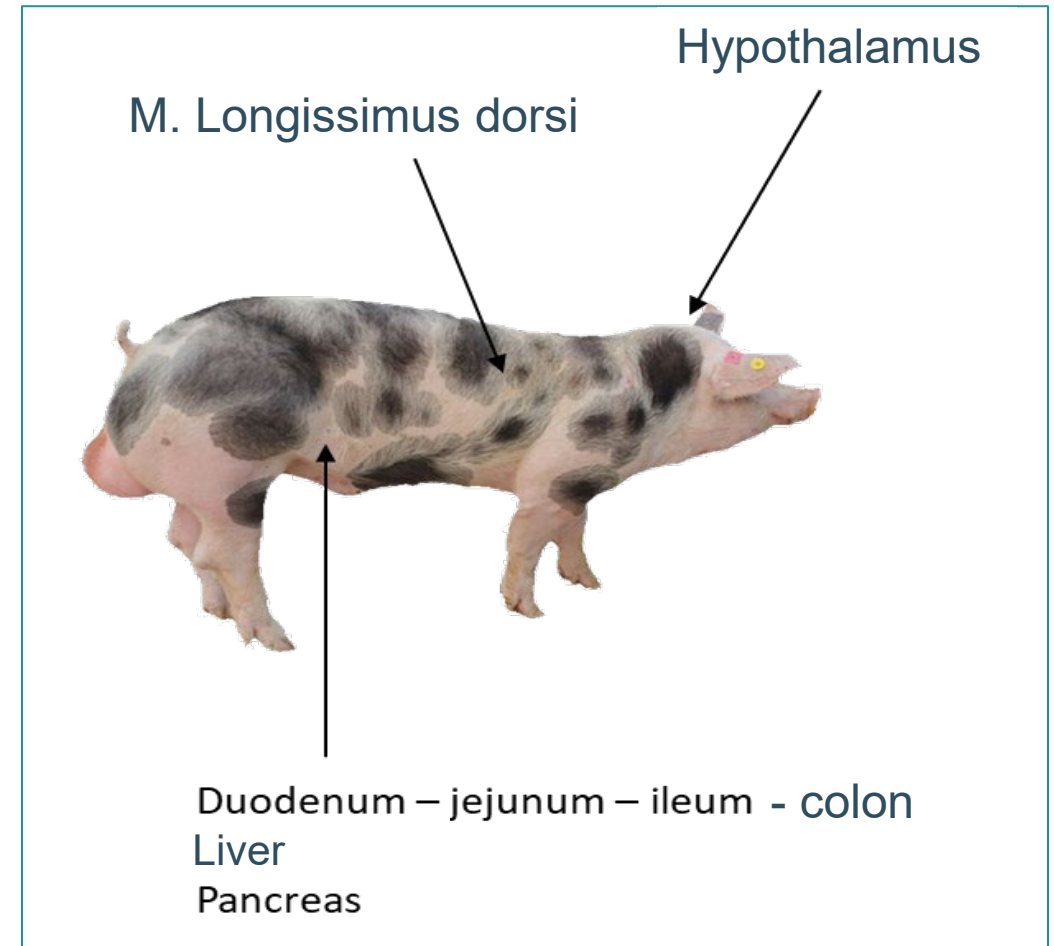
(EBV average = 110, sd = 20)

■ Tissues:

RNA was extracted from:

- **hypothalamus**
- pituitary
- M. longissimus dorsi
- liver
- pancreas
- **colon**
- **ileum**
- duodenum
- jejunum

RNA was extracted from tissues involved in the regulation of digestion. In the current study we focus on hypothalamus, colon and ileum



■ RNA extraction:

- RNAeasy Mini kit; ileum and colon
- RNAeasy Lipid Tissue Mini kit; hypothalamus; from QIAGEN
- The collected tissues were stored at -80 until RNA extraction
 - A260/A280 ratio indicator for purity, is ~ 2.0 for pure RNA,
 - A260/A230 ratio indicator for purity, is ~ 2.0 – 2.2 for RNA
 - RIN values using Agilent Bioanalyzer (RIN; ~ 7-8, > 5-7 and <5)

