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GERONIMO

Feed stress-induced transcriptomic changes in the liver of Krškopolje pigs reared in the outdoor system

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INTRODUCTION

Krškopolje pig – the only autochthonous breed in Slovenia

- reared in diverse production systems (extensive to intensive)
- low muscle and high fat deposition
- well adapted to local environmental conditions and (?) poor feed resources
- lower protein requirements

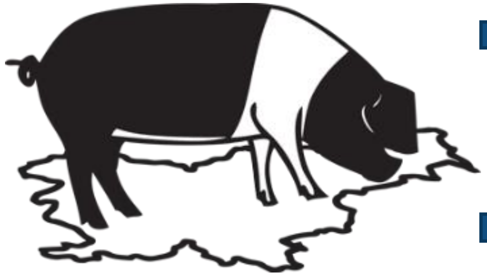
AIM OF THE STUDY

The present study aimed to evaluate the effect of feed stress on **liver** transcriptome of Krškopolje pig's reared in the outdoor production system.

The GEroNIMO project has received funding from European Union's Horizon 2020 research and innovation program under Grant Agreement No 101000236.

EXPERIMENTAL DESIGN

OUTDOOR SYSTEM



n = 20

Low protein diet (LP)[1]

(n = 10)

Standard protein diet (HP)[2]

(n = 10)

Isoenergetic diet

[1] 15% crude protein from 20 to 60 kg and 10% crude proteins from 60 kg to slaughter

[2] 15, 12.5 and 10% crude protein from 20 to 80 kg, from 80 to 100 kg and from 100 kg to slaughter, respectively

- Animals were slaughtered at the age of 330 ± 2 days (mean ± SE) and at the body weight of 166 ± 13.2 kg (mean ± SE)
- **LIVER** and adipose tissue were sampled for RNA extraction
- RNA-seq library was prepared - 10 samples per group
- Sequencing on Illumina NovaSeq 6000 (generation of 150 bp pair-end reads)
- Bioinformatic analysis (slaughter batch was included as a fixed effect)

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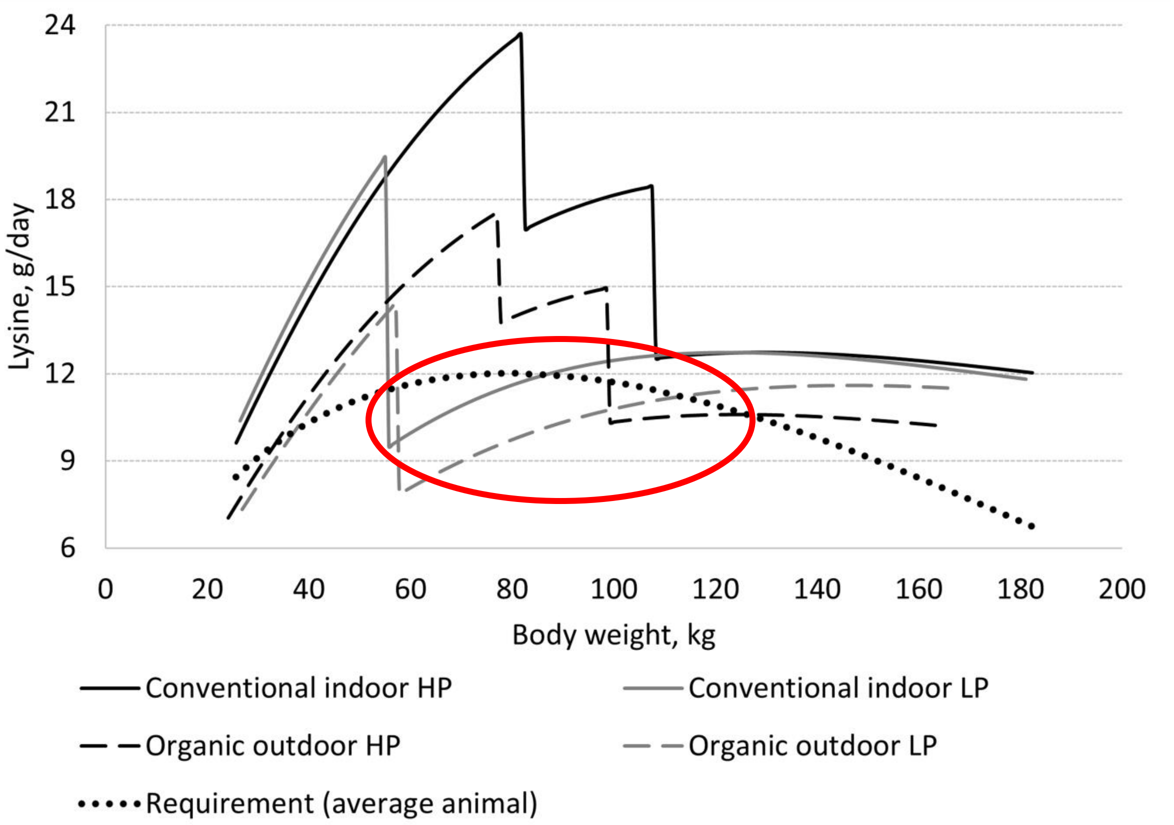
Previous results



