



# From muscle to meat: physiological and metabolic basis

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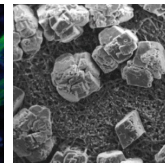
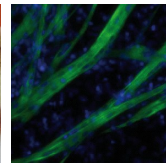
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# From muscle to meat: physiological and metabolic basis

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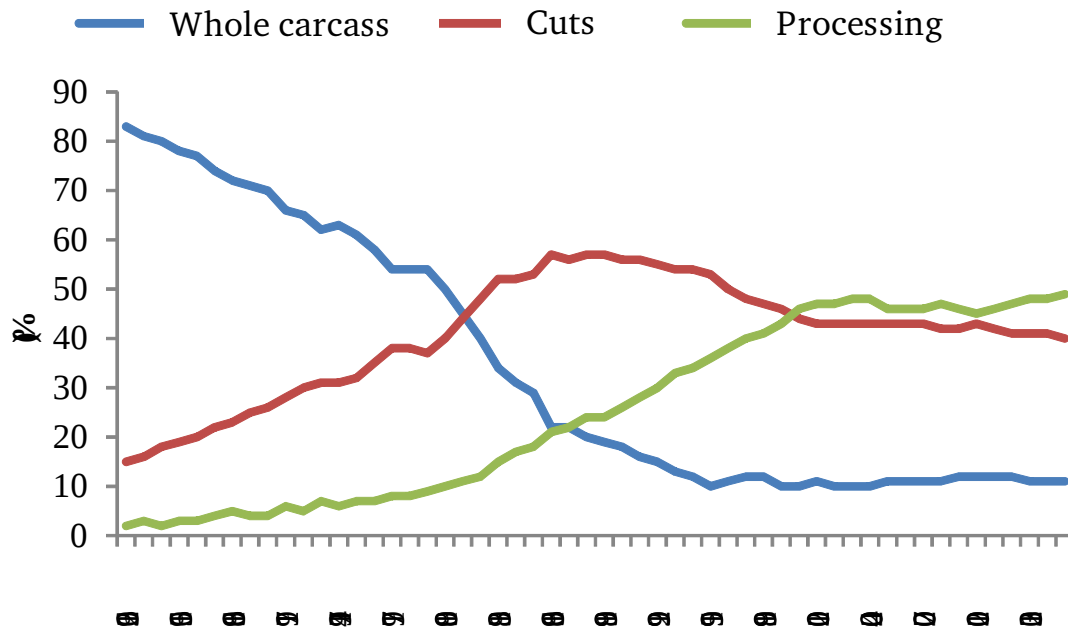
**URA, INRA, Nouzilly, France**



# Context

Increasing demand in poultry meat worldwide (+2%/year)  
Rapid evolution of the market share

