Stronger transcriptomic response to feed intake in the duodenum of pig with high feed efficiency from a divergent selection experiment

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Abstract

Feed efficiency is a trait of interest in pigs as it contributes to lowering the ecological and economical costs of pig production. A divergent genetic selection experiment from a Large White pig population was performed for 10 generations, leading to pig lines with relative low- (LRFI, more efficient) and high- (HRFI, less efficient) residual feed intake (RFI). The meals of pigs from the LRFI line are shorter and less frequent as compared to the HRFI line. We hypothesised that these differences in feeding behaviour could be related to differential sensing and absorption of nutrients in the intestine.

Here we investigated the duodenum transcriptomic response to short term feed intake in LRFI and HRFI lines (n=24). We identified 1106 differentially expressed genes between the two lines, notably affecting pathways of the transmembrane transport activity and related to mitosis or chromosome separation. The LRFI line showed a greater transcriptomic response to feed intake, with 2222 differentially expressed genes before and after a meal, as compared to 61 differentially expressed genes in the HRFI line. Feed intake affected genes from both anabolic and catabolic pathways in the pig duodenum, such as autophagy and rRNA production. We noted that several nutrient transporter genes were differentially expressed between lines and/or by short term feed intake.

Altogether, our findings highlighted that the genetic selection for feed efficiency in pigs changed the transcriptome profiles of the duodenum, and notably its response to feed intake.

Keywords

duodenum, transcriptomic profiling, postprandial transcriptome, Solute Carrier genes, nutrient transporter, feeding behaviours

Introduction

In monogastric livestock, feed efficiency is the ability to convert the greater part of ingested feed into food. It is a complex trait with many known influencing factors such as nutrition, physiology, genetics, microbiome, meteorological conditions, sanitary status, and gut microbiota. Feed efficiency is a trait of great interest for livestock since farming feed cost is a large and growing part of the production costs. Feed constitutes a large part of the environmental impacts of monogastric farming[1]. In addition to research into non-human-edible feed, improvement in the animal feed efficiency will reduce the amount of feed needed to raise livestock, thus contributing to the reduction of its environmental footprint[2].

Feed efficiency can be measured as Residual Feed Intake (RFI): the difference between the amount of feed one animal is consuming and the amount predicted for its maintenance and production requirements via multiple regression on several traits (average metabolic body weight, Average Daily Gain, and indicators of body composition). A negative RFI means that the animal is eating less (relative high efficiency), and a positive RFI means that the animal is eating more than the population average (relative low efficiency). RFI is a heritable trait in pigs[3].

Gilbert et al. created a genetic divergent selection experiment from a Large White pig breed nucleus, resulting in two pig lines of relative low (LRFI) and high (HRFI) feed efficiency[3]. The response to selection has led to multiple genetic and genomic changes between the lines[4]. A previous transcriptomic study has compared muscle, adipose tissues and liver transcriptome from generation 8 of selection[5], with pathways involved in immune response, response to oxidative stress and protein metabolism differentiating the two lines. These two lines also differ on their blood and muscle metabolism[6,7], with a lower insulin rate in the blood of LRFI pigs, and in their faecal microbiota[8]. Interestingly, the selection has led to distinct feeding behaviour between the lines: pigs from the LRFI line eat more per visit to the feeder, stay longer in the feeder, eat faster, and wait longer between two visits than pigs from the HRFI line[9].

The intestine is known to quickly adapt to various feeding challenges[10]. The duodenum is one of the key organs contributing to the satiety regulation, in relation with its proximal position in the gut[11,12]. Specialised enteroendocrine cells of the intestinal epithelium are responsible for the production of anorexygenic hormones such as Glucagon-like peptide-1 (GLP-1), Gastric inhibitory polypeptide (GIP), Cholecystokinin (CCK), and Peptide YY (PYY). To the opposite, cells from the stomach and the intestine secretes the orexygenic hormone Grhelin.

