

Novel tools and methods in aquaculture nutrition research: potential, achievements and caveats

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Aquaculture Development Beyond 2000: the Bangkok Declaration

ANSAD (Applied Nutrition for Sustainable Aquaculture Development)

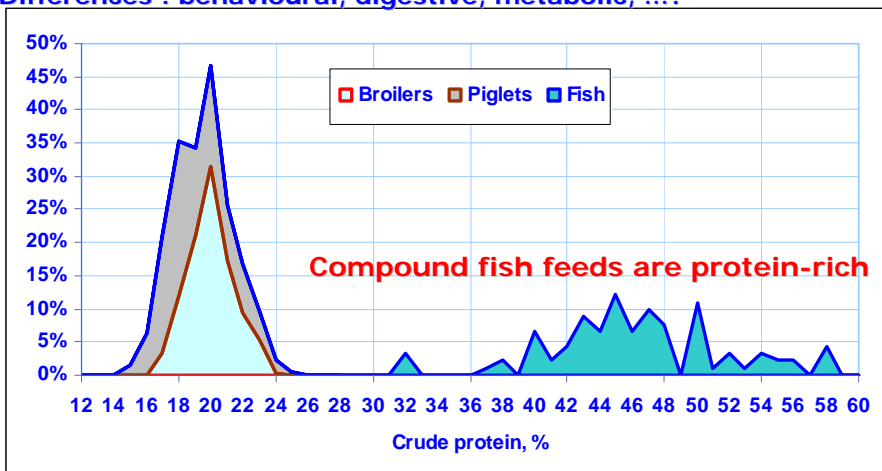
- understanding of dietary nutrient requirements ... application to practical culture
- diets that allow complete domestication and maximal reproductivity and larval quality
- larval nutrition ... reduce the need for live food;
- maximise nutrient retention efficiency;
- renewable feed ingredient sources
- nutrient bioavailability and interactions
- nutrient modulation of disease resistance
- minimise toxicity of feed origin
- selection and trade of raw materials for aquaculture feeds based on sound, documented scientific facts

Diverse challenges

- **Diversity of species, but basic nutritional principles**
 - Diverse trophic levels
 - Diversification : « New species » - Fast growers ?
 - Diverse farming conditions, objectives
 - Whole life cycle : from larvae to broodstock
- **Sustainability issues:**
 - Social, Environmental, Economic Issues
- **Fish as human food:**
 - health & safety issues
- **Advancing our knowledge on basic nutrition science**

Improving protein economy & utilisation efficiency

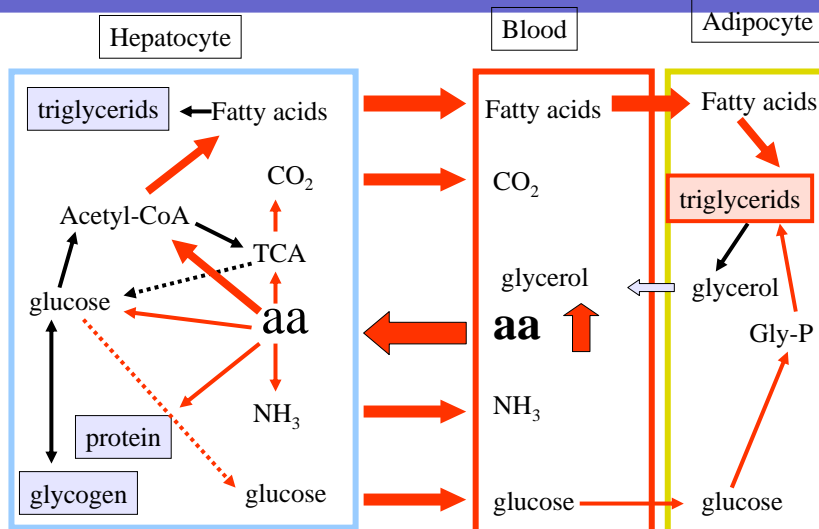
Piscivores, herbivores, frugivores, omnivores, filter-feeders...
Differences : behavioural, digestive, metabolic, ...?



Complementary tools: Molecule <-> Market

- Nutritional requirements
- Feed development
- Animal husbandry issues
- Biochemical / Metabolic
- Cellular / Molecular