A tendency to larger average backfat thickness in LP group.

* Modelling with INRAPorc®

Krškopolje pigs receiving the LP diet were deficient in lysine from 60 kg to 120 kg.

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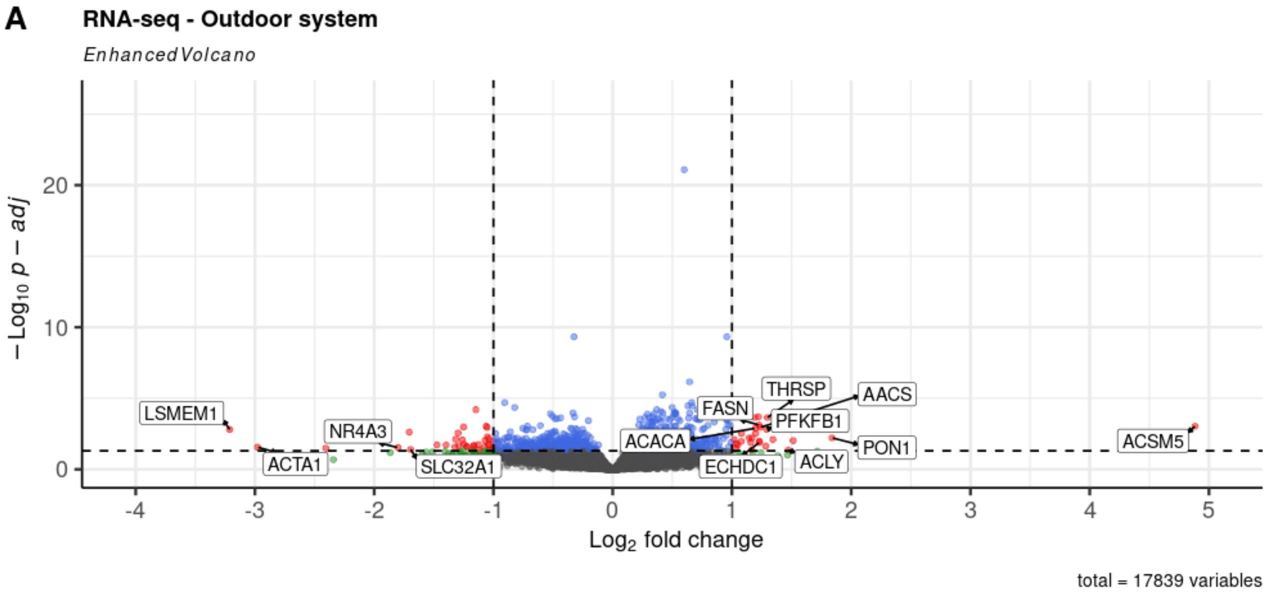
Previous results on adipose tissue

RNA-sequencing of subcutaneous adipose tissue (LP compared to HP diet)