The effect of feeding challenges on intestinal transcriptomes has been investigated by several groups. Zhang et al. reported that pigs submitted to different feeding frequencies, 1, 3 or 5 meals per day, have a different ileal and colonic mucosal transcriptome[13], although they only investigated transcriptomes from overnight fasted pigs. In mice, Yoshioka et al. have investigated the effect of a high-fat and low-fat diet on the duodenal mucosa compared to fasted mice[14]. They noted only a modest effect on the transcriptome, but highlighted the downregulation of *Slc5a1* after feed intake. *Slc5a1* is the gene encoding for the glucose transporter SGLT1. It is also documented that the gene *Slc15a1* encoding for the oligopeptide transporter PEPT1 is downregulated by feed intake in mice[15]. More generally, the Solute Carrier (SLC) gene family encodes transmembrane nutrient transporter, and is of great interest to understand the digestive physiology and beyond[16–18].

Since the divergent selection on feed efficiency has led to stark physiological differences between the LRFI and HRFI pig lines, we hypothesised that they may have very different intestinal responses to feed intake. We therefore investigated the transcriptome of the duodenum mucosa before or after a meal in pigs from the 10th generation of LRFI and HRFI lines, in a total of 24 animals.

Material & methods

Animal production and sampling

This experimentation was authorised by the French Minister of Higher Education, Research and Innovation under the number APAFIS#21107-2018120415595562 v10, after examinations by the animal experimentation ethic committee number 084.

Animals were raised using standard care in the INRAE pig experimental facility GENESI[19] up until the day before the procedure. Animals were from two French Large White lines of pigs that have been divergently selected on residual feed intakes (RFI) for 10 generations[3]. Pigs were from three litters in each line, and of balanced sexes within litter when possible (one litter was represented by 3 males and one female). They were 61 days old at the time of slaughter (min 60 days, max 62 days). At weaning (28 days old on average), animals were splitted into 2 enriched pens of 12 animals, full-sibs and sexes being equally distributed in the two pens (figure 1A). The day before sampling, feed access was removed at 5 p.m. in both pens. Animals had free access to water. At 8 a.m. the next day, feed access was introduced back in one pen, but not the other. Animals were slaughtered by electro-narcosis between 8.50 a.m. and 11.46 a.m., starting with animals left without feed access until 10.10 am where animals with unlimited feed access were also sampled (figure 1B). The gastro-intestinal tract was removed, and a 5 cm long section of the duodenum was sampled, opened longitudinally, and the mucosa was collected by scratching the inside duodenal section with a glass microscope slide. Samples were rinsed in PBS, and flash frozen in liquid nitrogen. Samples were preserved at -80°C until extraction.

Sample extraction, library preparation, and sequencing

Frozen duodenum samples were reduced to powder, and DNA and RNA were extracted from the powder using NucleoSpin TriPrep mini kit columns (Macherey-Nagel), as per manufacturer instructions. RNA-seq libraries have been prepared according to Illumina's protocols using the Illumina TruSeq Stranded mRNA sample prep kit to analyse mRNA. Briefly, mRNA were selected using poly-T beads. Then, RNA were fragmented to generate double stranded cDNA and adaptors were ligated to be sequenced. 11 cycles of PCR were applied to amplify libraries. Library quality was assessed using a Fragment Analyser and libraries were quantified by QPCR using the Kapa Library Quantification Kit. RNA-seq sequencing have been performed on an Illumina HiSeq3000 using a paired-end read length of 2x150 pb with the Illumina HiSeq3000 Reagent Kits, at the GeT-PlaGe core facility, INRAE Toulouse. Libraries were demultiplexed, and the resulting *fastq* files are available at the ENA database under the id PRJEB46060 / ERP130249.

RNA-seq bioinformatic processing

RNA-seq reads were processed using the nf-core/rnaseq pipeline[20] (version 3.0,), using Salmon[21] pseudo-alignment quantifications, Ensembl reference genome Sscrofa11.1, and the corresponding gene annotation file from version 102 of Ensembl[22]. Normalised counts were processed using {tximports}[23] to generate transcript per million (TPM) values, and gene-length normalised counts were used to carry over differential gene expression analysis with limma voom[24], using contrast matrices to test for different factors. The pig line (LRFI or HRFI), condition (fed or fasted) and sex (castrated male or female) were used to build a model matrix. Contrast matrices were constructed to compare the two lines, and to

compare the feeding effect within each line. A total of 13,738 genes were analysed, others having less than a total of 8 counts in our dataset. Gene ontology enrichment analysis was performed using {clusterProfiler}[25], using Sus scrofa Gene Ontology. It should be noted that not all genes annotated in the pig genomes are associated with a Gene Ontology term, leading to slightly lower gene numbers in the Gene Ontology Analysis. The set of genes used for differential gene expression analysis was used as a reference set. Enriched Gene Ontology were simplified for readability using the `simplify()` function, and simplified enriched ontologies were clustered using the `pairwise_termsim()` function. The upset plot was generated using the {UpSetR} package[26]. The list of substrates for each SLC transporter was obtained from the SLC tables at http://slc.bioparadigms.org/[17].

