

Glycemic index in finishing diet of Iberian pigs and its effect on muscle transcriptome

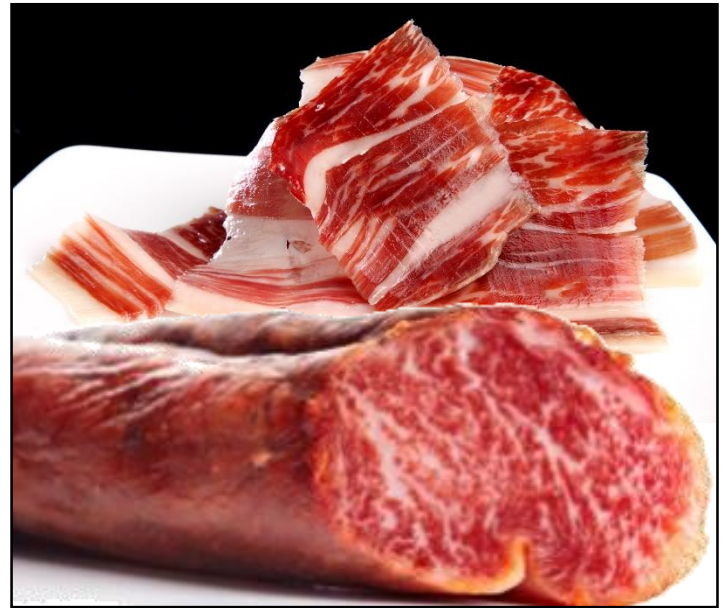
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IBERIAN & DUROC CROSSBREEDING



CROSSBREEDING

PRODUCTIVITY

FERTILITY ↑ ↓

WEIGHT GAIN ↑ ↓

FINAL PRODUCTS

QUALITY

INTRAMUSCULAR FAT (IMF)
QUANTITY & HOMOGENEITY

Nutritional strategies to improve IMF content and homogeneity

GENE EXPRESSION MAY BE MODIFIED BY DIET COMPOSITION



Animal Feed Science and Technology

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Meat Science












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Effect of dietary betaine supplementation on lipogenic enzyme activities and fatty acid synthase mRNA expression in finishing pigs

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Effects of dietary fat saturation on fatty acid composition and gene transcription in different tissues of Iberian pigs

R. Benítez ^a , Y. Núñez ^a , A. Fernández ^a , B. Isabel ^b , A.I. Fernández ^a , C. Rodríguez ^a , C. Barragán ^a , P. Martín-Palomino ^c , C. López-Bote ^b , L. Silló ^a , C. Óvilo ^a 

nature publishing group

ARTICLES
GENETICS

Sex-differential Expression of Metabolism-related Genes in Response to a High-fat Diet

Teresa Priego^{1,2}, Juana Sánchez^{1,2}, Catalina Picó^{1,2} and Andreu Palou^{1,2}

OBESITY | VOLUME 16 NUMBER 4 | APRIL 2008

NUTRIGENOMICS APPROACH → PIGOMIC Project
EFFECT OF GLYCEMIC INDEX (GI) MODIFICATION ON PHENOTYPE & TRANSCRIPTOME

EXPERIMENTAL DESIGN & DATA ANALYSIS

Experimental design

- 200 iberian crossbred pigs
- **FATTENING DIETS:**
 - Isoproteic + Isoenergetic
 - **Low** and **High** Glycemic Index (GI)

Nutrient (%)	Low GI	High GI
Starch	45.439	48.030
Sugar + Starch	47.906	51.576
Brute Fibre	5.042	3.959
Glycemic Index	13.000	16.000

Phenotypic analysis

Measures

- Growing
- Weight / Yield: Carcass, Premium cuts
- Meat composition (LD)
- IMF content

Sampled muscles (IMF)

- Ham
- *Quadriceps* (Quad)
- *Biceps femoris* (BF)
- *Semimembranosus* (SM)
- *Longissimus dorsi* (LD)