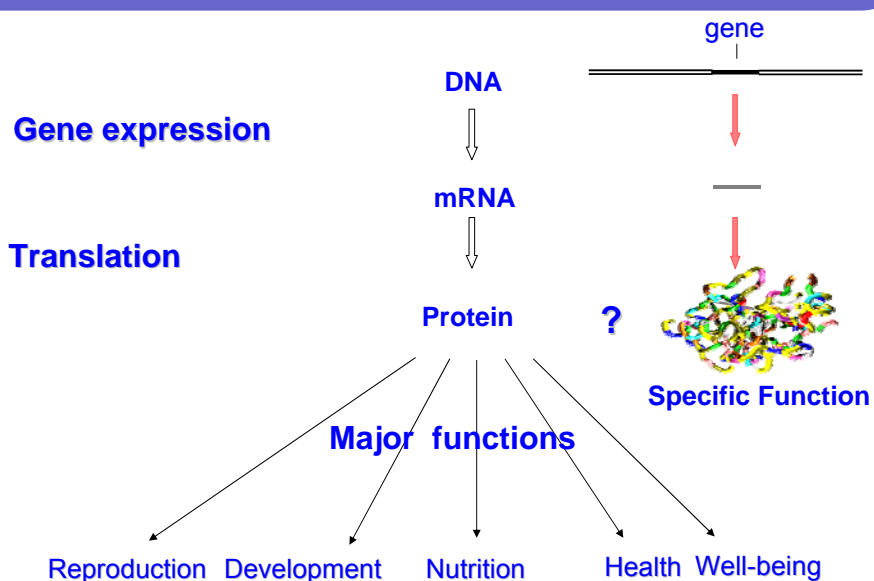
Study metabolic pathways



Cellular & molecular biology

- **A cell is a smallest structural unit of an organism that is capable of independent functioning**
- **All Cells have common cycles: Born, eat, replicate, and die**
- **All Life depends on 3 critical molecules:**
 - **DNAs: Hold information on how cell works**
 - **RNAs: Act to transfer short pieces of information to different parts of cell; Provide templates to synthesize into protein**
 - **Proteins: Form body's major components (e.g. hair, skin, etc.); Form enzymes that send signals to other cells and regulate gene activity**

Molecular bases of biological functions



Diet induced changes in gene Expression

- Target genes
 - **Post-absorptive changes**
 - Hepatic level, Target tissues
 - **Proteins / amino acids, Carbohydrates, Fats / Fatty acids, Micronutrients**
 - Endogenous potential
 - Digestive enzymes
 - Metabolic potential
- Nutrigenomics
 - Dietary FM / FO
- Proteomics

Example : Poor utilisation of dietary glucose in fish

- **Low insulin release after dietary cbh intake**
- **Low number of insulin receptors in muscle**
- **Absence of glucose transporters in muscle**
- **Poor regulation of hepatic glucose metabolism**

Nutritional control of enzymes : glycolysis and gluconeogenesis in the liver

Regulation

Biochemical level

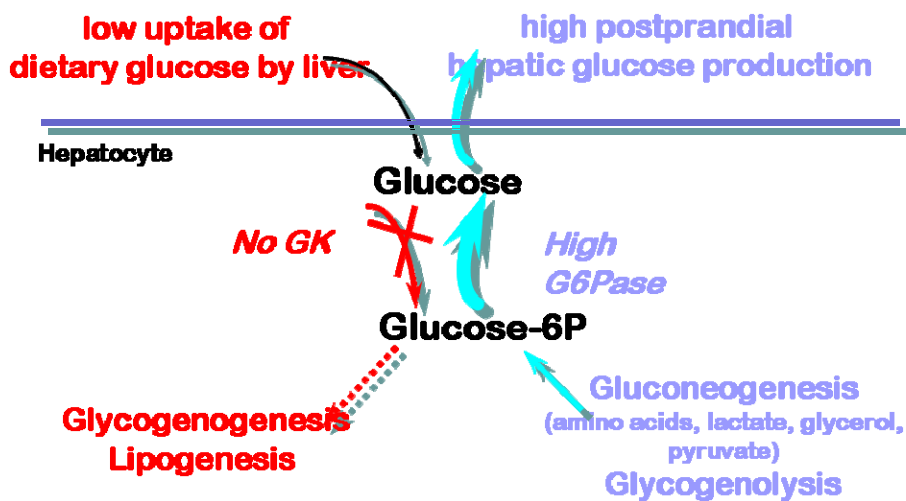
Molecular level

Approaches

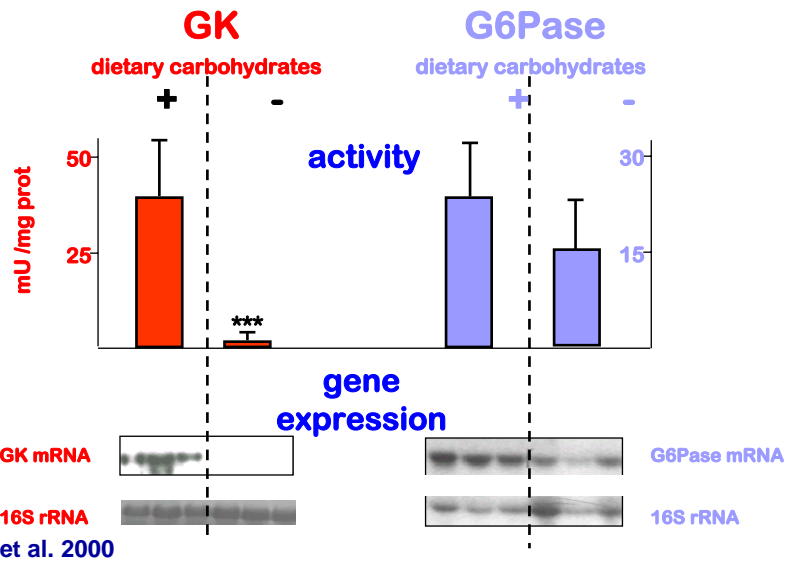
Enzyme activities

Clone the gene(s)
Expression of gene(s)