Upregulated genes in LP group were involved in:

- lipogenesis
    - FASN, ACACA, ACLY
    - THRSP - energy storage
    - MOGAT2
- fatty acid beta oxidation (e.g., ECHDC1)
- response to oxidative stress (e.g., PON1, XDH)

Downregulated genes in LP group were involved in:

- adipogenesis (e.g., NR4A3) - regulate lipid and carbohydrate metabolism, reduce deposition of white fat
- negative regulation of oxidative stress (e.g., TRPV4)

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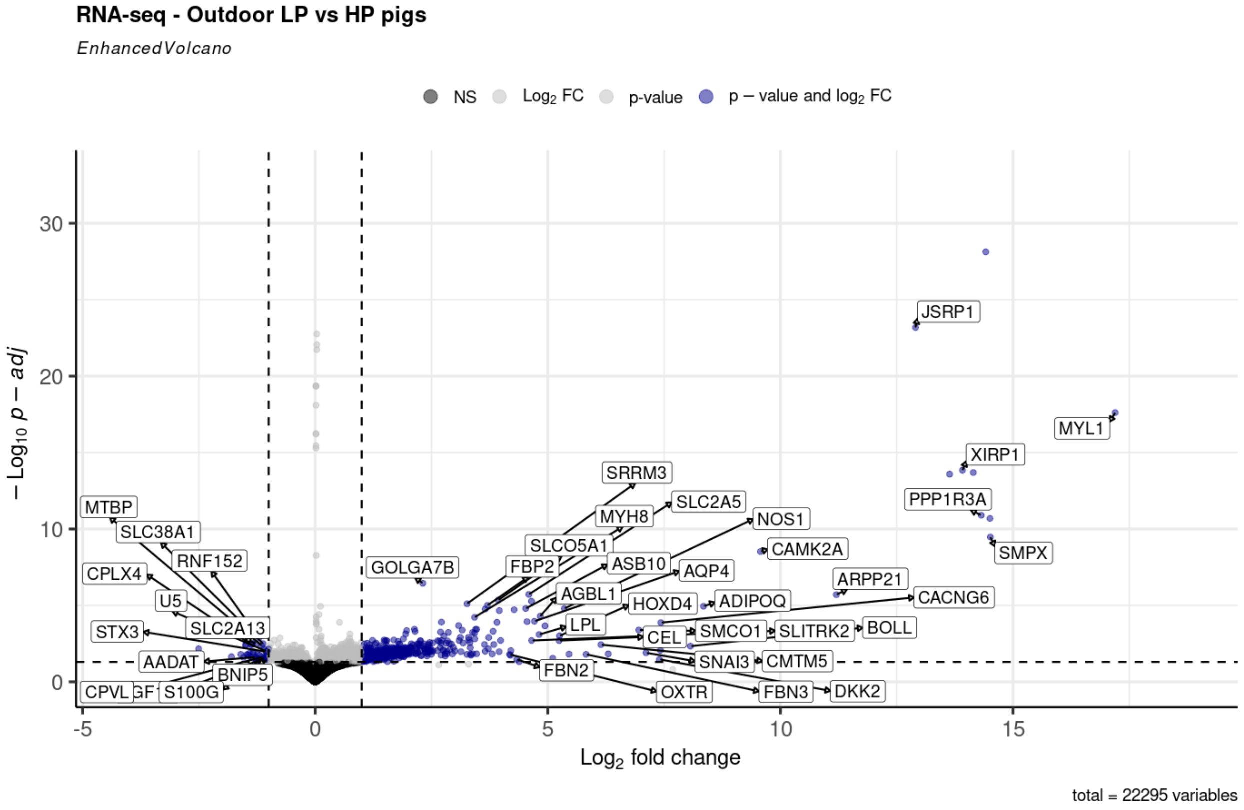
Differential gene expression analysis - Liver

643 upregulated and 41 downregulated genes in LP compared to HP group.

