Hepatic molecular changes induced by a high-fat high-fiber diet in growing pigs

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Increasing demand for feed supply and food production

Thinking to the incorporation of new ingredients in animal diets

Partial substitution of cereals
by co-products (rich in fibers)

Contribution of fibers to dietary energy supply is minor in non-ruminants such as pigs

Adding crude fat to fiber diets may be recommended to restore the dietary energy content

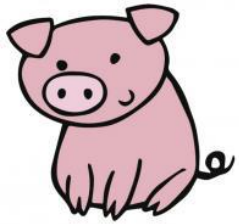
Feed energy

Carbohydrates (starch from cereals) ➡ Lipids (oils)

Fibers may interact with dietary fat on body composition and tissue metabolisms *(pigs: Yan et al., 2013)*

Liver plays a central role in energy metabolism, with additional roles in inflammation, immunity, health, etc.

Many hepatic genes encoding key enzymes in glycogenesis, *de novo* fatty acid synthesis and oxidation, were regulated in chicken fed of a high-fat high-fiber diet *vs.* low-fat low-fiber diet *(Désert et al. 2018)*



Liver is not the primary site of *de novo* fatty acid synthesis in pigs

Defining the changes in hepatic molecular pathways in pigs fed diets with contrasting sources of energy and nutrients

Experimental design

Large White barrows

Diets fed ad lib. during 10 wks (n= 24 pigs/diet)
(a grower diet during the first 7 wks, then a finisher diet)

Diet LF

Wheat, barley & corn starch

Diet HF

Crushed wheat straw – 11%
Oils (rapeseed & soybean) – 7%

Composition of grower diets

Diets	LF	HF
ME (MJ/kg)	**12.9**	**12.9**
NE (MJ/kg)	9.6	9.8
Ingredients, %		
Crude proteins	**17.0**	**17.0**
Fat	**2.1**	**7.2**
Starch	46	33
NDF	12.0	19.0
ADF	3.6	8.2

Pigs killed at 132 d of age => Liver, adipose tissues and the loin muscle were sampled for molecular & biochemical analyses

Results: Performance during the test period

	Diets			
	HF	**LF**	**RSD**	***P*-value**
ADG, g/d	770 ↘	907	73	<0.001
ADFI, kg/d	2.2 ↘	2.3	0.1	0.004
End BW, kg	70.9 ↘	80.2	5.5	<0.001
Liver, kg **Liver , % BW**	**1.6** ↘ **2.1** ↘	**1.7** **2.3**	**0.1** **0.2**	**0.03** **0.09**
Perirenal fat, % BW	0.6 ↘	0.8	0.1	<0.001
Backfat, % CW	5.9 ↘	7.3	0.9	<0.001
Loin, % CW	**28.8** ↗	**27.8**	**0.73**	**<0.001**

Abbreviations used: ADG: average daily gain; ADFI: average daily feed intake; BW: body weight; CW: chilled carcass weight

Results: Number of differentially-expressed genes

Transcriptomic analyses

(porcine microarray, 60 K, Agilent)

Biological pathways (DAVID tool & IPA®)

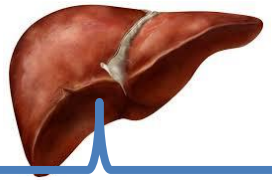
Differentially-expressed (DE) probes and genes *($p < 0.01$; $FC > |1.1|$)*

Up-regulated probes		
Diets	**HF**	**LF**
Liver	**721** (355 genes)	**950** (451 genes)
Perirenal fat	**3 313**	**5 820**
Loin muscle	**504**	**844**

Pivotal regulators (Influence graph analysis of gene networks; *Blavy et al. 2014)*

Gene expression related to phenotypes (Partial Least Square)

Results: Biological pathways



Up-regulated by **HF** diet

Protein AA phosphorylation (29 DEG)

Serine/threonine kinase signaling pathway (11 DEG)

Cell cycle phase (16 DEG)

Cell growth (10 DEG)

Cell death (24 DEG)

Cell adhesion (25 DEG)