Glucose homeostasis: low hepatic glucose phosphorylation, persistent hepatic glucose production



Hepatic GK/G6Pase expression long-term effects (10 wks of adaptation with specific diets)



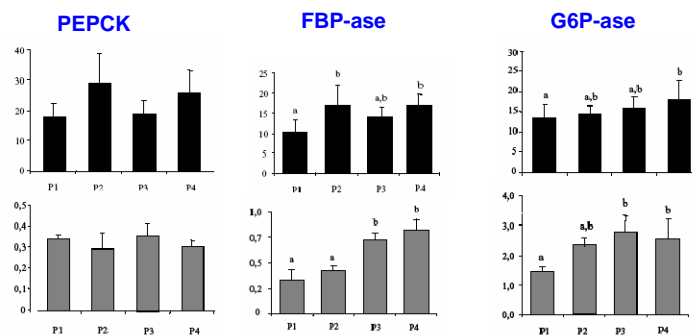
Hepatic GK expression in rainbow trout short-term effects (a single meal, pair-fed)



Protein & AA metabolism

- **Kirchner et al. 2003: Protein intake & glucose homeostasis in trout : activities vs mRNA levels**
- **Gomez-Requeni et al. 2004: Somatotropic responsiveness to dietary protein sources in gilthead seabream**
- **Hevroy et al. 2007: High vs low dietary Lysine on expression of somatotropic factors in Atlantic salmon**
- **Seilliez et al. 2007: Protein degradation**
- **Skiba et al. 2008: Amino acids / interaction with insulin : cell signalling pathways**

Protein intake & glucose homeostasis in trout : activities vs mRNA levels

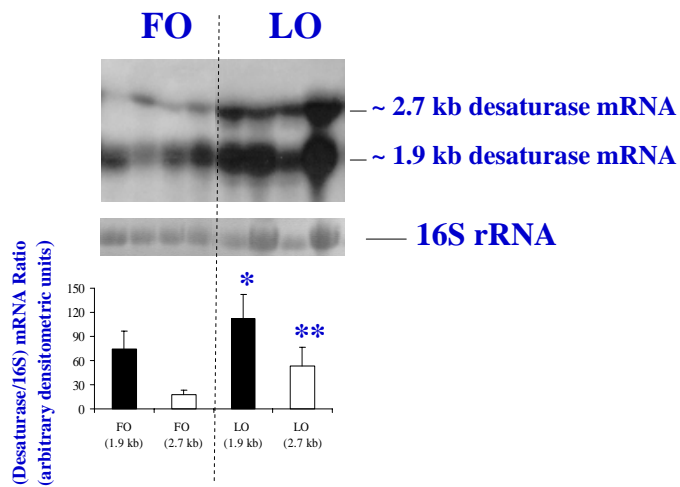


Kirchner et al. 2003

Lipid metabolism

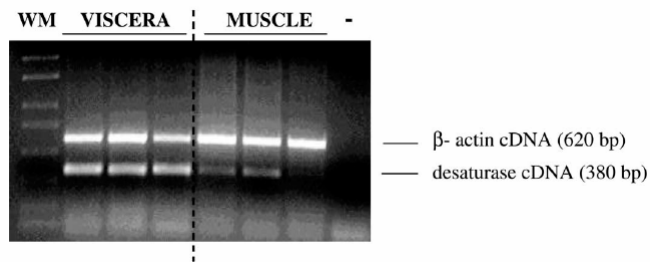
- Fatty acid bioconversion capacities
- Lipogenesis
- Transport
- Catabolism / oxidation

Can fish oil replacement affect potential FA bioconversion capacity ?
FA desaturase expression in RBT fed fish oil or linseed oil



Seiliez et al. 2001

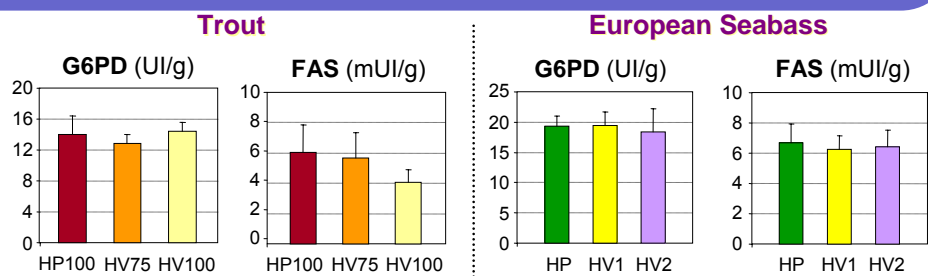
FA desaturase expression in gilthead seabream larvae fed HUFA-free diet



Shown in a number of teleosts
Zebrafish, tilapia, salmon, tilapia....

