Protein restriction differentially modifies liver transcriptome at different stages of the growing period of Duroc x Iberian crossbred pigs

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INTRODUCTION

Iberian pigs
- Fat depots → High quality meat and dry cured-products
- Low growth rates

Production based on → Duroc x Iberian crossbred
- Maintaining meat quality
- Increase growth rate

Production Rule establishes Slaughter age >10 months & Carcass weight > 115 kgs

⬇

Difficult achievement : animals raise 115 kgs at 8 months

Possible solution

Low protein diet during growing period → Decrease growth rate

INTRODUCTION

Changes in diets could alter gene expression

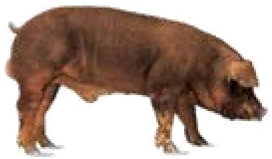
Identification of gene pathways and networks whose expression is modified

↳ To improve understanding of the **physiological** and **biochemical** processes involved in **growth** and **fat deposition**

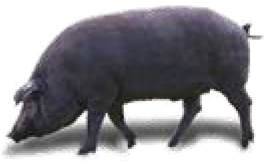
OBJECTIVE

To analyse the effect of a low protein diet in the **transcriptome** of **liver** of Duroc x Iberian crossbred pigs

MATERIAL AND METHODS



Duroc



Iberian

20 castrated males
with **differential isoenergetic diet** at 25 kg

Ad libitum feeding

Two slaughter batches
↗ 45 kg: 5 C vs 5 LP
↘ 90 kg : 5 C vs 5 LP

Diet composition	**Control (C)**	**Low protein (LP)**
Ingredients (%)		
Barley	30.00	44.37
Corn	20.00	20.00
Wheat	18.17	18.17
Wheat bran (23% starch)	5.00	5.00
Soy flour. 44% PC	21.20	6.50
Fat	1.60	1.60
L-Lys 50	0.24	0.05
Calcium Carbonate	1.28	1.22
Bicalcium Phosphate	1.00	1.20
Sodium chloride	0.40	0.40
Binder	1.00	1.00
Corrector Micro	0.30	0.30
Nutrients (%)		
Raw protein	16.5	11.5
Raw fat	3.8	3.7
Raw fibre	4.0	3.8
Lysine	0.83	0.58
Energetic content (kcal/kg)		
Metabolizing energy	3101	3095

MATERIAL AND METHODS

RNA isolation and sequencing

- RNA extraction from liver samples
- Pair-end libraries sequenced on Illumina Hi-Seq 2000: 5 samples per lane
- Quality assessed with **FastQC** and trimmed with **Trim-galore** (paired-end read pairs > 40 bp)
- Mapping against the pig reference genome (**Sscrofa11.1**) with **TopHat v2.1.0** through the alignment of the reads first to the **ENSEMBL (11.1)** transcriptome annotation
- Transcripts assembled and quantified in FPKM using **Cufflinks v2.2.1**
- Identification of novel isoforms with **Cuffcompare**

MATERIAL AND METHODS

Differential Expression Analysis, Gene Functional Classification and Network Analyses

- Differential expression with **Cuffdiff**
  - 45 kg: 5 C vs 5 LP
  - 90 kg: 5 C vs 5 LP
- Genes and novel isoforms filtered
  - >0.5 FPKM per group
  - >1.2 Fold change between C and LP
- R package ***q*-value** to adjust multiple testing
- GO enrichment with **FatiGO**
- Network analyses with **STRING**