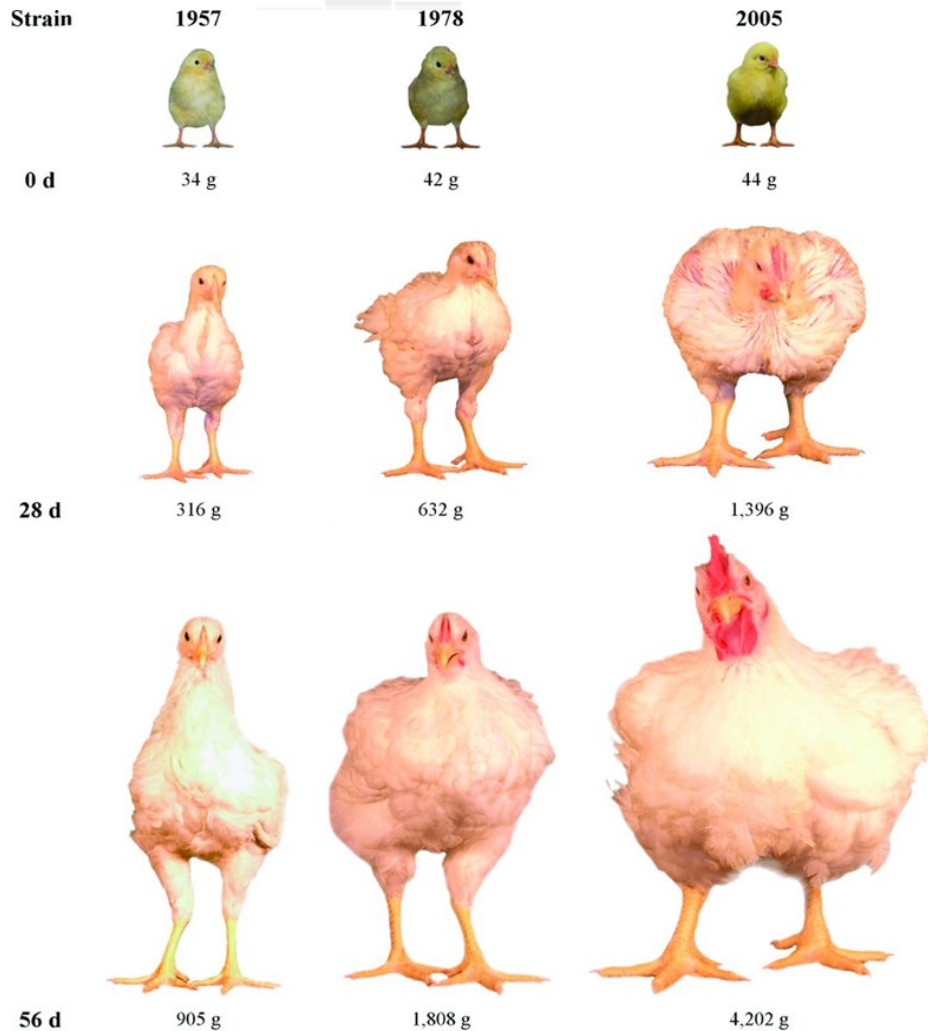
Market share, NCC (2012)



## Many quality expectations:

- Meat yields
- Functional properties
- Storage ability
- Sensory perception
- Nutritional value
- Image (animal welfare)

# Consequences for broiler production

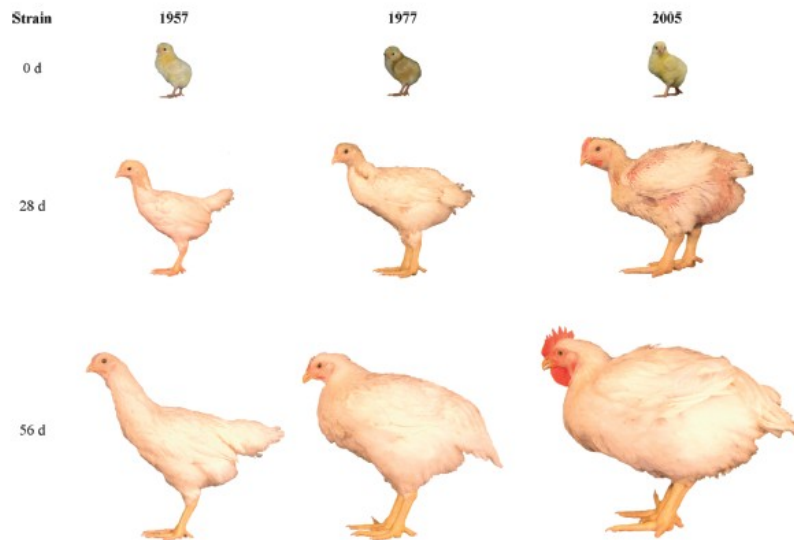


- Feed efficiency ↑
- Growth rate ↑
- Breast meat yield ↑

Source: Zuidhof *et al.*, 2014

# Consequences for broiler production

Year	Strain	Live weight, g	Age, j	Breast, g	Breast, % LW
1957	Athens	2078	57	280	13,5
2001	Ross 308	2207	43	349	15,8
2007	Ross 308	2200	36	410	18,6
2012	Ross 308	2200	35	464	21,1