Seiliez et al. 2003

Dietary fat sources on lipogenesis



	FO100	VO75	VO100
G6PD (UI/g)	→	↗	→
AGS (UI/g)	→	↗	→
Gene expression G6PD	→	→	→
Gene expression FAS	→	↗	→

Richard et al, 2006

Dietary fat sources on Lipid transport

Feeding VO: decrease in plasma cholesterol, LDL levels

Expression of LDL-receptors in trout VO vs FO diets

Adipose tissue	↘ ÷ 1,6
White muscle	→
Liver	↘ ÷ 3,9

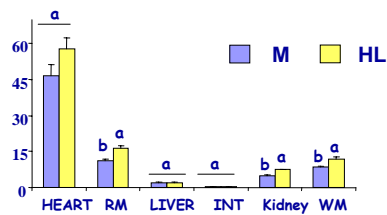


Does not explain low LDL in VO fed fish

Richard et al, 2006

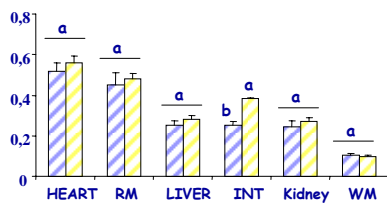
Beta oxydation as affected by dietary fat levels

mRNA levels



↗ in mRNA in white / red muscle, kidney

Activité CPT I (mUI/mg prot)

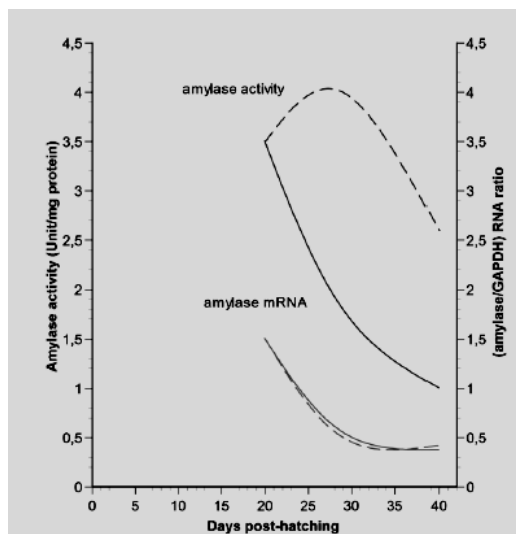


↗ in specific activity in the intestine

Gene Expression

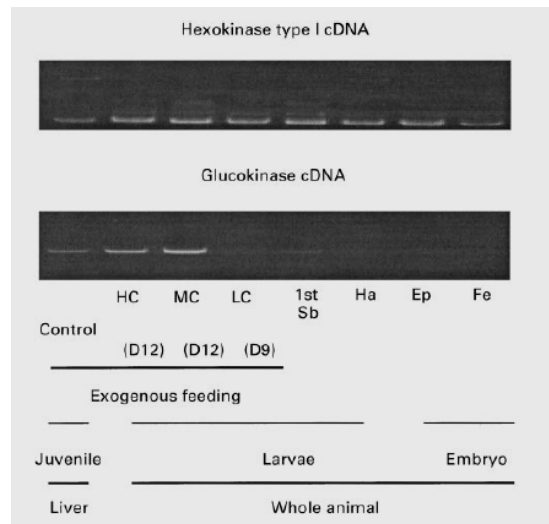
- Target genes
 - **Endogenous potential**
 - **Digestive enzymes**
 - **Metabolic potential**
 - Post-absorptive metabolism
 - Hepatic level, Target tissues
 - Proteins / amino acids, Carbohydrates, Fats / Fatty acids, Micronutrients
- Nutrigenomics
 - Dietary FM / FO
- Proteomics

Example : Digestive enzymes during larval development of European seabass



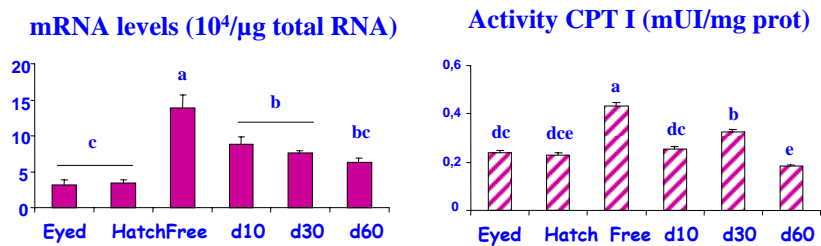
Zambonino & Cahu, 2001

Expression of glucokinase and hexokinase-I genes during carp development



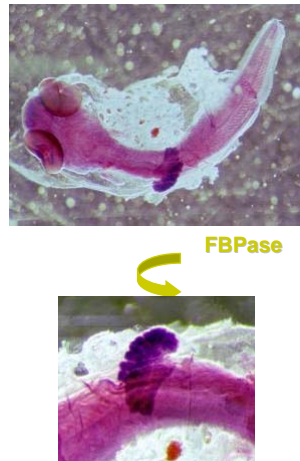
Panserat et al. 2001

Activity & Expression of CPT I during ontogenesis in trout



Gutieres, 2003

Expression of FBPase during early development



Fishmeal replacement :