Data and script availability

RNA-seq reads are available through the ENA database, id PRJEB46060 / ERP130249.

Scripts used to process and analyse RNA-seq reads are available through a public gitlab repository at the address: forgemia.inra.fr/genepi/analyses/rosepigs. Gene expression count as well as lists of differentially expressed genes and sample metadata tables are available in this repository (forgemia.inra.fr/genepi/analyses/rosepigs/-/tree/master/processed_files).

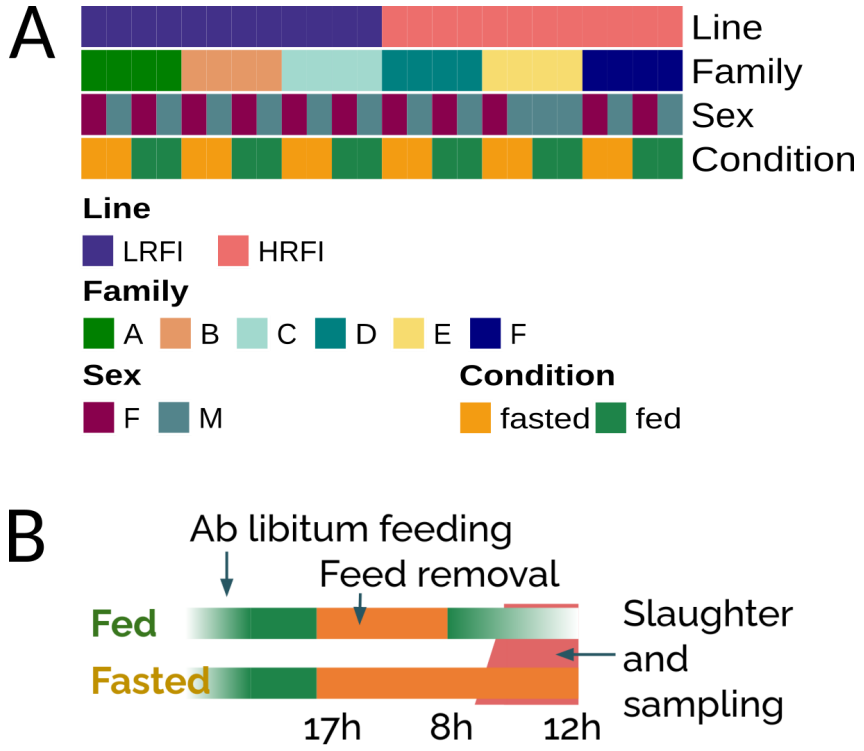

Figure 1: Experimental setup. **A.** 24 pigs from two pig lines (12 LRFI and 12 HRFI) were used, distributed in 3 litters of 4 pigs for each line, with two males and two females in each litter (to the exception of litter E). Half of the pigs were fasted while the other half was fed before sampling. **B.** Fasting procedure. Pigs were slotted into two pens with ab libitum feeding. Feed was removed from the two pens overnight, and reintroduced into one of the pens a few hours before sampling in the morning.

Results

Transcriptomic differences between the duodenum of high and low feed efficiency pig lines

To compare pigs from the 10th generation of selection, 12 animals for each line were taken from 3 litters in the same breeding strip trying to balance sex ratios (figure 1A). Around the 60th day of age after conventional weaning and post-weaning care, animals were removed from feed access around 5 p.m. the day before the sampling, while maintaining unrestricted access to water. At 8 a.m. the day of sampling, feed was reintroduced to 6 HRFI and 6 LRFI animals in a single pen (balancing litters and sex, figure 1B). Duodenal mucosa samples were then collected, between 2 to 3 hours after feed re-introduction, or between 12 to 14 hours of feed restriction.