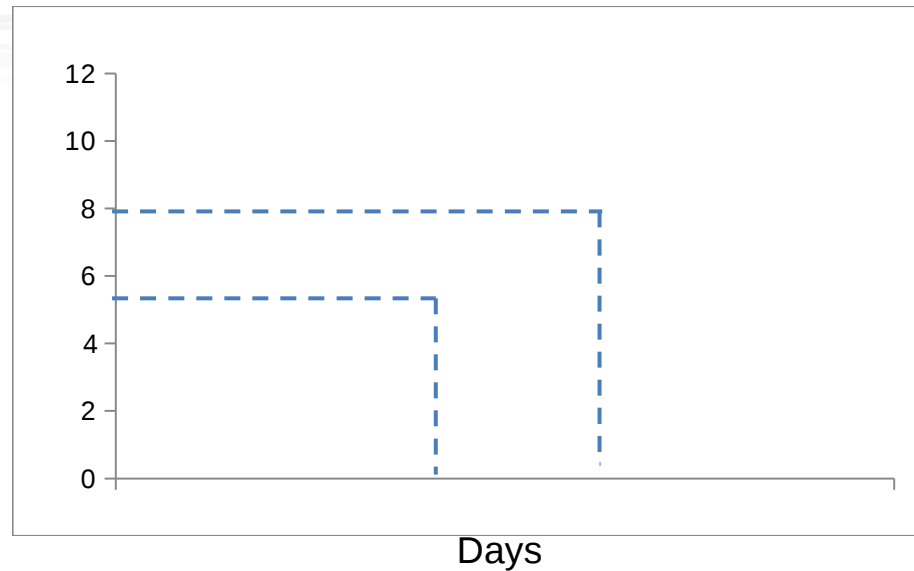


(Petracci et al., 2015)

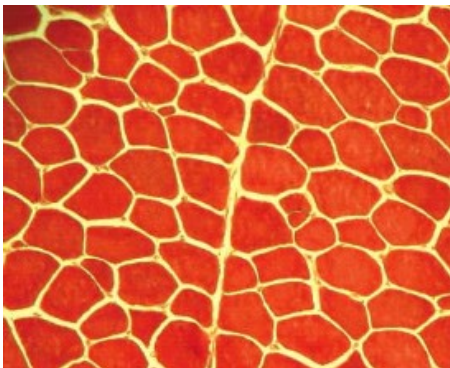
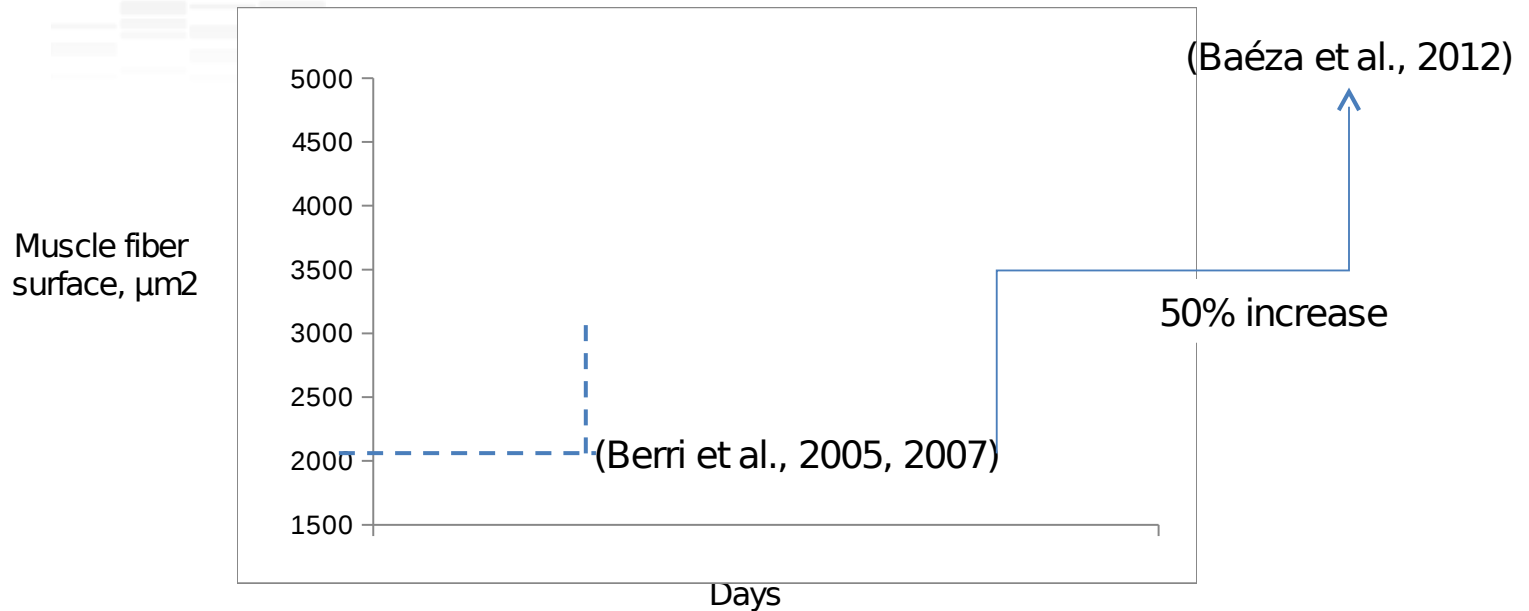
Source: Zuidhof *et al.*, 2014