Statistics

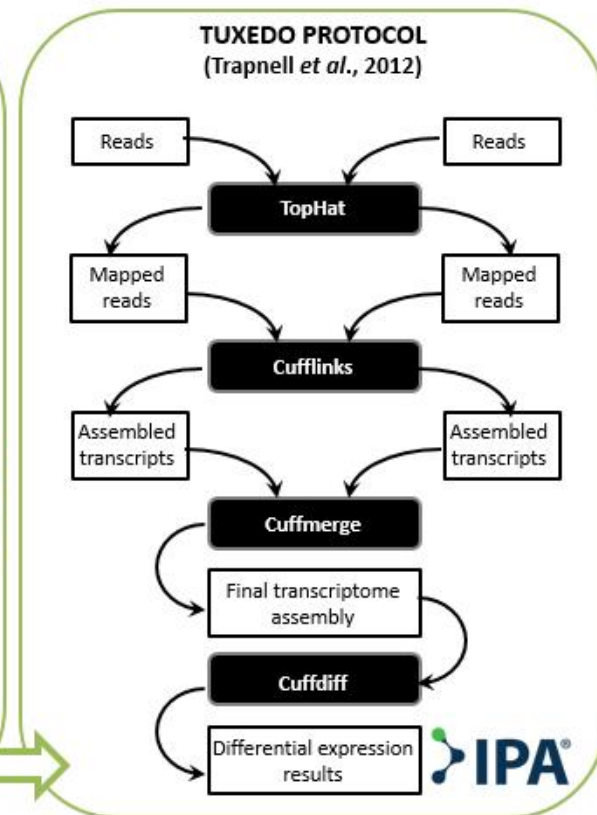
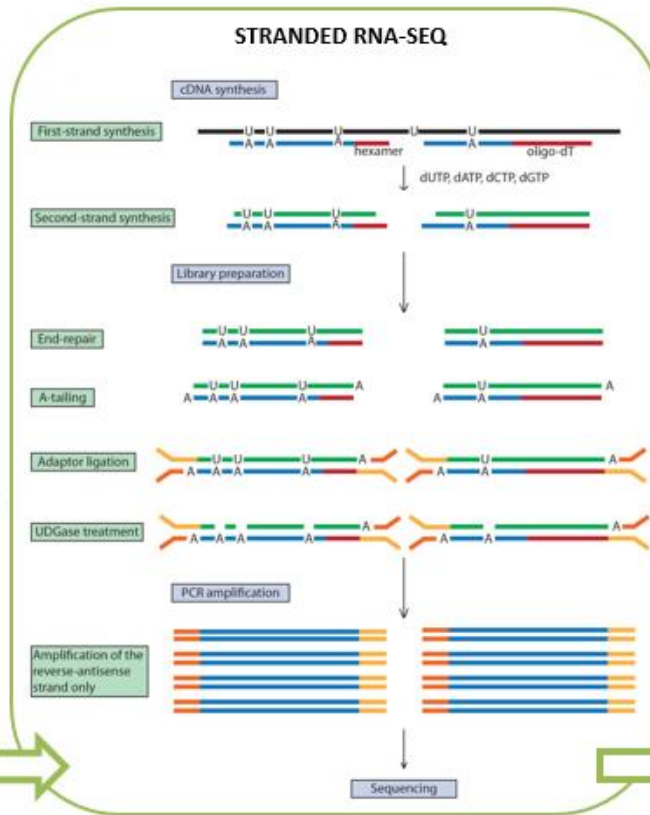
- R environment
- 2-WAY ANOVA
- Post-Hoc Tukey test

LINEAR MODEL: $y \sim D + S + W + I(D, S) + e$

D = diet (L, H); **S** = sex (M, F); **W** = pre-fattening weight (kg)