- **Producing fish with fish alone is a short-sighted approach**
- **Replacement of Fishmeal is possible**
 - provided the ANFs are reduced / deactivated
 - is already practised for a number of warmwater species
 - has been shown to be possible for all salmonids and marine teleosts studied so far
- **Substitution levels can vary between species : there is much hope even for “carnivorous” species**
 - non- or low-fishmeal diets are possible

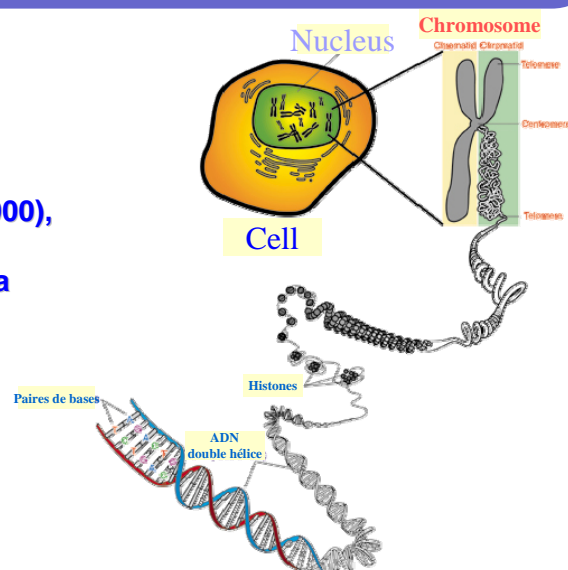
Fish oil replacement

- In fish meal based diets, it is possible to replace large amounts of fish oil
- Shown in turbot, brown trout, Atlantic salmon, gilthead seabream, European sea bass
- Finishing with fish oil can bring the flesh fatty acid profile back to reasonable levels, to maintain the nutritional value of aquaculture products

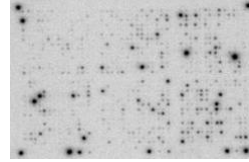
Genome level changes

Genomics:

Data on changes in expression of a large number of genes (>10 000), with known or hitherto unknown functions, in a single study



« Nutrigenomics »



Diets contain different ingredients which provide nearly 40 different nutrients

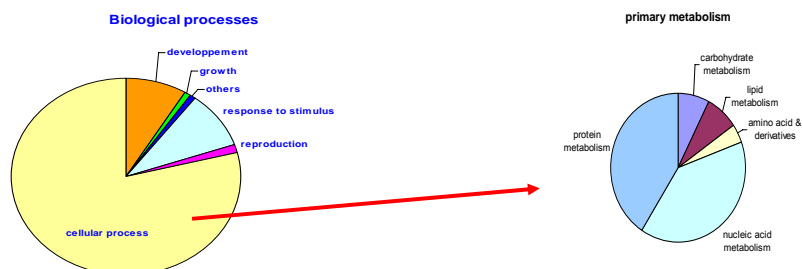
Dietary changes have multiple physiological responses

An integrative approach is needed to analyse such multiple responses

From « Nutrition & Single Gene Expressions » to « Analysis of Expression of Network of Genes »

Trout multi tissue microarrays

9023 trout cDNAs (INRA Sigenae)
<http://www.sigenae.org/>

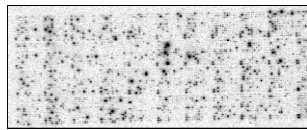


Microarrays: Examples

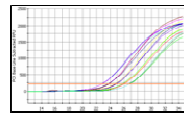


Diets :

- Fish meal (FM) vs plant proteins (PP) (52 weeks of feeding)
- Fish oil (FO) vs vegetable oils (VO) (62 weeks of feeding)

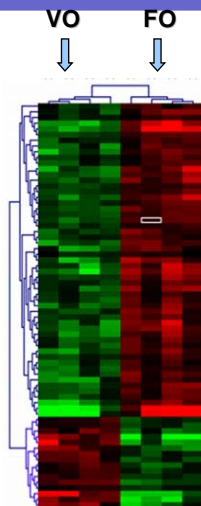


Trout cDNA microarray (10K) hybridization



Data validation :
Real time PCR

Replacement of Fish oil by Veg oils



71 differentially expressed genes
($p < 0.01$; t-test):