Duodenal transcriptomes were obtained by RNA-sequencing after poly-A purification, enriching for messenger RNA. We detected 1106 genes differentially expressed between the LRFI and HRFI lines, independent of the feeding status of the animals (figure 2A). In detail, 464 genes were identified as upregulated in the LRFI line, and 642 genes were identified as up-regulated in the HRFI line.

Functional enrichment analysis reveals that genes upregulated in HRFI are notably involved in various aspects of cell division, including spindle checkpoint signalling, chromosome segregation, and DNA-templated DNA replication (figure 2B). Fewer processes were functionally enriched in genes upregulated in the LRFI lines (figure 2C), including glycosphingolipid metabolic process and transmembrane transporter activity.

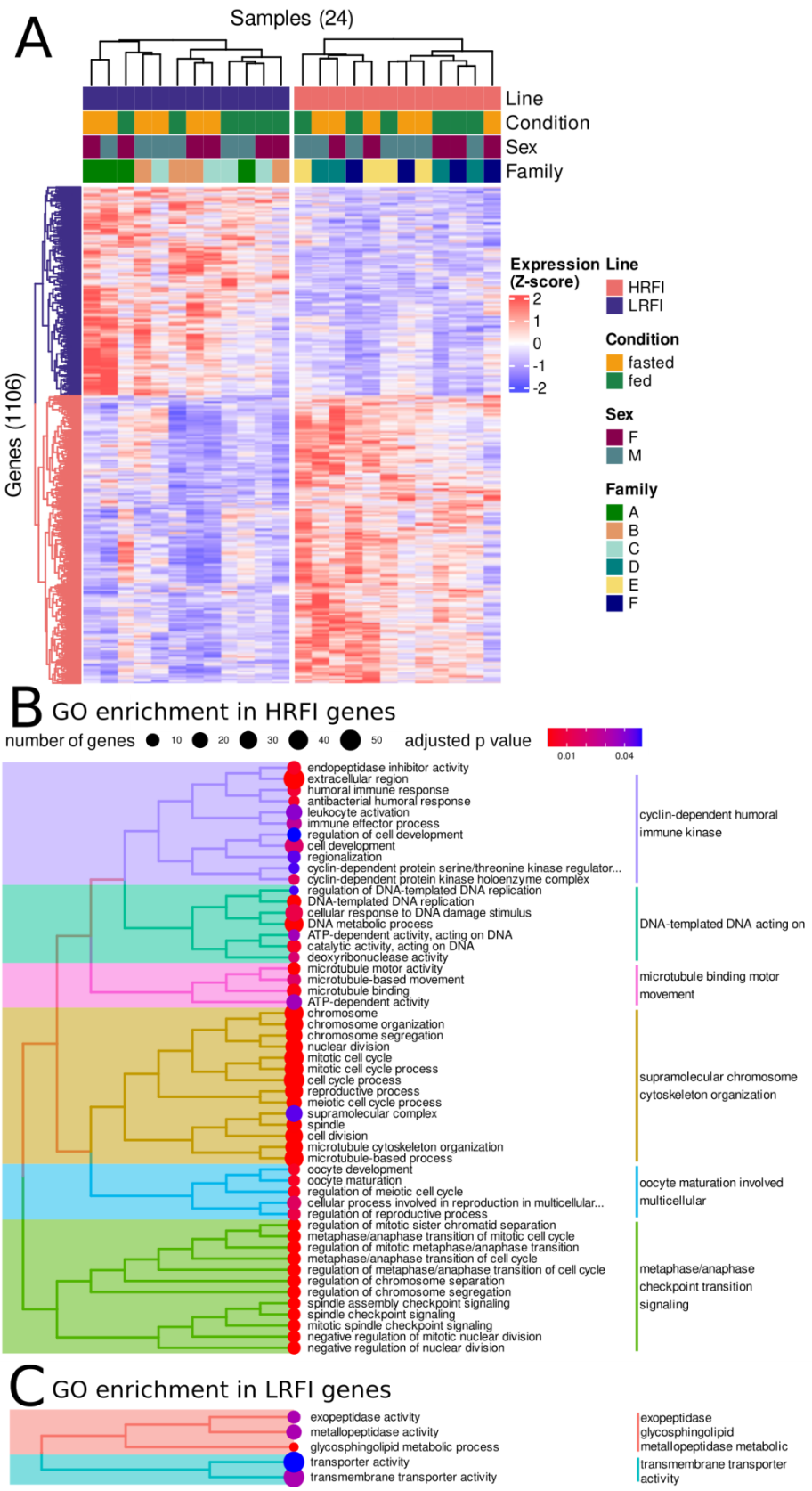

Figure 2: Differential gene expression between the duodenum of LRFI and HRFI lines. **A.** 1106 genes were detected differentially expressed between LRFI and HRFI, including 464 genes upregulated in LRFI (top) and 642 genes upregulated on the HRFI line (bottom). **B & C.** Gene Ontology - Biological Process (GO-BP) enrichment analysis. GO-BP terms statistically enriched in the 642 genes upregulated on the HRFI line (**B**) and the 464 genes upregulated in LRFI line (**C**) are displayed as a tree using jaccard similarity between each pair of GO-BP. GO-BP clustering is indicative, and cluster labels are the most frequent world in each GO-BO cluster.

The pig duodenum transcriptome response to feed intake is stronger in LRFI pigs than in HRFI pigs