RNA-seq PROTOCOL

Sample: *Biceps femoris* $\left\{ \begin{array}{l} \text{L group: 12 animals (6 } \text{♂} + 6 \text{ ♀)} \\ \text{H group: 12 animals (6 } \text{♂} + 6 \text{ ♀)} \end{array} \right.$



REFERENCE GENOME: *Sscrofa 11.1*



PHENOTYPIC ANALYSIS

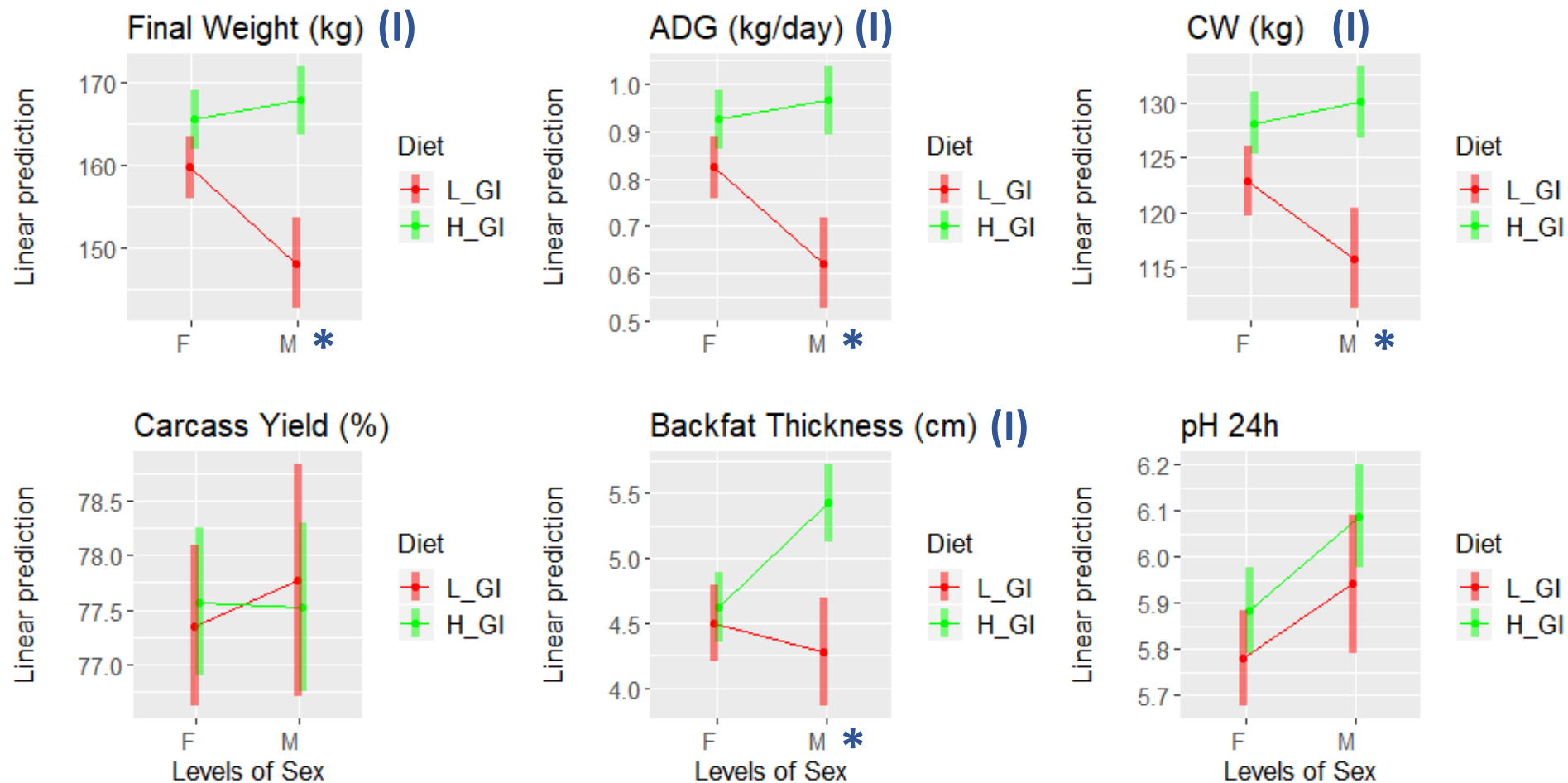
2-way ANOVA: Means for diet groups (L, H), standard error mean (SEM) and ANOVA p-value for diet effect in measured traits (W.=Weight; Yd.=Yield; Th.=Thickness).