- 16 up-regulated in VO
- 55 down-regulated in VO

MAFK_MOUSE
RC3HA_MOUSE
DHSB_HUMAN
SYN_HUMAN
TERNA_RAT
SGO3_HUMAN
F3BP_HUMAN
AND1_HUMAN
ZNF1_HUMAN
GPI3_ONCMY
PRRX_HUMAN
RNO1_HUMAN
HSP7_CHICK
FAB_CHICK
UNKNOWN
LGF4_RAT
UNKNOWN
EGL1_HUMAN
ELCQ_MOUSE
SARA_HUMAN
UNKNOWN
ARIA_HUMAN
TRG1_HUMAN
PRSE_YEAST
PUB1_SCHPO
YCD1_HUMAN
KOP2_DIC
YMD1_YEAST
PES2_HUMAN
UNKNOWN
TBA_ONDKE
NFAT1_HUMAN
THAT_MOUSE
HSP7_CHICK
F2B_HUMAN
SMD2_HUMAN
VATL_HUMAN
FAS_HUMAN
DLX3_MOUSE
ICP3_MOUSE
GDI3_MOUSE
YBGA_SCHPO
PNSD_MOUSE
RBP6_CHICK
GSP3_MOUSE
GPI3_ONCMY
GSP7_HUMAN
NEXT_HUMAN
MPS1_HUMAN
HPOB_RAT
UNKNOWN
GATD_GLYMA
KL74_MOUSE
LPI1_HUMAN
CRSA_DROME
BYO1_HUMAN
K1CG_HENLA
AGS1_RHIME
PGBM_HUMAN
SHO2_HUMAN
GZA_MOUSE
UCR2_HUMAN
UNKNOWN
YARS_YEAST
GATE_CHICK
MLB3C_HUMAN
ACT11_FUSRU
UNKNOWN

CYP1A
Proteolytic system :
proteasome

FAS

Proteolytic system :
proteasome

FAS

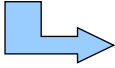
CYP3A

Proteolytic system :
Lysosome
(cathepsin)

Proteolytic system :
Lysosome
(cathepsin)

Fish oil replacement by vegetable oils confirmatory measures

	Genes	Fold variation (in VO)
71 differential expressed genes (p<0.01; t-test):	Fatty acid synthase	-10.1
	Cathepsin B	-2.3
	Ubiquinol	
	cytochrome c reductase	-3.1
	Cytochrome P450 CYP1A3	-2.7
16 up-regulated in VO	Cytochrome P450 CYP3A27	+2.8
55 down-regulated in VO		


qRT-PCR
 (focus on 5 specific genes)

EU projects RAFOA & AQUAMAX

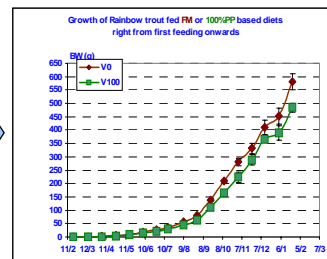
Fish meal replacement by plant protein sources

Diets

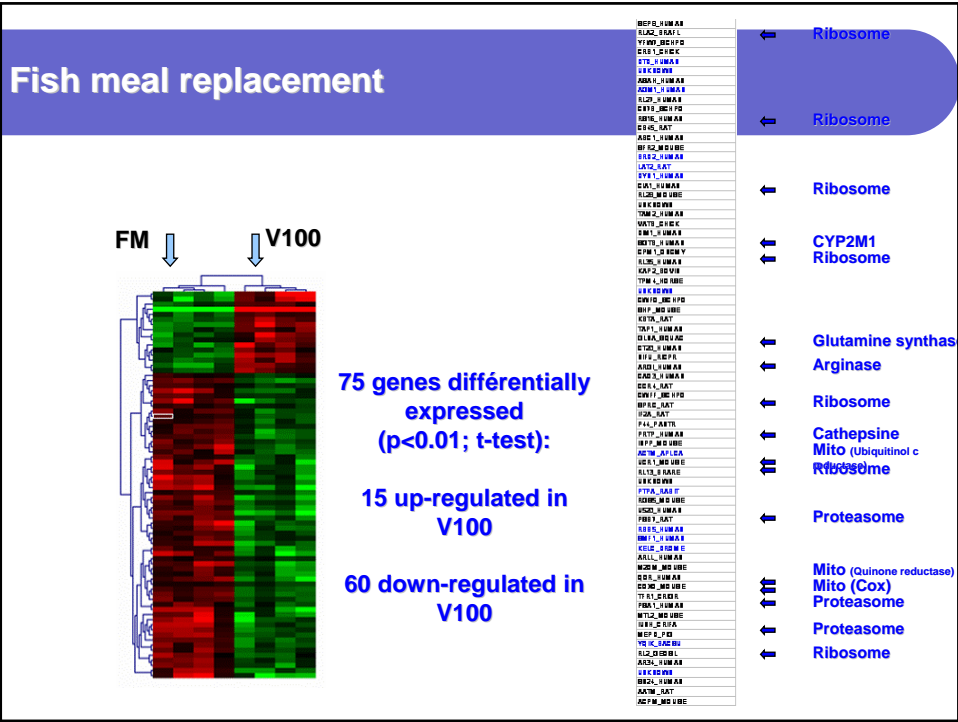
Fish meal
Fish meal LT94

Plant proteins
Corn gluten meal
Wheat gluten
Lupin meal

%P: 40; %F: 22%
52 weeks



EU projects : PEPPA & AQUAMAX



Fish meal replacement confirmatory measures

75 differentially expressed genes
($p < 0.01$; t-test):