Upregulated genes in LP group were involved in:

- **lipolysis** (e.g., LIPE, PNPLA2, LPL, CEL, GPIHBP1).
- **heat shock proteins** (e.g., CRYAB, HSPB1, HSP70.2, DNAJB5)


RNA-seq - Outdoor LP vs HP pigs

EnhancedVolcano

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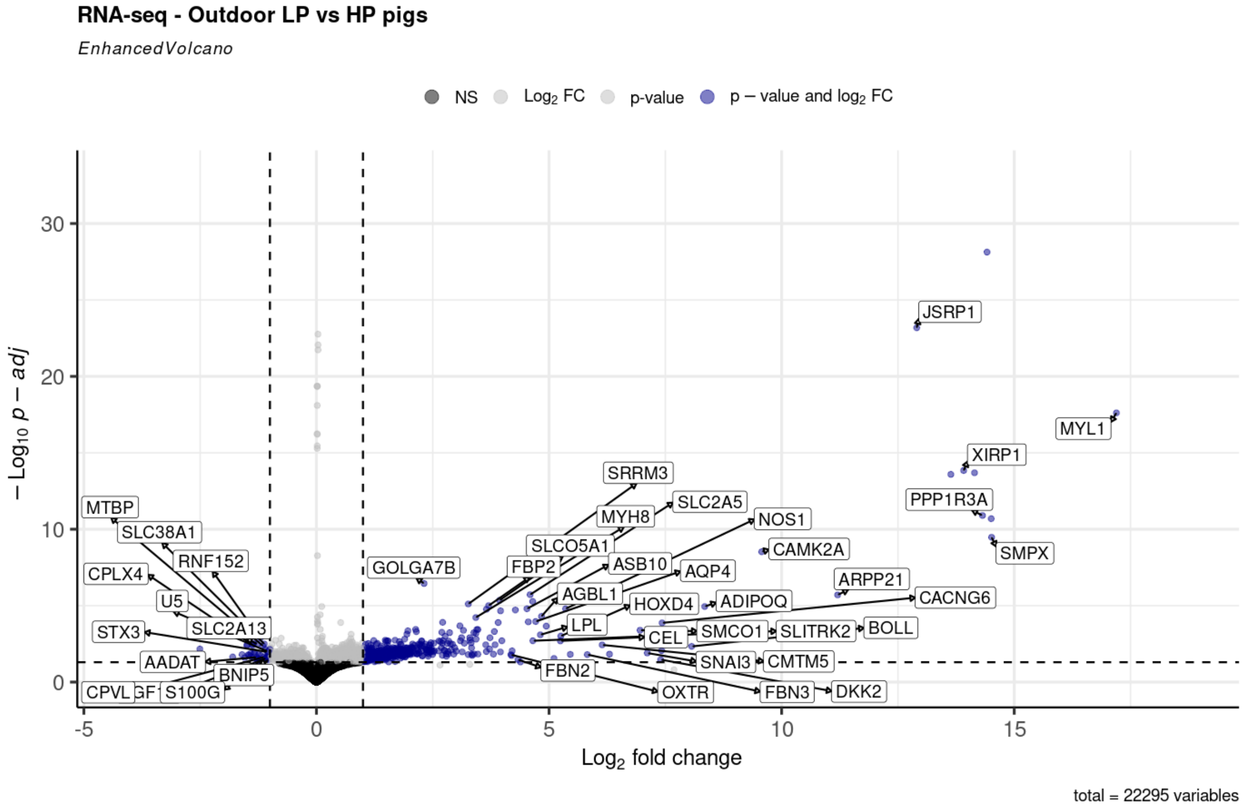
Differential gene expression analysis - LIVER

643 upregulated and 41 downregulated genes in LP compared to HP group.