Total RNA qualities from ileum, colon and hypothalamus

Sample	A260/A280	A260/A230	Concentration (ng/μl)	RIN	tissues mass (mg)	RNA yield (ug)
Ileum	2.023	2.090	1310	8.7	25	52
Colon	2.013	2.098	1189	8.7	27	48
Hypothalamus	1.940	1.960	577	7.1	86	23

■ Differential gene expression analysis

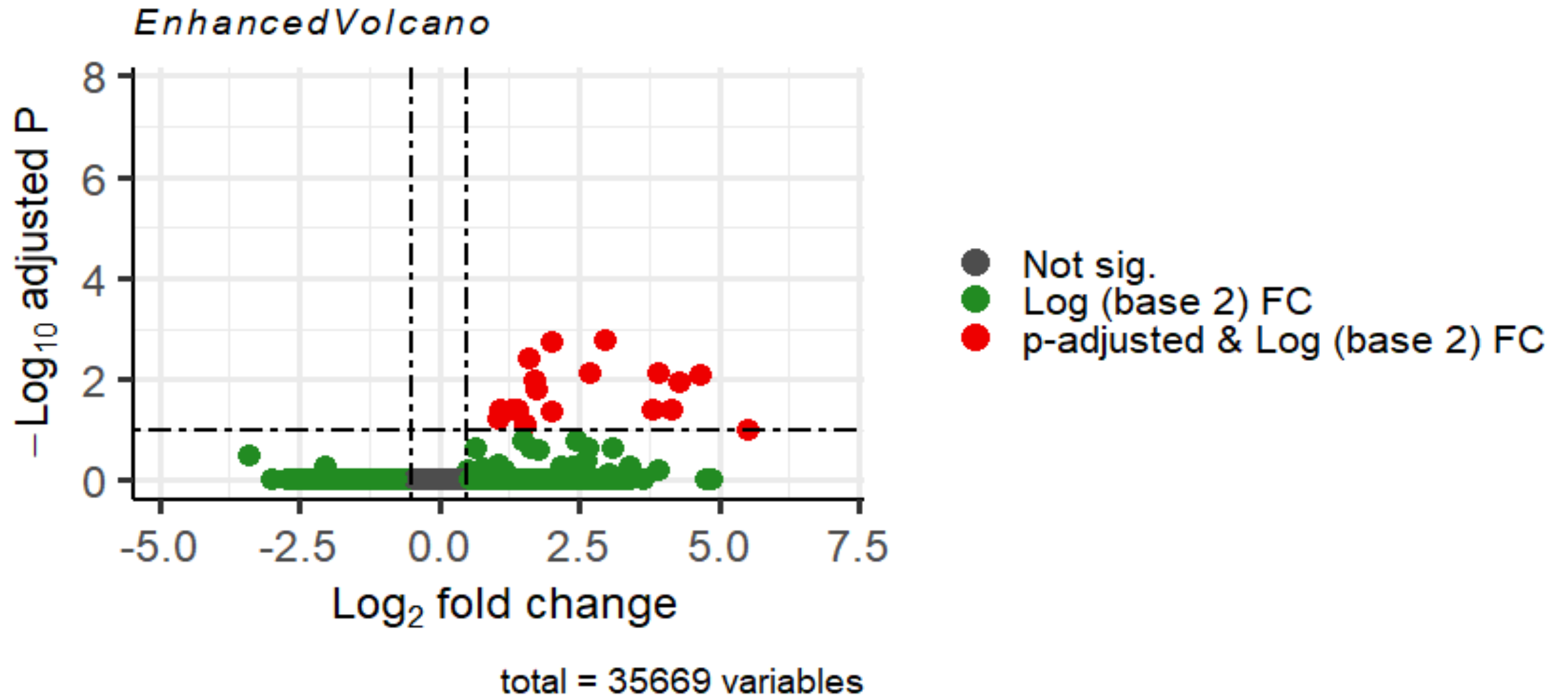
- Differential expression of genes was tested on the 3 main factors:
 - comparison of high and low EBV for feed intake
 - comparison of control and High-fat high-fibre diet
 - comparison of barrow and gilt
- Using DESeq2 in R package
 - DEGs based on $\log_2FC = 0.5$ and $p_{adj} = 0.1$
 - A positive $\log_2FoldChange$ implies an upregulation of the gene in condition 2.
In other words, the gene in question is more expressed in condition 2
- ShinyGO v0.741: Gene Ontology Enrichment Analysis