# Consequences for broiler production

Live weight, g

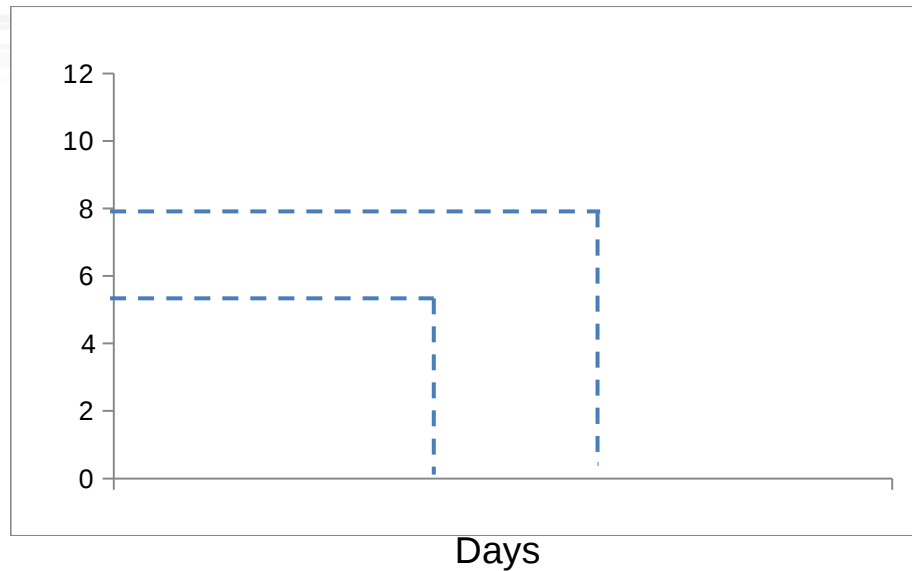


# Muscle hypertrophy : fiber size increase



# Consequences of older broiler production

Live weight, g

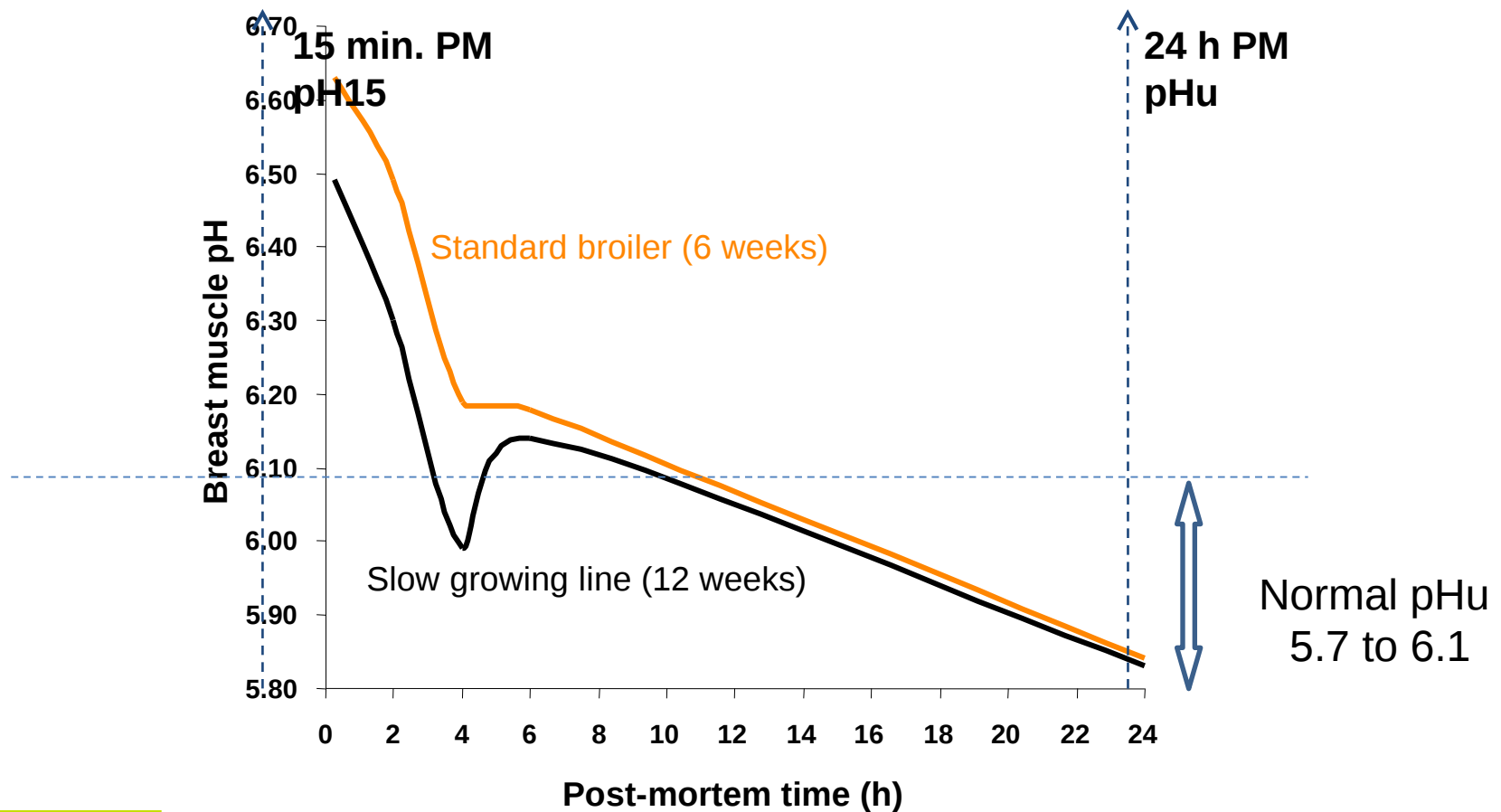