Trait	L_GI	H_GI	SEM	p	Trait	L_GI	H_GI	SEM	p
Final W. (kg) *	157.03	164.69	1.17	0.000	pH 1h	6.20	6.16	0.03	0.510
ADG (kg/day) *	0.72	0.95	0.02	0.000	pH 5h	5.88	5.93	0.03	0.419
CW (kg) *	121.47	127.67	0.94	0.000	pH 24h *	5.81	5.99	0.03	0.001
Carcass Yd (%)	77.61	77.49	0.18	0.732	LD Collagen	0.72	0.74	0.02	0.686
Backfat Th. (cm) *	4.44	4.91	0.09	0.002	LD Moisture	69.65	69.41	0.20	0.572
Ham W. (kg) *	14.31	14.78	0.10	0.006	LD Protein	21.35	20.99	0.10	0.088
Shoulder W. (kg)	9.84	9.95	0.06	0.309	LD IMF	6.99	7.52	0.26	0.311
Loin W. (kg)	2.65	2.61	0.03	0.407	SM IMF	4.58	4.97	0.21	0.327
Ham Yd. (%)	23.59	23.15	0.13	0.064	BF IMF	5.51	5.33	0.28	0.758
Shoulder Yd. (%) *	16.24	15.59	0.10	0.001	Quad IMF	2.97	3.17	0.10	0.332
Loin Yd. (%) *	4.38	4.09	0.05	0.004	Ham IMF	4.39	4.49	0.19	0.799

Blue: Significant interaction between Sex and Diet effects.