For each line, we then compared duodenal transcriptomes before and after feed intake. We detected 2222 differentially expressed genes in the LRFI pig line, but only 61 differentially expressed genes in the HRFI line (figure 3A, B), leading to a total of 2225 genes affected by feed intake in one or both pig lines. Visualisation of expression profiles revealed that in the LRFI line, all 6 fasted pigs were showing stark differences of expression as compared to the 6 fed pigs in the differentially expressed genes, while 3 fasted HRFI pigs and 2 fed HRFI pigs did not have a transcriptomic signature matching the feeding status signature of the LRFI line (figure 3A).

In the LRFI line, the 1050 genes overexpressed in the fasted state were enriched in GO-BP categories linked to autophagy, cell junction and plasma membrane receptors, Wnt signalling pathway, and protein catabolic processes (figure 3C). The 1172 genes overexpressed in the fed state were enriched in GO-BP categories linked to protein folding, ribosome biogenesis and the sterol biosynthetic process (figure 3D). In the HRFI line, the 41 genes overexpressed in the fasted state were enriched in GO-BP categories linked to ubiquitin ligase complex, and with mitochondrial envelope to a lesser extent (figure 3E). No GO-BP category was detected as enriched in the 20 genes overexpressed in the fed states in HRFI pigs.

Among the genes impacted by the feeding status in one or both lines, 185 genes were also detected as differentially expressed between the two lines (figure 3B). Visual inspection of these genes revealed that they are mostly genes that respond to feed intake in the LRFI line, but not in the HRFI line, resulting in differences in average expression levels between the lines (figure 3F).