## Production of heavy and high-yielded broilers (> 3kg)

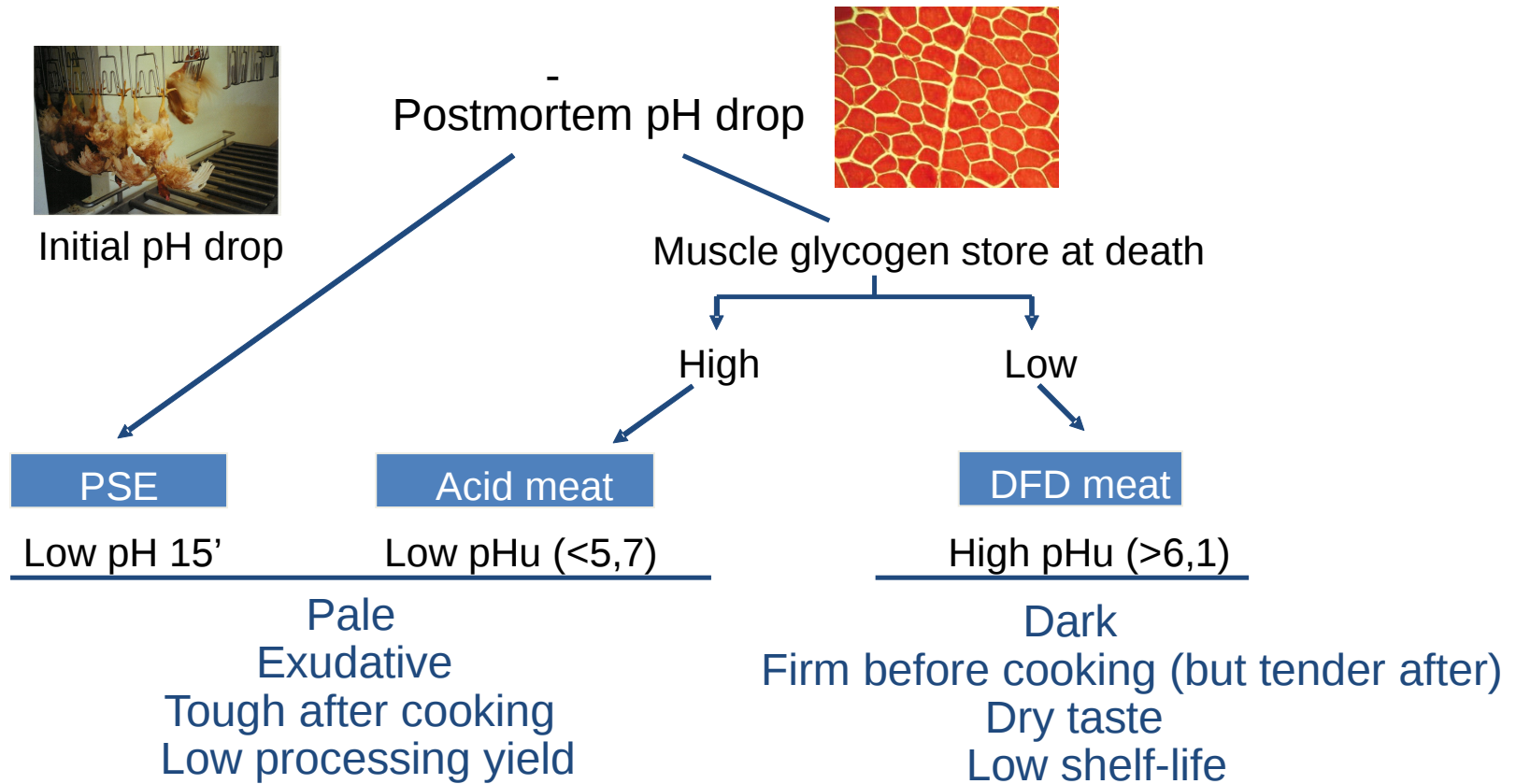
- Increased muscle fiber size (Baéza et al., 2012)
- Increased lipid and protein content (Baéza et al., 2012; Janisch et al., 2013)
- Modified post-mortem metabolism (Baéza et al., 2012)