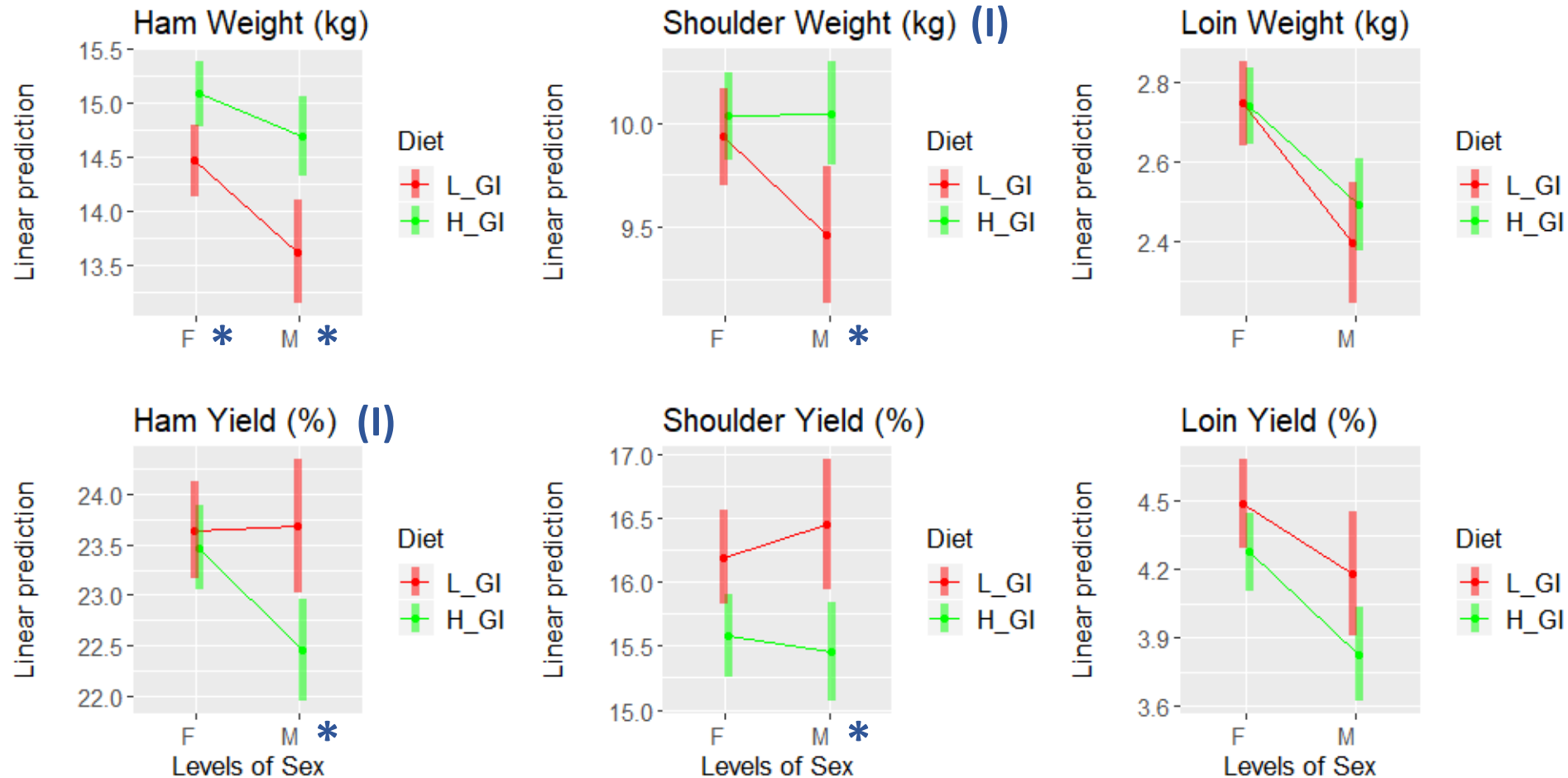
** $p < 0.05$*

SEX-DIET INTERACTIONS & POST-HOC TESTS



Post-hoc test: Tukey method for comparing a family of 4 estimates; $\alpha = 0.05$
(I) = Significant sex-diet interaction; * $p < 0.05$

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RNA-seq ANALYSIS RESULTS

Number of paired reads per sample = 37M

Overall mapping % of paired reads = 96 %

43 differentially expressed (D.E.) genes found (FDR < 0.10; FC > 1.5)

	D.E. genes (FC rank)		
	T	L	H
Males	4	2 (~ 2.7x)	2 (3x -5x)
Females	39	25 (2x - 21x)	14 (2x - 80x)
TOTAL	43	27	16

*Number of D.E. genes (and Fold Change ranks) when comparing males and females from L and H diets. **T**: total D.E. genes; **L**: D.E. genes **overexpressed** in **low GI** diet; **H**: D.E. genes **overexpressed** in **high GI** diet.*

RNA-seq: D.E. GENES (FEMALES & MALES)

D.E. genes up-regulated in low GI diet; F: D.E. in females group; M: D.E. in males group.

ID	Sex	FC	Entrez Gene Name
ACTC1	F	4.022	<i>actin, alpha, cardiac muscle 1</i>
AMPD3	F	2.333	<i>adenosine monophosphate deaminase 3</i>
ANKRD1	F	3.245	<i>ankyrin repeat domain 1</i>
AQP3	F	2.313	<i>aquaporin 3 (Gill blood group)</i>
CDKN1A	F	2.751	<i>cyclin dependent kinase inhibitor 1A</i>
CHI3L1	F	3.392	<i>chitinase 3 like 1</i>
CRABP2	F	2.433	<i>cellular retinoic acid binding protein 2</i>
CSRP3	F	5.213	<i>cysteine and glycine rich protein 3</i>
FAM83G	F	3.048	<i>family with sequence similarity 83 member G</i>
FOXO6	F	2.466	<i>forkhead box O6</i>
HMOX1	F	2.299	<i>heme oxygenase 1</i>
HOXD1	F	5.067	<i>homeobox D1</i>
HOXD8	F	2.439	<i>homeobox D8</i>
HOXD9	F	3.067	<i>homeobox D9</i>