RESULTS

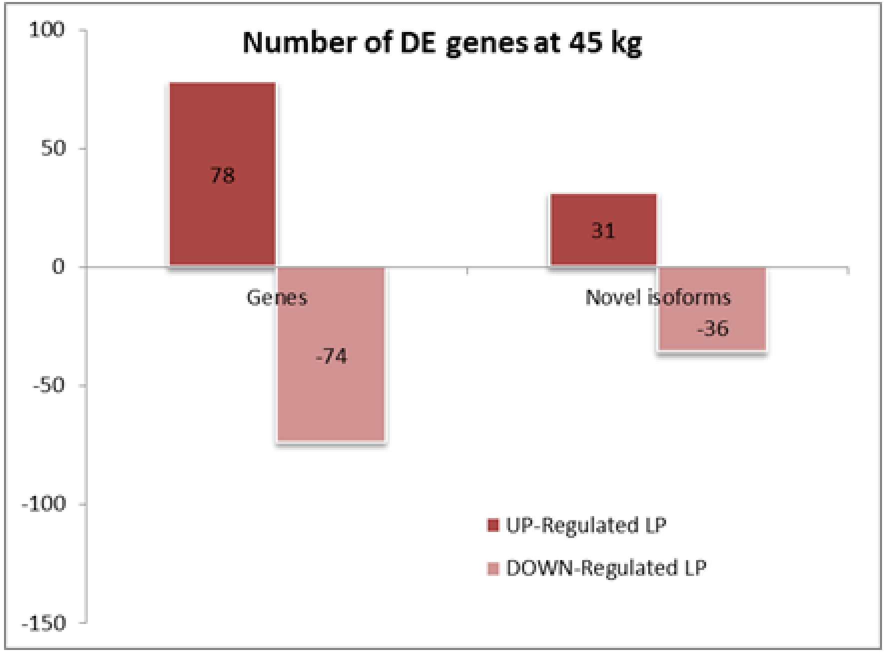
	45 kg	90 kg
Reads after trimming (millions)	828.93	898.15
Mapped against Sscrofa11.1 (%)	93.79	94.48
Complete match of intron (transcripts %)	6.83	8.21
Contained in the reference (transcripts %)	4.53	5.80
Potentially novel isoform (transcripts %)	37.58	38.69
Pre-mRNA (transcripts %)	3.51	3.39
Exon falling into an intron (transcripts %)	26.03	21.48
Generic overlap (transcripts %)	1.41	1.61
Polymerase fragment (transcripts %)	1.23	1.45
Intergenic fragment (transcripts %)	9.30	9.45
Exonic overlap with reference on opposite strand (transcripts %)	1.36	1.42
Multiple classifications (transcripts %)	8.23	8.50
Total number of transcripts	184,79	152,335