15 up-regulated in V100

60 down-regulated in V100

	Genes (Glutamine synthase isoforms)	Fold variation (in PP)
	GS01	-6.9
	GS02	2.1
	GS03	-7.1

qRT-PCR
 (focus on 1 specific gene)

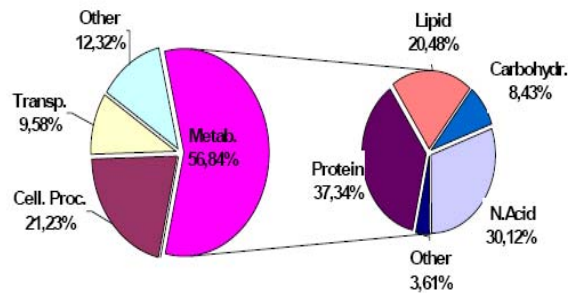
Combined FM & FO replacement

176 genes were differentially expressed

A large majority of genes were involved in metabolism (57%)

- 96 genes over-expressed in fish fed plant-based diets (vegetable diet)
- 80 genes under-expressed in fish fed plant-based diets (vegetable diet)

Repartition of differentially expressed genes (Go Miner analysis)



Rainbow trout: nutrigenomic data

14 genes specific to fish fed plant diet

Over-expressed

Carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1 (EC 3.1.3.16)
 Probable ATP-dependent RNA helicase DDX49 (EC 3.6.1.-)
 Coagulation factor XIII A chain precursor (EC 2.3.2.13)
Glycerol kinase (EC 2.7.1.30)
 Histone H2A
 Kinetochores protein Hec1 homolog
 Zinc-finger protein ubi-d4
 RING finger protein 122

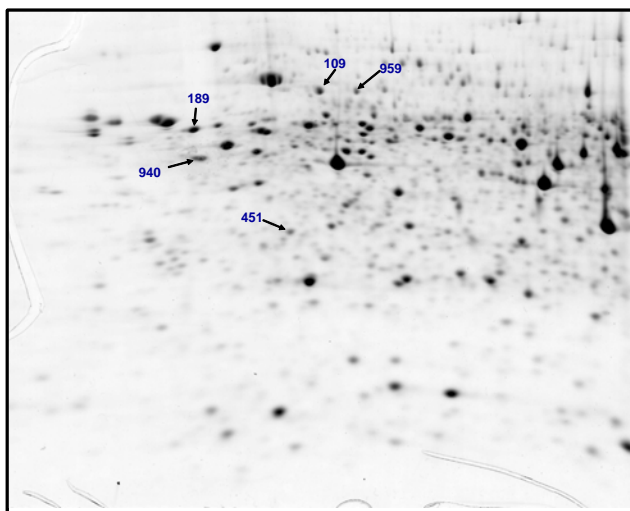
Under-expressed

ATP-citrate synthase (EC 2.3.3.8)
 Dystrophin
 Eukaryotic translation initiation factor 2-alpha kinase 4 (EC 2.7.11.1)
 Transcription cofactor HES-6
Phosphoenolpyruvate carboxykinase [GTP], mitochondrial precursor (EC 4.1.1.32)
 RING finger protein 4 (SNURF)

Proteomics

- Individual protein abundance is modulated by dietary factors
- Protein profiles may be used as diagnostic tool for studying dietary effects on metabolism
- Proteomics may tell us that the message (mRNA) does not always result in the product (protein).
- Salmonids still have poorly characterised genome in comparison to model species
- Need for a common format