Upregulated genes in LP group were involved in: - **regulation of glucose/energy homeostasis** and **lipid metabolism**

- PRKAG3 – an energy sensor protein kinase,
- PPAR – key regulator of adipocyte differentiation and glucose homeostasis,
- CPT1B – rate-controlling enzyme of the fatty acid beta-oxidation pathway,
- FITM1 – binding to triglyceride and diacylglycerol, and hydrolysis of fatty acyl-CoA,
- APOO – fatty acid transport,
- PPP1R1A – hormonal regulation of glycogen metabolism,
- GYS1 – glycogen synthesis


RNA-seq - Outdoor LP vs HP pigs

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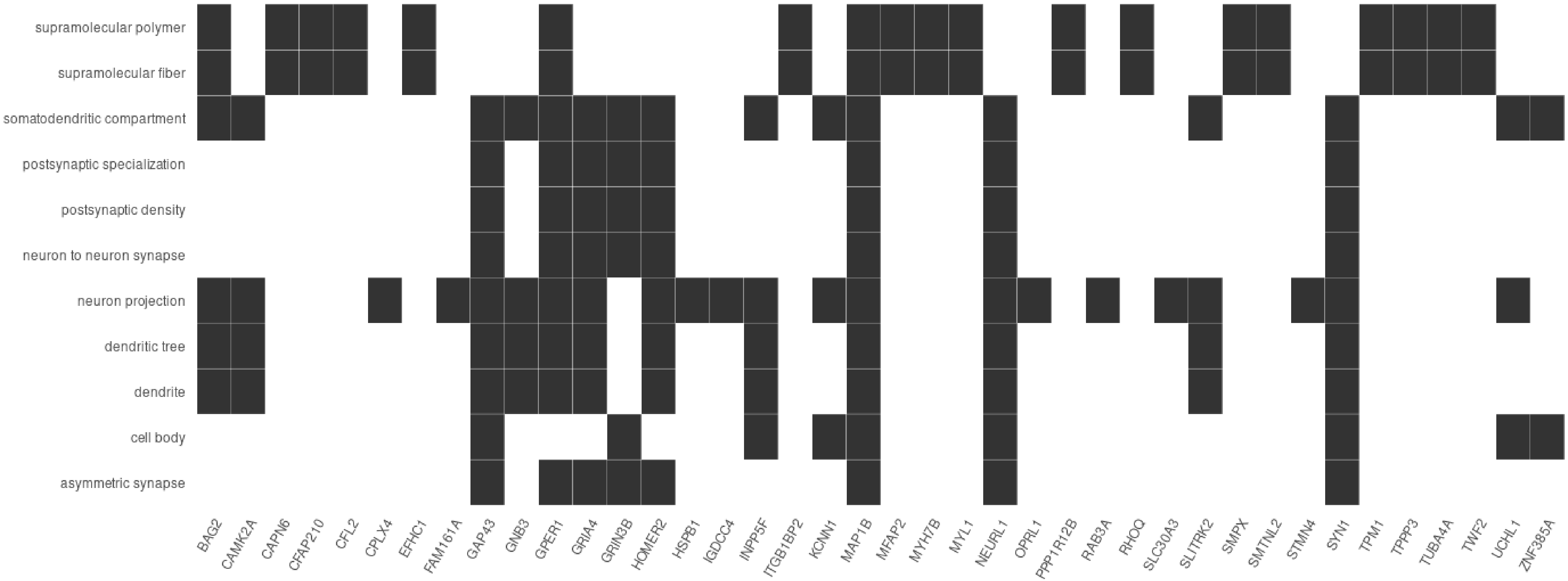
Gene ontology analysis