Results

- Differential gene expression analysis
- Total number of DEGs in hypothalamus, colon and ileum

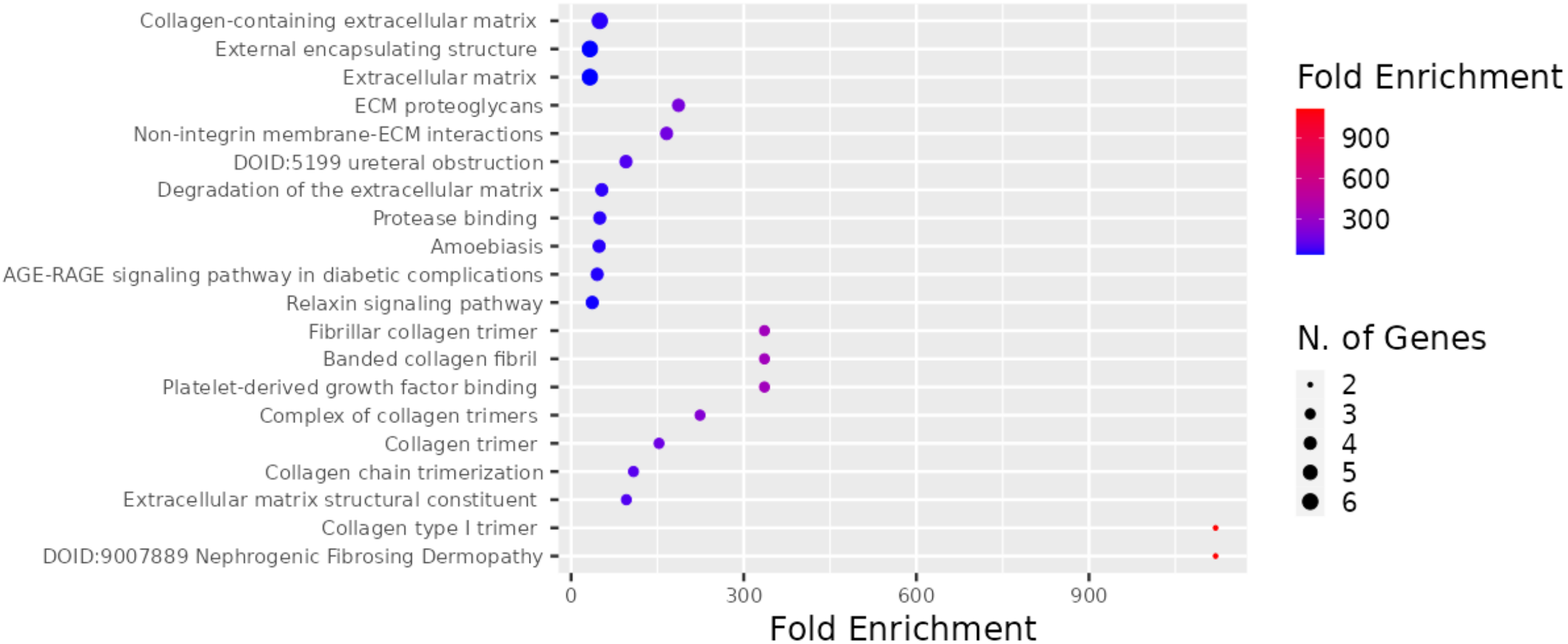
Tissue	Feed Control vs HFF	EBV High vs low	Sex Male vs female
Hypothalamus	20 (0↓ 20↑)	0	0
Colon	0	18 (10↑ 8↓)	17 (3↑ 14↓)
Ileum	0	3 (1↑ 2↓)	36 (20↑ 16↓)

Volcano plot of High-fat-high-fibre diet vs control diet



Functional Enrichment Analysis of Differentially Expressed Genes

Biological process regulated by the of differentially expressed genes in the hypothalamus: feed



Conclusion

- One week feed change:
 - 20 genes were significantly differentially expressed in hypothalamus of Piétrain pigs as a result of the feed shift
 - Several biological processes were regulated by the differential expressed genes playing roles in feed resilience
 - In colon and ileum no differential expression of genes was found
- In addition, sire type and sex had effect on differential gene expression in
 - colon (18 and 3, respectively)
 - and ileum (17 and 36, respectively)

Acknowledgements

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