Protein spots identified as differentially expressed



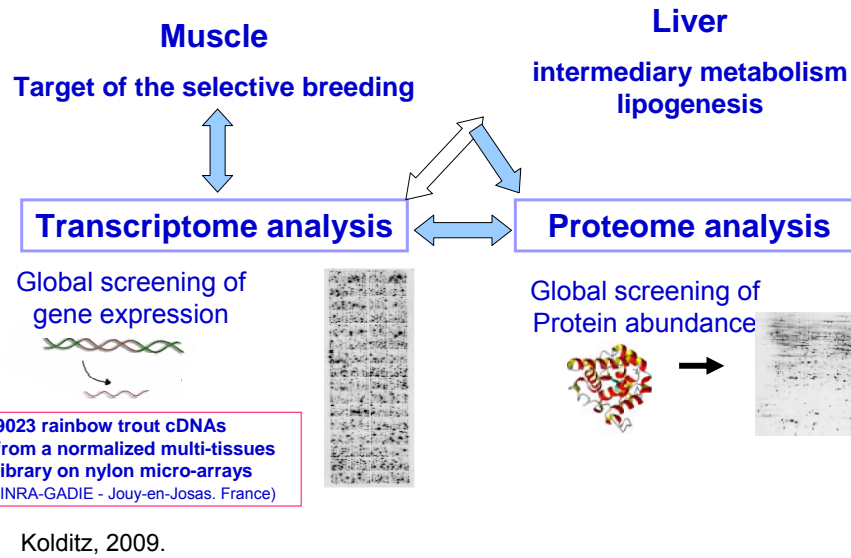
Increased in diet TM40

109
451
959

Increased in diet TM50

189
940

Genotype-Nutrient interactions: diet-induced metabolic changes in two lines of trout



Fish oil removal (FO-) Trout liver transcriptome and proteome

Transcriptome analysis

- ↓ **Fatty acid catabolism**
(Arachidonic acid epoxigenase)
- ↓ **Glycolysis**
(GK (HK IV), GAPDH (2))
- ↑ **Protein & amino-acid catabolism**
(Proteasome subunits, Cathepsin L, GOT (2) ALAT...)
- ↑ **Lipogenesis**
(G6PD, FADS, ICDH, 6PGD...)
- ↑ **Lipid transport**
(H-FABP (2), ACBP, LIPE, CETP)
- ↑ **Cholesterol biosynthesis**
(DHCR24, ERG25, ERG28, EBP...)
- ↑ **Betaine synthesis**
(BADH, TFHFD (2), BHMT)

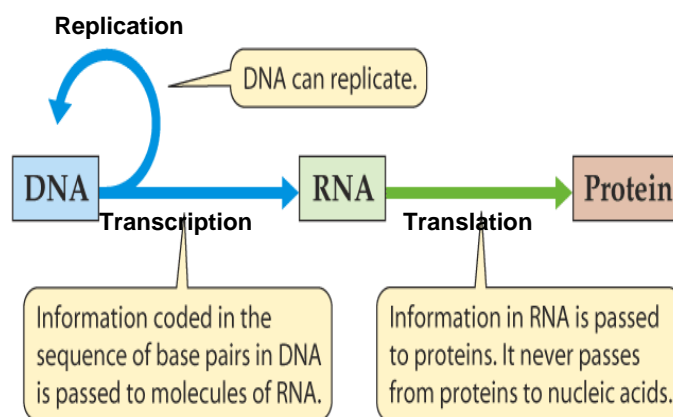
Proteome analysis

- ↓ **Glycolysis**
(GAPDH, α-1 enolase)
- ↑ **Amino-acid catabolism**
(GDH, GOT (2), ALAT)
- ↑ **Lipid transport**
(H-FABP (2), ACBP)
- ↑ **Betaine synthesis**
(BADH, TFHFD (2), BHMT)

Other integrative tools

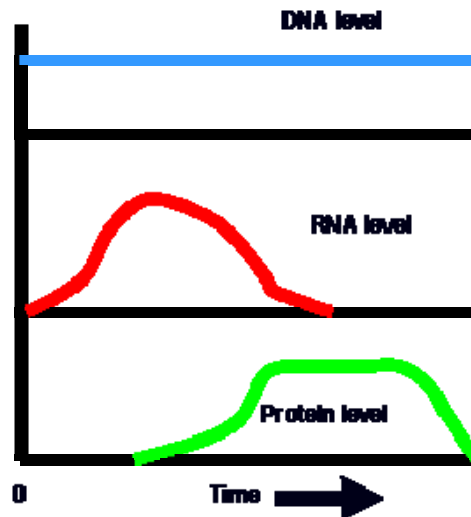
- **Metabolome :**
 - The quantitative analysis or description of all low molecular weight metabolites in specified cellular, tissue or biofluid compartments. (Metabolomics: Numbers, chemical classes, structures, concentrations: < 1KDa)
 - To identify, measure and interpret the complex time-related concentration, activity and flux of endogenous metabolites in biological samples (cells, tissues, blood, urine or saliva...)
- **Metabonome :**
 - Quantitative measurement of the dynamic multiparametric metabolic response of living systems to environmental stimuli or genetic modification
 - The sums, products & interactions of all the individual compartments/metabolomes (including extra-genomic sources) dispersed in a complex organism...The 'Global' System.

DNA, RNA, and the Flow of Information



A gene is expressed in two steps
Transcription: RNA synthesis; Translation: Protein synthesis

Caution : Time course of changes



Conclusions / Issues

- **Molecular biological tools are extremely useful to understand / validate / complement biological data**
- **So far, no dose-response studies**
- **Quantitative nature of changes difficult to assess**
- **Nutrients have specific effects on a number of individual (network of) genes**
- **Genomic and proteomic databases are available for some species and tissues**
- **These tools are currently descriptive; but pathway-specific metabolic microarrays / chips can become useful**
- **Metabolomic tools for profiling**