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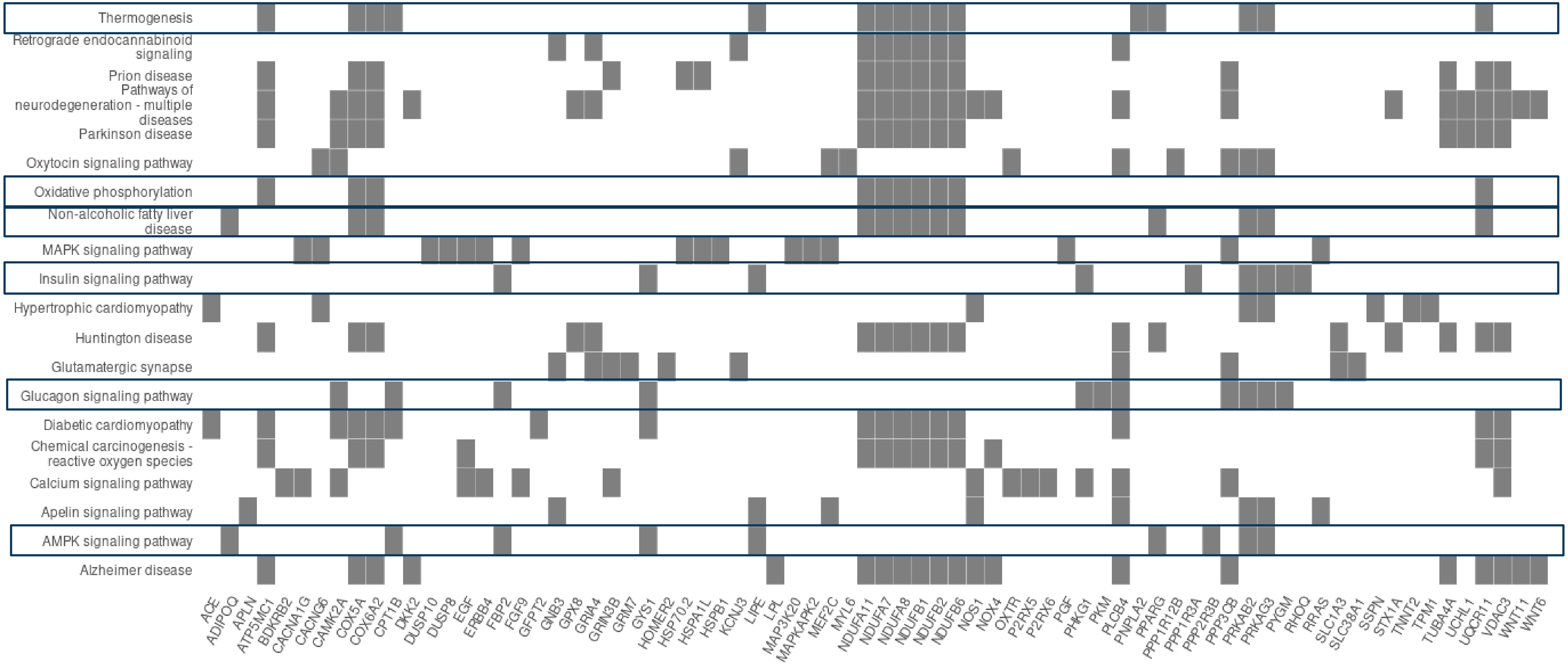
KEGG pathways



	ACE	ADIPOQ	APLN	ATP5MC1	BDKRB2	CACNA1G	CACNG6	CAMK2A	COX5A	COX6A2	CPT1B	DKK2	DUSP10	DUSP8	EGF	ERBB4	FBP2	FGF9
Thermogenesis				■					■	■	■							
Retrograde endocannabinoid signaling																		
Prion disease				■					■	■								
Pathways of neurodegeneration - multiple diseases				■				■	■	■		■						
Parkinson disease				■				■	■	■								
Oxytocin signaling pathway							■	■										
Oxidative phosphorylation				■					■	■								
Non-alcoholic fatty liver disease		■							■	■								
MAPK signaling pathway						■	■						■	■	■	■		■
Insulin signaling pathway																	■	
Hypertrophic cardiomyopathy	■						■											
Huntington disease				■					■	■								
Glutamatergic synapse																		
Glucagon signaling pathway								■			■						■	
Diabetic cardiomyopathy	■			■				■	■	■	■							
Chemical carcinogenesis - reactive oxygen species				■					■	■					■			
Calcium signaling pathway					■	■		■							■	■		■
Apelin signaling pathway			■															
AMPK signaling pathway		■									■						■	
Alzheimer disease				■					■	■		■						