Regulation of catabolic process (7 DEG)

Response to organic substances (23 DEG)

Up-regulated by **LF** diet

Glucose & glycogen metabolic process (13 DEG)

Generation of precursor metabolites & energy (17 DEG)

Response to nutrients (9 DEG)

Phosphorylation (30 DEG)

Translation/regulation of translation (19 DEG)

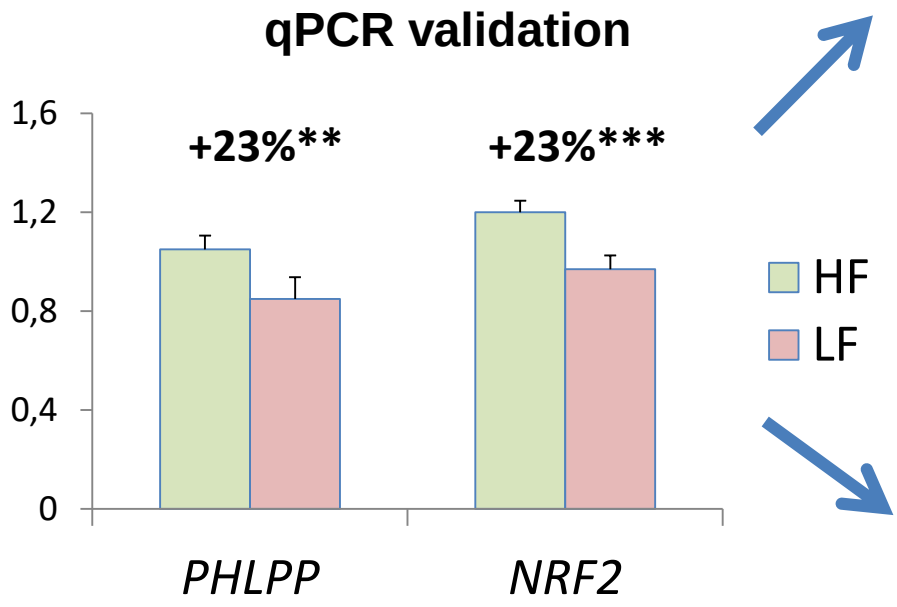
PPAR signaling pathway (19 DEG)

IPA analysis: Proliferation, hepatic fibrosis, liver necrosis and mitochondrial dysfunction as top-toxicity functions responding to diet

Cell proliferation/cell apoptosis may be modulated by HF diet

Pivotal molecular actors revealed by automatic graph inference of genes networks:

- *PHLPP* : serine/threonine phosphatase
- *NRF2* : redox homeostasis



PHLPP : the encoded protein promotes apoptosis and functions as a tumor suppressor

Activated *Nrf2* induces apoptosis and delays proliferation

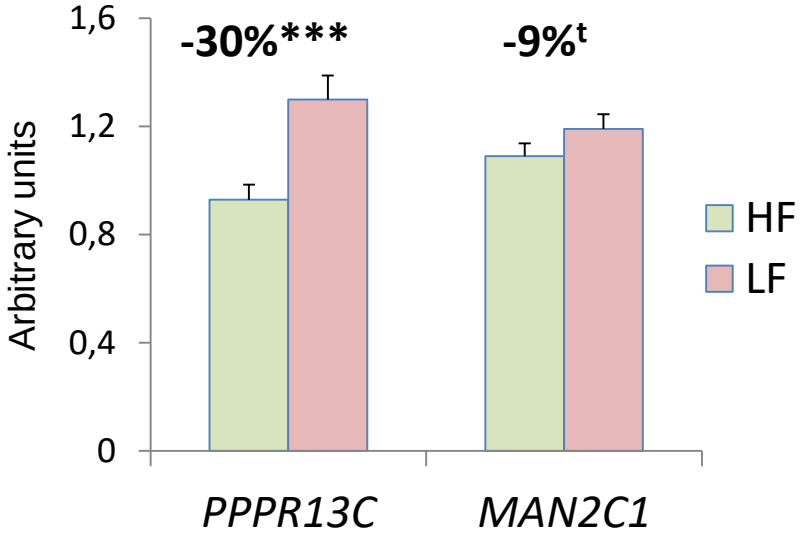
SMAD3 ↗ by HF diet : a transcriptional modulator activated by transforming growth factor (TGF)-beta playing a role in the regulation of liver fibrosis