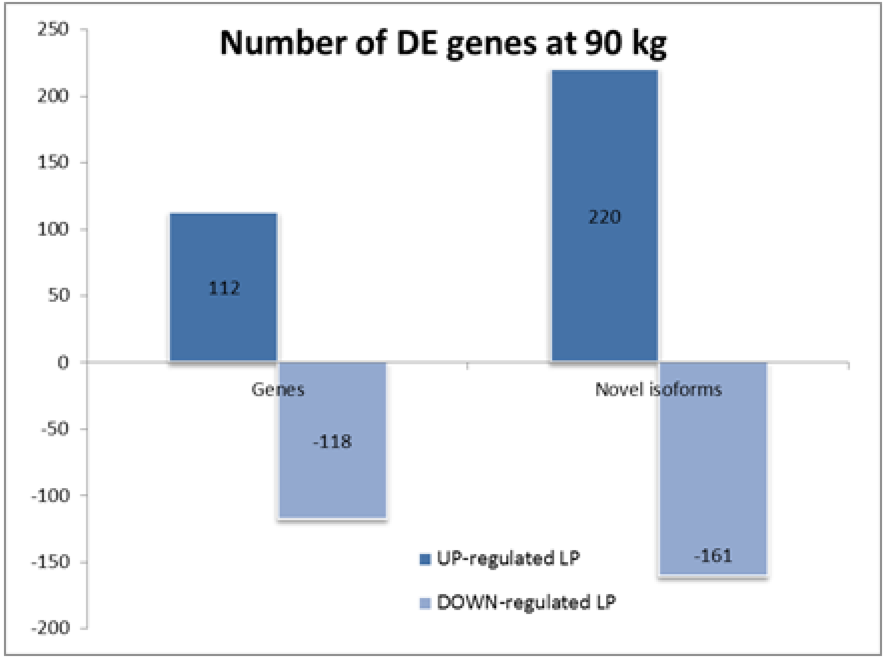
Higher number of transcripts at 45kg

RESULTS

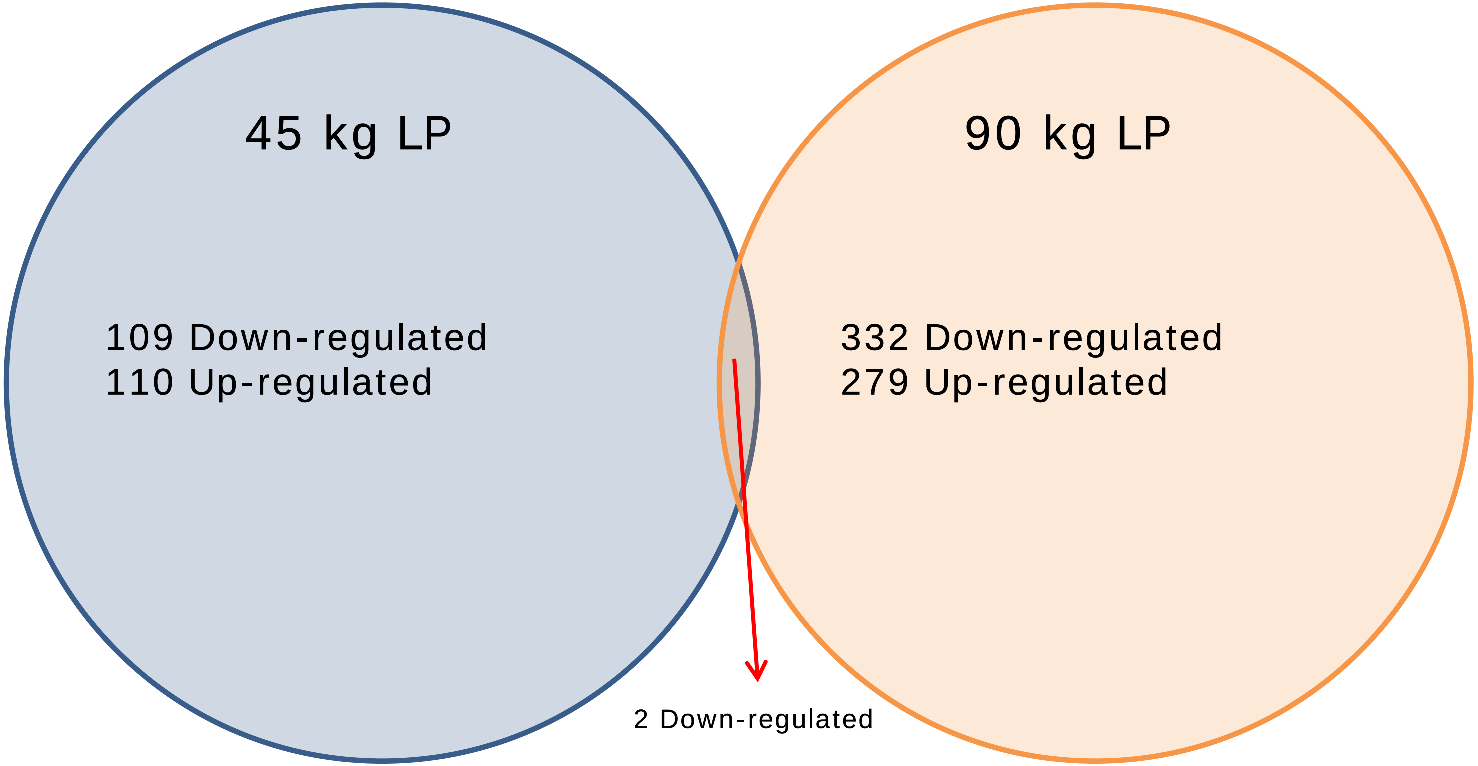
Number of differentially expressed genes or new isoforms (DE)