ID	Sex	FC	Entrez Gene Name
KLHL40	F	2.667	<i>kelch like family member 40</i>
LHX6	F	3.776	<i>LIM homeobox 6</i>
MREG	F	3.516	<i>melanoregulin</i>
MUSK	F	2.342	<i>muscle associated receptor tyrosine kinase</i>
NYX	F	2.402	<i>nyctalopin</i>
PEG10	F	2.375	<i>paternally expressed 10</i>
PFKFB3	F	2.495	<i>6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3</i>
SLC7A5	F	2.535	<i>solute carrier family 7 member 5</i>
SLPI	F	20.735	<i>secretory leukocyte peptidase inhibitor</i>
SNORD12	F	6.873	<i>small nucleolar RNA, C/D box 12</i>
SRXN1	F	2.652	<i>sulfiredoxin 1</i>
APLN	M	2.661	<i>apelin</i>
OLFM1	M	2.817	<i>olfactomedin 1</i>

RNA-seq: D.E. GENES (FEMALES & MALES)

D.E. genes up-regulated in **high GI** diet; **F**: D.E. in **females** group; **M**: D.E. in **males** group.

ID	Sex	FC	Entrez Gene Name
AQP4	F	2.813	<i>aquaporin 4</i>
B3GALT1	F	2.341	<i>beta-1,3-galactosyltransferase 1</i>
CAV2	F	2.338	<i>caveolin 2</i>
CCDC82	F	2.415	<i>coiled-coil domain containing 82</i>
GOLGA4	F	2.362	<i>golgin A4</i>
KITLG	F	2.504	<i>KIT ligand</i>
MICU3	F	2.315	<i>mitochondrial calcium uptake family member 3</i>
NFKBIZ	F	2.509	<i>NFKB inhibitor zeta</i>
PTGFR	F	2.586	<i>prostaglandin F receptor</i>
SLC4A7	F	2.462	<i>solute carrier family 4 member 7</i>
SNORD107	F	80.393	<i>small nucleolar RNA, C/D box 107</i>
SPART	F	2.422	<i>spartin</i>
TEX9	F	2.828	<i>testis expressed 9</i>
TFRC	F	2.435	<i>transferrin receptor</i>
SPP1	M	5.311	<i>secreted phosphoprotein 1</i>
TNNT2	M	3.195	<i>troponin T2, cardiac type</i>