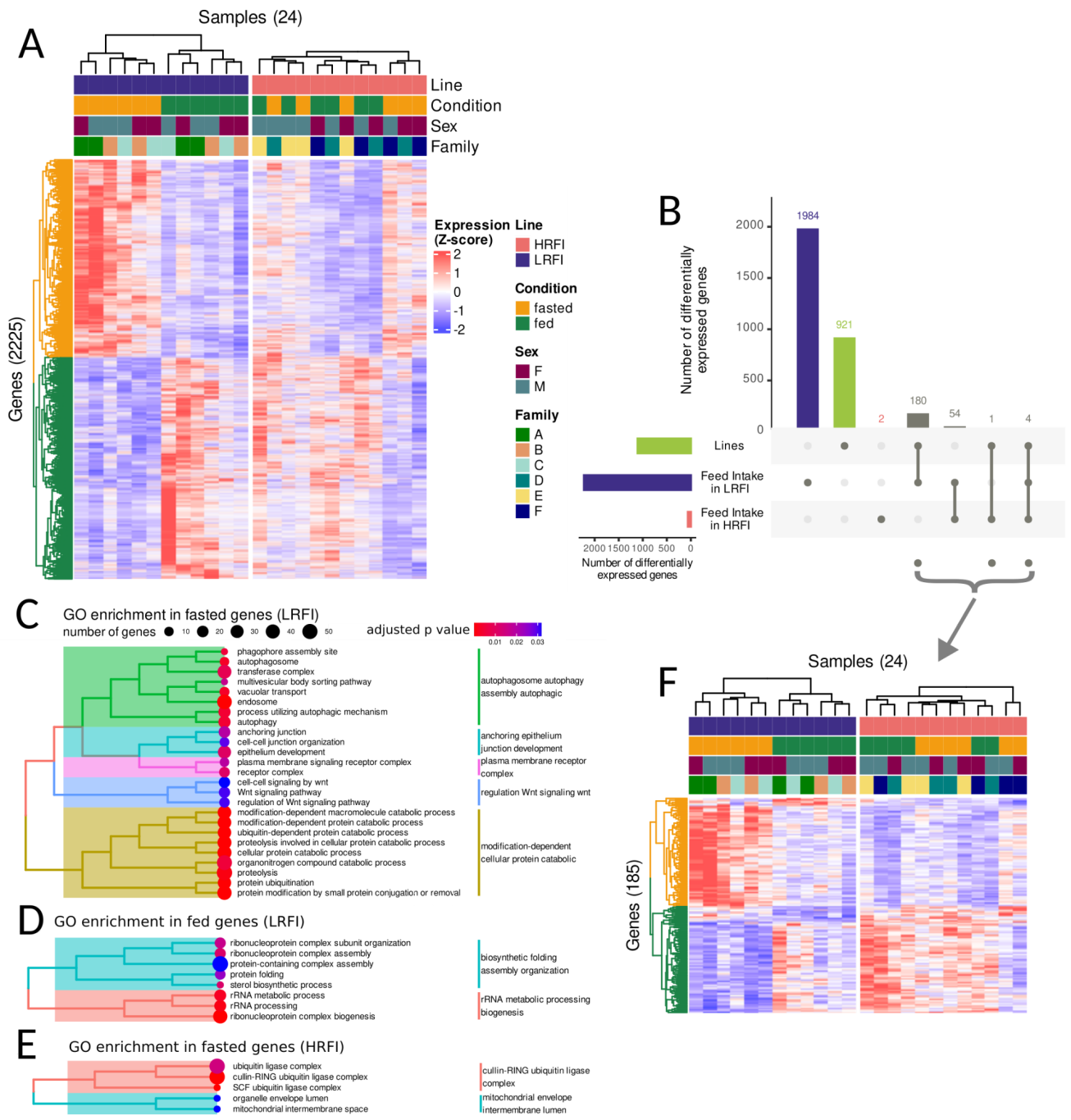

Figure 3: Duodenum transcriptomic response to feed intake. **A.** Gene expression heatmap of the 2225 genes differentially expressed by feed intake in LRFI (2222 genes), in HRFI (61 genes, including 58 genes also affected by feed intake in LRFI). **B.** Upset diagram showing the overlap between differentially expressed gene lists, between LRFI and HRFI (green), and due to feed intake in LRFI pigs (blue) or HRFI pigs (red). **C, D & E.** Gene Ontology - Biological Process (GO-BP) enrichment analysis. GO-BP terms statistically enriched in the 1050 genes upregulated upon fasting in the LRFI line (**C**), the 1172 genes upregulated in fed pigs from the LRFI line (**D**), and the 41 genes upregulated in fed pigs from the HRFI line (**E**) are displayed as a tree using jaccard similarity between each pair of GO-BP. GO-BP clustering is indicative, and cluster labels are the most frequent word in each GO-BO cluster. **F.** Gene expression heatmap of the 185 genes differentially expressed both by line and by feed intake in LRFI and/or HRFI pigs.

Duodenal expression of nutrient transporter

The “transmembrane transporter activity” ontology term is overrepresented in genes with higher expression in LRFI than in HRFI (figure 2C). The Solute Carrier (SLC) gene family encodes for transmembrane transporters, including some nutrient transporters present in the intestinal epithelium[17,18]. We therefore focused our analysis on the expression patterns of SLC genes in our experimental setup. In the duodenum mucosa, 28 SLC genes are differentially expressed between the LRFI and HRFI lines: 17 are more expressed in the LRFI line, such as the folate transporter *SLC25A32*, 11 are more expressed in the HRFI line such as the glucose and galactose transporter *SLC2A10*. 35 SLC genes are differentially expressed by short term feed intake in the duodenum of LRFI pigs: 18 are more expressed during short term fasting, such as monosaccharides transporters *SLC5A1* and *SLC2A2* and the aspartate and glutamate transporter *SLC25A13*, 17 are more expressed after feed intake, such as the glutamate transporter *SLC17A8* (figure 4). No SLC gene was detected as differentially expressed by feed intake in the duodenum of HRFI pig.