Filters: FPKM>0.5 in both groups, FC>1.2, *p-value*<0.05 & *q-value*<0.10





RESULTS



45 kg LP

109 Down-regulated
110 Up-regulated

90 kg LP

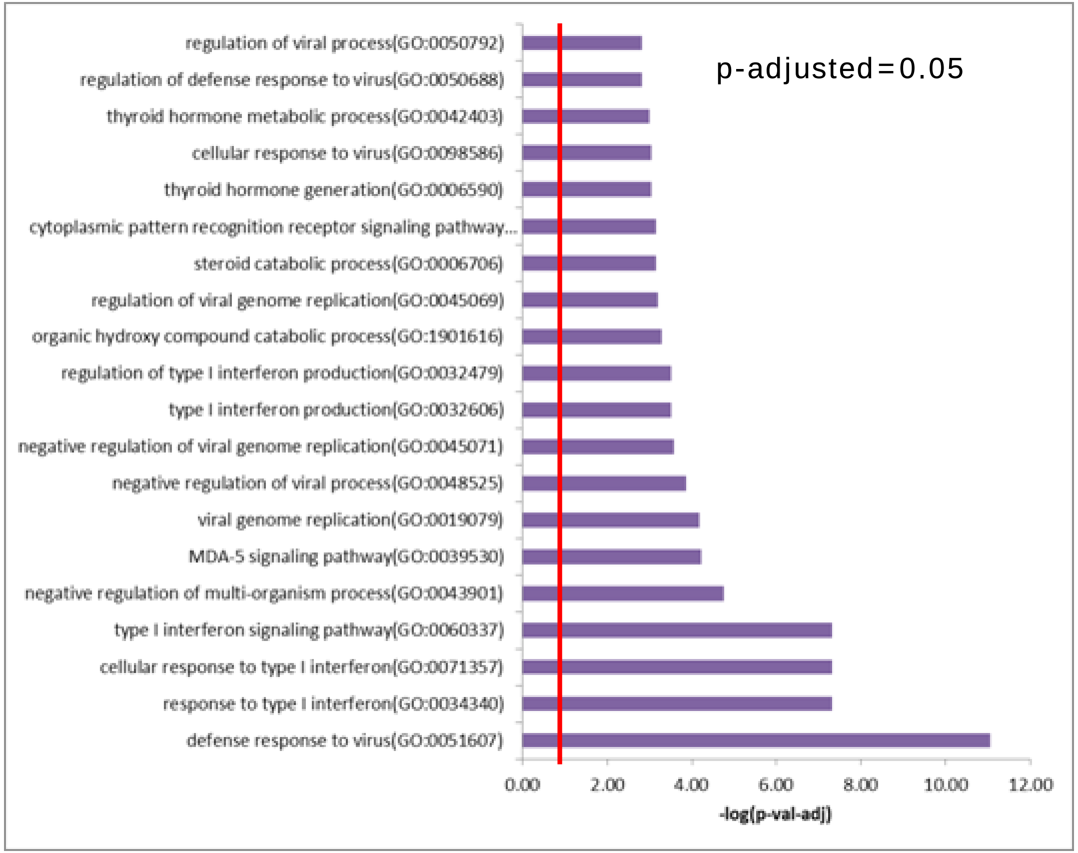
332 Down-regulated
279 Up-regulated

2 Down-regulated

Most of the DE genes/new isoforms are different at different ages

RESULTS

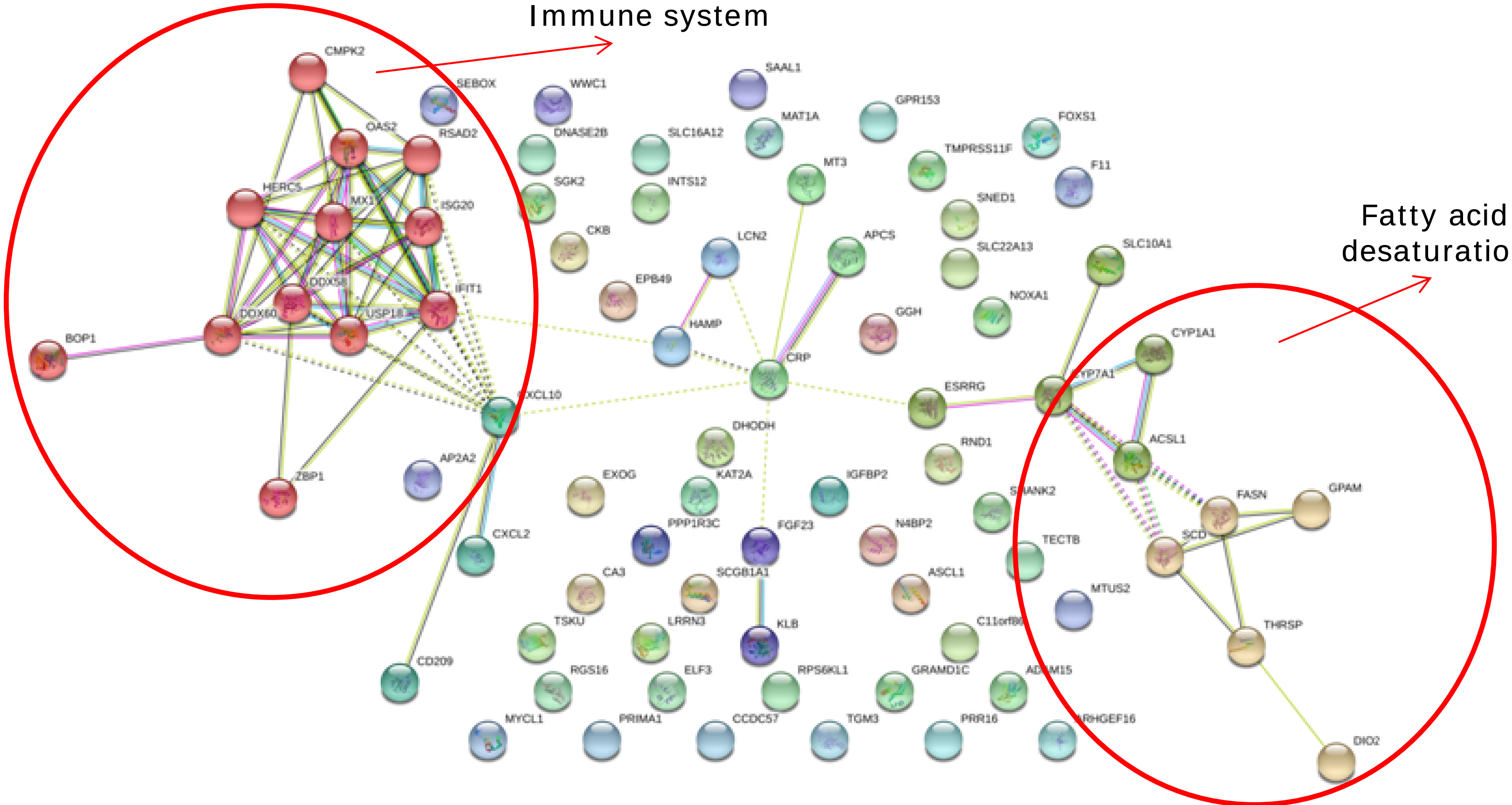
GO Enrichment Analyses at 45 kg



20 most significant GO_{BP} terms out of 46