# The post-mortem pH fall: a key element for meat quality



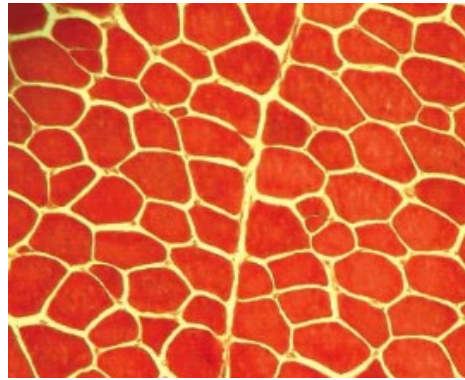
# Postmortem metabolism and meat quality



# Muscle growth and meat quality

Age at slaughter  
Selection  
Nutrition

Increase in muscle  
fiber size



(Berri et al., 2007)

Rg: -0.52



Glycogen

Rg: -0.97

pHu



Lightness

Rg: -0.65



Drip loss

Rg: -0.89



Shear force

Rg: -0.81



Reduced  
occurrence  
of acid meat

Improved  
color, WHC,  
tenderness

# Impact of increasing muscle growth on meat pH

(Berri et al., 2001)

Selection

pHu

9.9  
9  
8.1  
7.2  
6.3  
5.4  
4.5  
3.6  
2.7  
1.8  
0.9  
0

Genotype

BW: 1.3kg → 3.0 kg  
BMY: 11.5% → 18.5%

(Baéza et al., 2012)

Age at  
slaughter

pHu

12  
10  
8  
6  
4  
2  
0

Age at slaughter

BW: 1.9 kg → 4.2 kg  
BMY: 18.7% → 22.2%

(Berri et al., 2008)