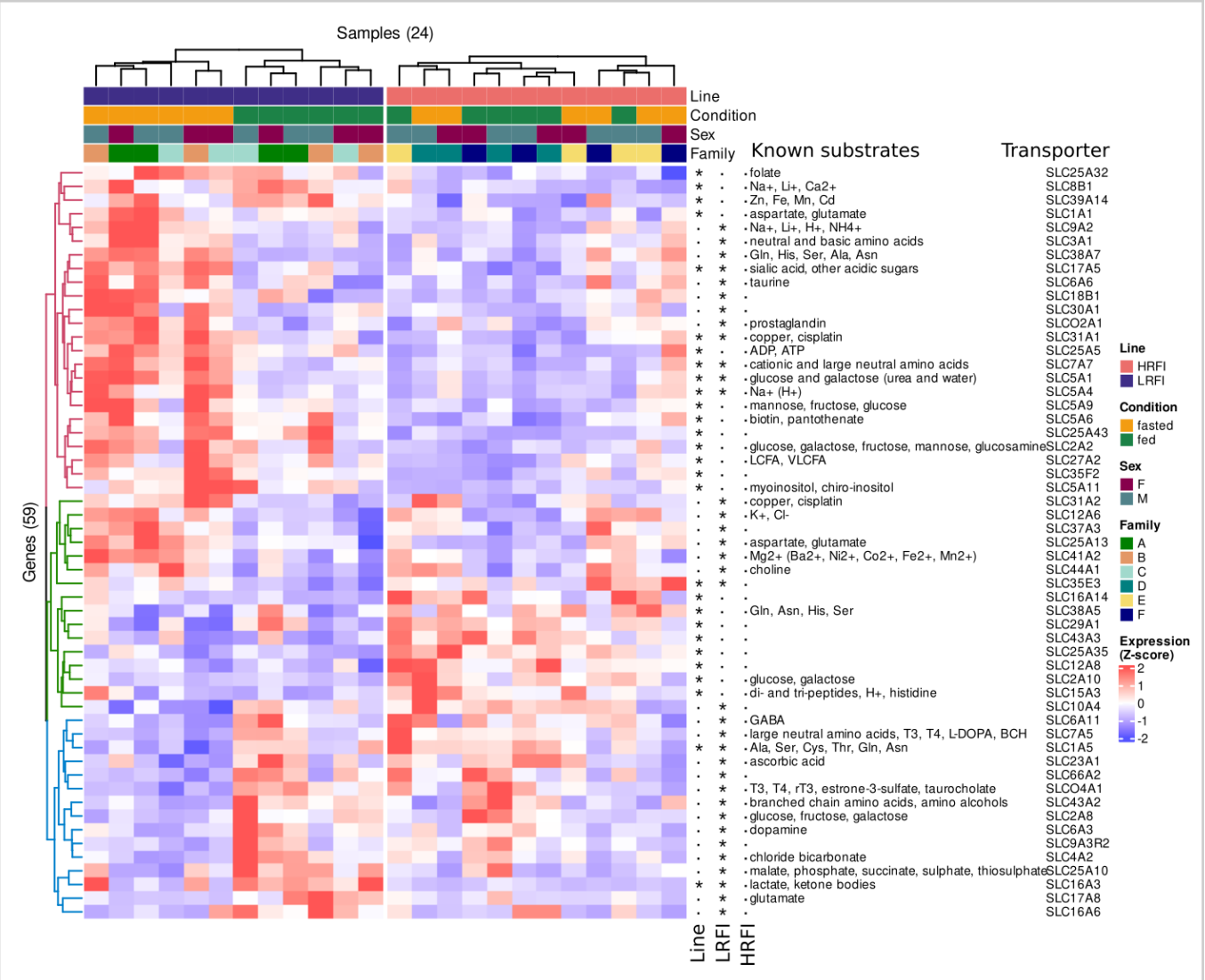

Figure 4: Gene expression heatmap of the 59 *SLC* genes that are differentially expressed either between the LRFI and HRFI lines, or by feed intake in each line. Transporter names and known substrates are noted on the right of the Heatmap. Three symbolic columns indicates if each transporter is significantly differentially expressed (marked with “*”) or not (marked with “·”) between the two lines (Line column), or by feed intake in the LRFI line (LRFI column), or by feed intake in the HRFI line (HRFI column).