RESULTS

Interaction Network Analyses at 45 kg

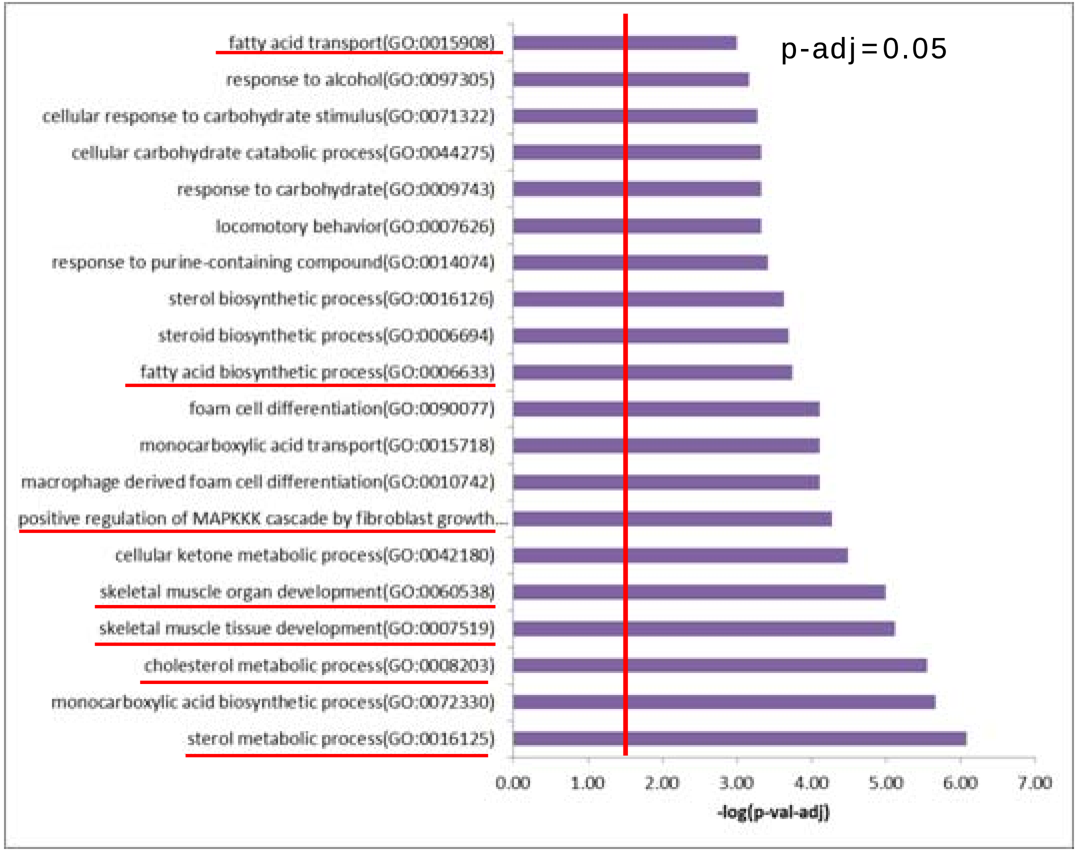


Immune system

Fatty acid desaturation

RESULTS

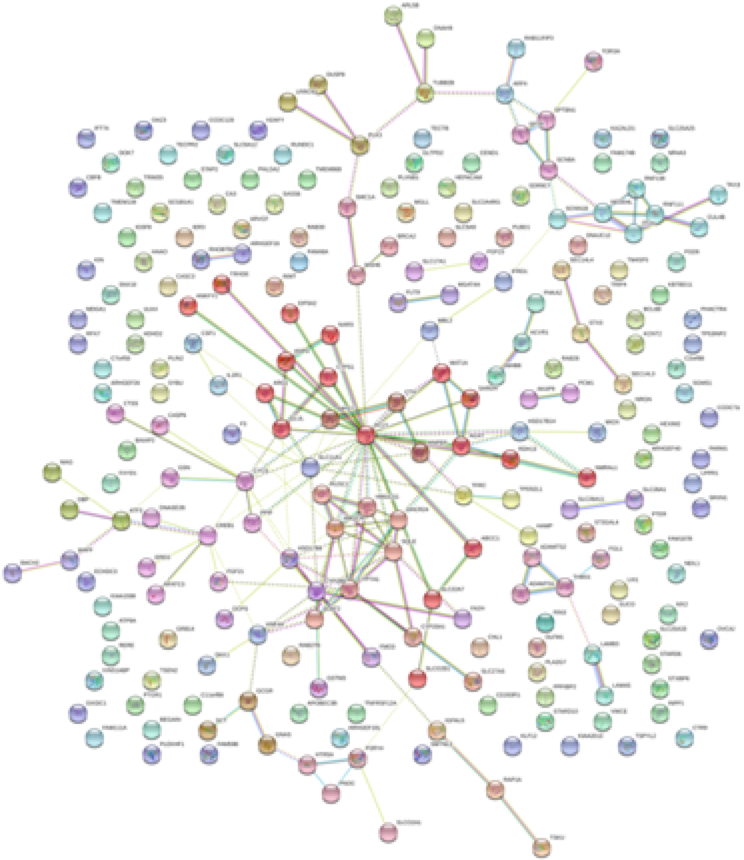
GO Enrichment Analyses at 90 kg

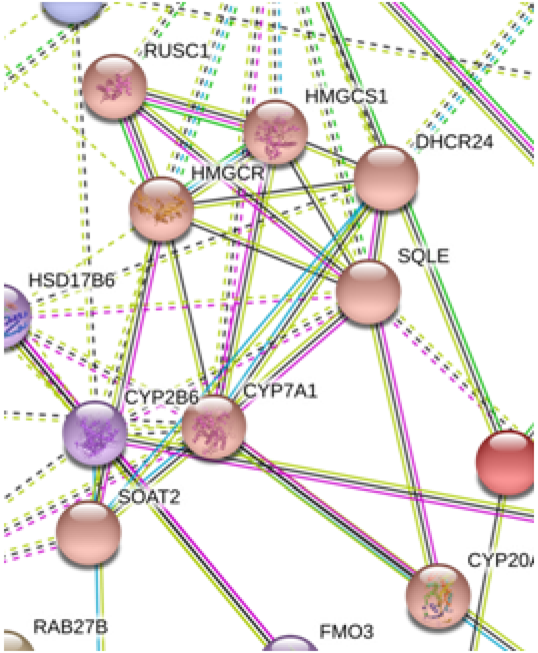


20 most significant GO_{BP} terms out of 134

RESULTS

Network Analyses at 90 kg



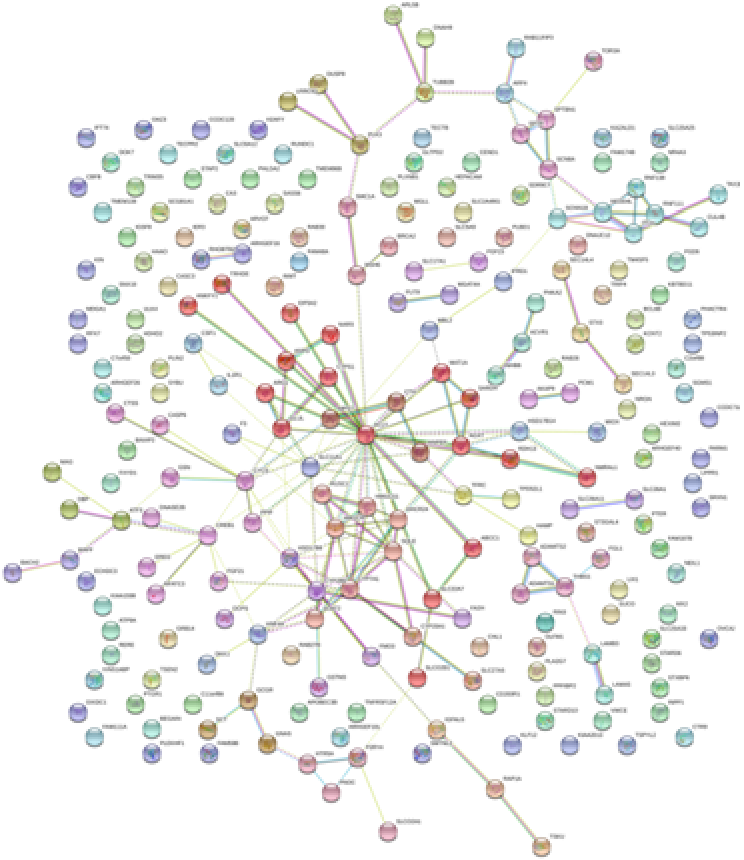


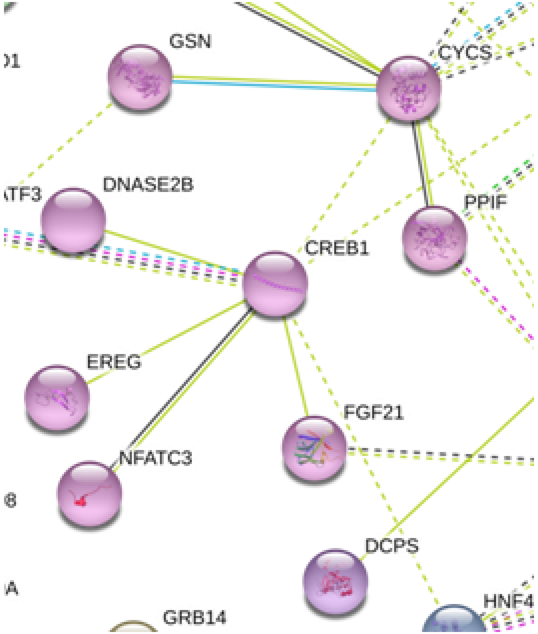
Steroid metabolism

Cholesterol metabolism

RESULTS

Network Analyses at 90 kg





fibroblast growth factor receptor signaling pathway

CONCLUSIONS

- Low protein diets during growing period modify gene expression in **liver** at 45 and 90 kg

- Most of the genes expressed and pathways altered by low protein diets are different at 45 and 90 kg

    - ➡ 45 kg: Pathways related with immune system and fatty acid desaturation
    - ➡ 90 kg: Pathways related with growth and cholesterol synthesis

- Search of **candidate genes** for **growth** and **fat** deposition based on **transcriptome** analyses should be **sequentially** carried out at **different growth** stages

ACKNOWLEDGMENT

Ibéricos Puros de Extremadura S.L.

Slaughter house: *Jamones y Paletas del Sur S.A. (Mazafra)*

Work funded by *RTA2013-00063-C03-01* grant

Thank you for your attention