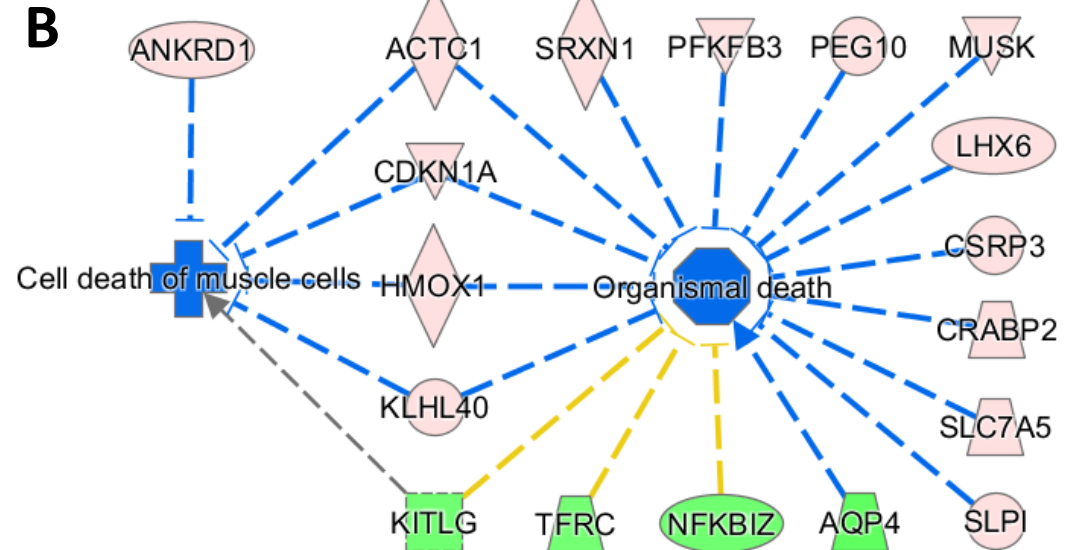
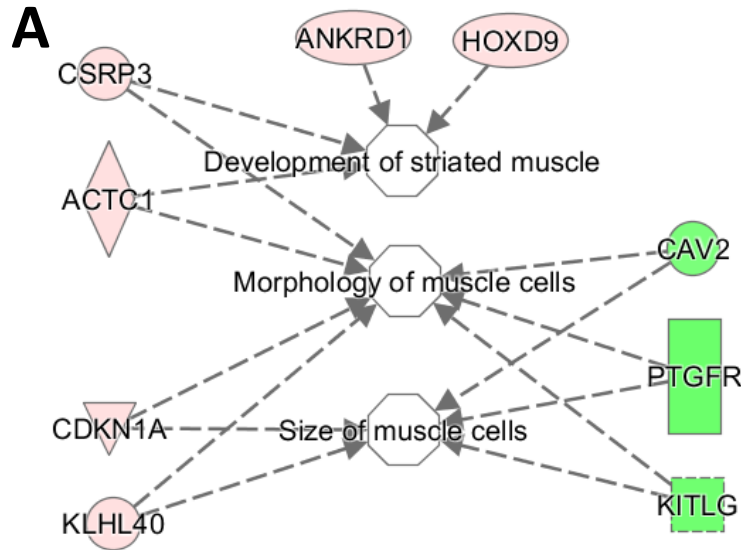
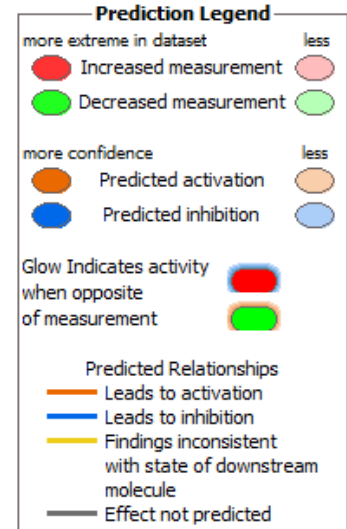
RNA-seq: RELATED BIOLOGICAL FUNCTIONS

Top Diseases and Bio Functions

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cell Morphology	1,53E-02 - 6,44E-06	19
Cell Death and Survival	1,40E-02 - 3,85E-05	17
Cellular Development	1,53E-02 - 9,60E-05	17
Cellular Growth and Proliferation	1,53E-02 - 9,60E-05	14
Cellular Function and Maintenance	1,28E-02 - 2,29E-04	18






1 2 3 4 5 8 7 8 9 >



IPA function networks: A) Skeletal and muscular system development and function; B) Organismal and muscle cell death.