Down-regulation of glucose metabolism in the liver of HF pigs

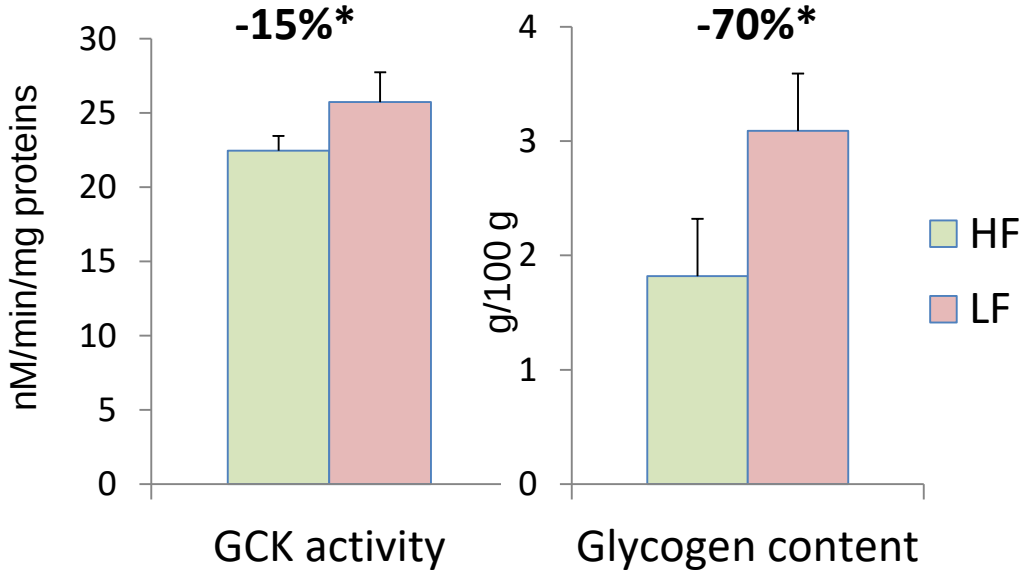
Pivotal molecular actors revealed by automatic graph inference of genes networks:

- *PPP1R3C* : Glycogen biosynthesis
- *MAN2C* : Glycogen metabolism
- *GCK* : GDP-glucose biosynthesis

qPCR validation



Functional studies



HF pigs : Reduction of the glucokinase (GCK) activity and lower glycogen content in liver

Modulation of hepatic fatty acids composition by PPAR signaling pathway

Pivotal molecular actors revealed by automatic graph inference of genes networks:

- *PPARA* : a major regulator of lipid metabolism (oxidation, lipogenesis) in the liver
- *CPT1C* and long-chain fatty-acid-coenzyme A ligases were modulated

But no changes in enzymatic rates controlling FA oxidation, because HAD and CS enzymes activities did not differ between HF and LF diets

GC analysis : changes in fatty acids (FA) composition in the liver of HF *vs.* LF pigs

FA, %	HF diet
C16:0	↘
C16:1	↘
C18:0	↘
∑PUFA	↗

Partial-least square (PLS) analysis to relate gene expression levels to FA composition in the liver

19 genes, among which many of them participate to **PPAR-signaling pathway & lipogenesis**, were identified as VIP explaining variations in hepatic FA composition

Conclusions

❖ Feeding pigs a high fiber high fat (HF) diet changed the molecular and biochemical profiles of liver, adipose tissue, and muscle in the growing pigs

=> Liver was however not the most affected tissue

❖ Modulation of cell development and metabolic alterations including repressed glucose-related pathways, were suggested as the primary responses to HF vs LF diet in the liver

❖ Modulation of PPAR signaling pathway may also affected fatty acid composition in the liver when pigs were fed a HF diet

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