	GFPT2	GNB3	GPX8	GRIA4	GRIN3B	GRM7	GYS1	HOMER2	HSP70.2	HSPA1L	HSPB1	KCNJ3	LIPE	LPL	MAP3K20	MAPKAPK2	MEF2C	MYL6
Thermogenesis													■					
Retrograde endocannabinoid signaling		■		■								■						
Prion disease					■				■	■								
Pathways of neurodegeneration - multiple diseases			■	■														
Parkinson disease																		
Oxytocin signaling pathway												■					■	■
Oxidative phosphorylation																		
Non-alcoholic fatty liver disease																		
MAPK signaling pathway									■	■	■				■	■	■	
Insulin signaling pathway							■						■					
Hypertrophic cardiomyopathy																		
Huntington disease			■	■														
Glutamatergic synapse		■		■	■	■		■				■						
Glucagon signaling pathway							■											
Diabetic cardiomyopathy	■						■											
Chemical carcinogenesis - reactive oxygen species																		
Calcium signaling pathway					■													
Apelin signaling pathway		■											■				■	
AMPK signaling pathway							■						■					
Alzheimer disease														■				

	NDUFA11	NDUFA7	NDUFA8	NDUFB1	NDUFB2	NDUFB6	NOS1	NOX4	OXTR	P2RX5	P2RX6	PGF	PHKG1	PKM	PLCB4	PNPLA2	PPARG	PPP1R12B	PPP1R3A
Thermogenesis	■	■	■	■	■	■										■	■		
Retrograde endocannabinoid signaling	■	■	■	■	■	■									■				
Prion disease	■	■	■	■	■	■													
Pathways of neurodegeneration - multiple diseases	■	■	■	■	■	■	■	■							■				
Parkinson disease	■	■	■	■	■	■													
Oxytocin signaling pathway									■						■			■	
Oxidative phosphorylation	■	■	■	■	■	■													
Non-alcoholic fatty liver disease	■	■	■	■	■	■											■		
MAPK signaling pathway												■							
Insulin signaling pathway													■						■
Hypertrophic cardiomyopathy							■												
Huntington disease	■	■	■	■	■	■									■		■		
Glutamatergic synapse															■				
Glucagon signaling pathway													■	■	■				
Diabetic cardiomyopathy	■	■	■	■	■	■									■				
Chemical carcinogenesis - reactive oxygen species	■	■	■	■	■	■		■											
Calcium signaling pathway							■		■	■	■		■		■				
Apelin signaling pathway							■								■				
AMPK signaling pathway																	■		
Alzheimer disease	■	■	■	■	■	■	■	■							■				

	PPP2R3B	PPP3CB	PRKAB2	PRKAG3	PYGM	RHOQ	RRAS	SLC1A3	SLC38A1	SSPN	STX1A	TNNT2	TPM1	TUBA4A	UCHL1	UQCR11	VDAC3	WNT11	WNT6
Thermogenesis			■	■												■			
Retrograde endocannabinoid signaling																			
Prion disease		■												■		■	■		
Pathways of neurodegeneration - multiple diseases		■									■			■	■	■	■	■	■
Parkinson disease														■	■	■	■		
Oxytocin signaling pathway		■	■	■															
Oxidative phosphorylation																■			
Non-alcoholic fatty liver disease			■	■												■			
MAPK signaling pathway		■					■												
Insulin signaling pathway			■	■	■	■													
Hypertrophic cardiomyopathy			■	■						■		■	■						
Huntington disease								■			■			■		■	■		
Glutamatergic synapse		■						■	■										
Glucagon signaling pathway		■	■	■	■														
Diabetic cardiomyopathy																■	■		
Chemical carcinogenesis - reactive oxygen species																■	■		
Calcium signaling pathway		■															■		
Apelin signaling pathway			■	■			■												
AMPK signaling pathway	■		■	■															
Alzheimer disease		■												■		■	■	■	■

Conclusions

Protein restriction affects the expression of liver genes involved in:

- glucagon, insulin signalling pathways
- fatty acid oxidation and transport
- regulation of energy homeostasis

Feed and environmental stress

Potential risks of LP diet?

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