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1 2 3 4 5 8 7 8 9 >

Other functional categories

p-value range

Molecules

Carbohydrate metabolism

1.03E-02 – 1.53E-02

4

↳ ↓AQP3, ↓PFKFB3, ↓SLPI, ↑SLC4A7

Lipid metabolism

2.57E-03 – 1.28E-02

3

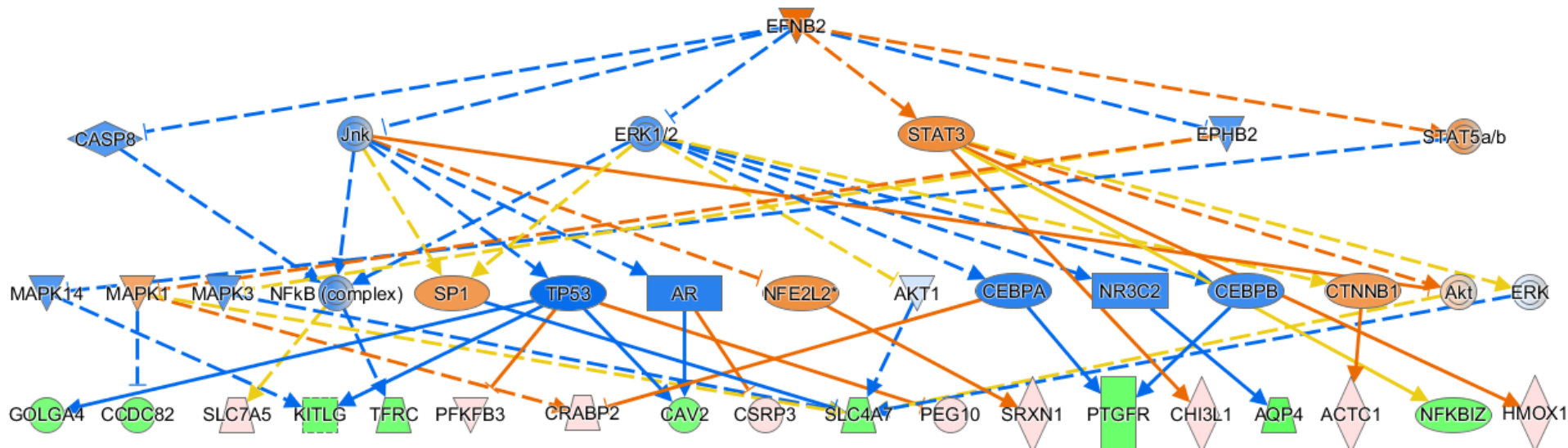
↳ ↓SLPI, ↑B3GALT1, ↑KITLG

↓: Up-regulated in **L_GI**; ↑: Up-regulated in **H_GI**.

RNA-seq: TOP CAUSAL NETWORKS

Causal networks for D.E. genes in **females** with significant prediction of **activation** (z-score > 2).

Master regulator	Genes	p-value	Master regulator participation in biological processes
EFNB2	18 (22)	3.38E-06	<i>anatomical structure morphogenesis; angiogenesis; cell adhesion, differentiation, migration & proliferation; cell-cell signaling</i>
SOD2	17 (13)	6.53E-06	<i>cell proliferation; regulation of blood pressure; response to DNA damage, cellular & oxidative stress; response to nutrient & ion</i>
FANCG	14 (11)	3.76E-06	<i>cell cycle; DNA repair; mitochondrion organization; response to DNA damage stimulus</i>
ATF4	4 (1)	7.79E-05	<i>apoptosis; cellular response to aminoacid & glucose starvation; circadian regulation of gene expression; gluconeogenesis</i>



REMARKS

- Phenotypic analysis:

- Significant **differences** between fattening **diets** for animal and carcass **weight, ADG** and **premium cut yield**.
- **Interaction** between **sex** and **diet** present for some traits.

- RNA-seq analysis:

- Evidence of **muscle transcriptome modification** when changing **GI** in fattening diet.
- Huge variations on **gene differential expression** between **sexes**, pointing to a strong interaction between sex and diet.
- Found **D.E. genes** related with interesting **biological functions**:
 - Muscular development
 - Muscular cell growth / survival
 - Lipid / carbohydrate metabolism



THANK YOU FOR YOUR ATTENTION!