Discussion

Here we demonstrated that the divergent selection on feed efficiency changed the duodenum mucosal transcriptome in pigs, identifying 1106 genes differentially expressed between the LRFI and HRFI lines. Genes overexpressed in LRFI were enriched in gene ontologies relevant to glycosphingolipid metabolic process, transmembrane transport, and exopeptidase and metabopeptidase activity. These functions do not directly mirror results from the transcriptome of muscle, liver and adipose tissues[5], or in blood, muscle and liver metabolism[6]. Therefore, it is likely that genetic differences due to selection have led to differences in gene expression that are distinct from tissues to tissues, and are not systematic. Functions enriched in HRFI pigs were overwhelmingly related to mitosis related processes, such as chromosome separation, DNA replication, and mitosis checkpoint. It is not known if it is due to issues with cell division in the HRFI line that could be frequently failing, or if it simply reflects a higher cell division rate in the duodenum mucosa of HRFI pigs. Histological examination of the duodenum slices from HRFI and LRFI pigs might be very informative, especially if coupled with measures of cell divisions. Due to the limited number of animals in this study, we did not investigate how genomic differences between the animals[4] could explain the line distinct duodenum transcriptomes. Expression QTL analyses would require more samples.

The LRFI line shows a very strong transcriptomic response to feed intake in its duodenum mucosa, with 2222 differentially expressed genes. This is in contrast to the weak response observed in HRFI pigs, with only 85 differentially expressed genes. It is not known if selection has increased the transcriptomic response in the LRFI line, suppressed the transcriptomic response in the HRFI line, or both at the same time. In mice, only a modest transcriptomic response was observed by Yoshioka et al.[14], but it might be due to a relatively poor sensitivity of the SAGE method. Gene expressed before feed intake were enriched in catabolic functions and autophagy, while genes expressed after feed intake were more anabolic.

We observed that several nutrient transporters had their expression level increase or decrease by feed intake in the LRFI line. We notably confirmed in pigs the previous observation of the downregulation of *Slc5a1* in fasted mice[14]. *SLC5A1* encodes for the glucose transporter SGLT1. Different glucose absorption dynamics between LRFI and HRFI lines might lead to different insulinemia, as it has been observed in the same pig lines[6,7]. More generally, it is tempting to hypothesise that the lack of transcriptomic response to feed intake in the duodenum of the HRFI line might explain some feeding behaviours observed in the HRFI line. For example, the higher frequency of feeder visit in the HRFI line might be partially explained by a reduced satiety regulatory loop.

While the transcriptomic response to feed intake was very different between LRFI and HRFI lines, we did not detect any *line x feeding* statistical interactions, neither using limma nor edgeR (data not shown). Our hypothesis is that detecting these statistical interactions would require more samples to gain statistical power.

The pig is thought to be a good model of human digestive physiology[27–29], due to its closest diet, diurnal rhythm and size when compared to rodent models. The dataset produced here might prove useful to better understand the transcriptomic response to feed intake of the human duodenum. Therefore our dataset is publicly available, at the sequencing read levels, in the form of gene expression tables, and in lists of differentially expressed genes. In addition, better understanding of the biological mechanisms of feed efficiency may contribute to improvements of feed efficiency in pig farming, leading to a more sustainable production.

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Acronyms

RFI: Residual Feed Intake
HRFI: Pig line selected for a High RFI (low feed efficiency)
LRFI: Pig line selected for a low RFI (high efficiency)
SAGE: Serial analysis of gene expression
SLC: Solute Carrier, a gene family of transmembrane nutrient transporter
TPM: Transcript per million, a normalised expression value.
GO-BP: Gene Ontology - Biological Process

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