Nutrition

pHu

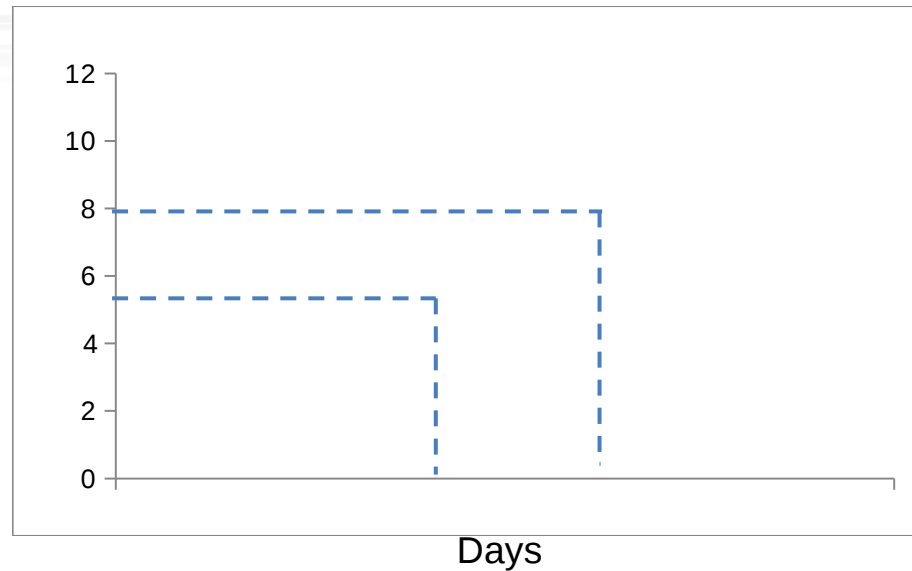
9.9  
9  
8.1  
7.2  
6.3  
5.4  
4.5  
3.6  
2.7  
1.8  
0.9  
0

Digestible Lysine

BW: 2.6 kg → 2.7 kg  
BMY: 22.0% → 22.9%

# Consequences for broiler production

Live weight, g

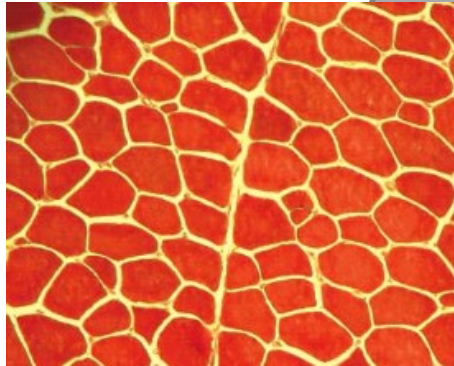


## Production of heavy and high-yielded broilers (> 3kg)

- Increased muscle fiber size (Baéza et al., 2012)
- Increased lipid and protein content (Baéza et al., 2012; Janisch et al., 2013)
- Modified metabolism (Baéza et al., 2012)

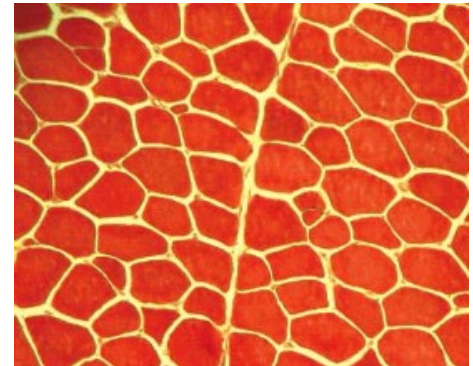
# Metabolic Changes

(Berri et al., 2001)

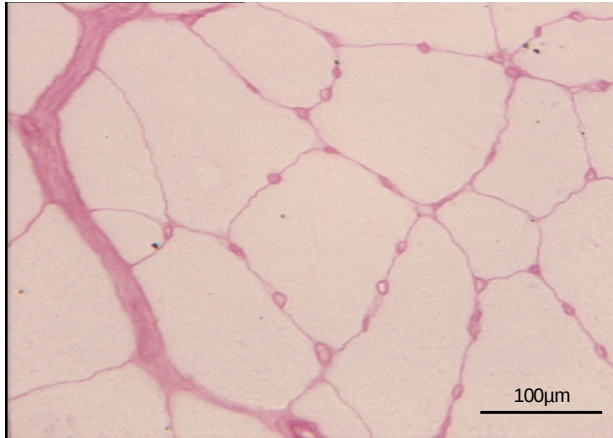


128 $\mu\text{M}$	Glycogen	87 $\mu\text{M}$
2.4 ppm	Iron content	1.8 ppm
5.8 mM/g	HAD activity	4.7 mM/g

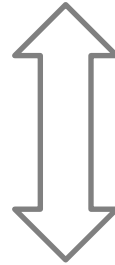
Lower carbohydrate reserve  
Lower blood supply  
Lower oxidative activity



# Muscle blood supply and Metabolism

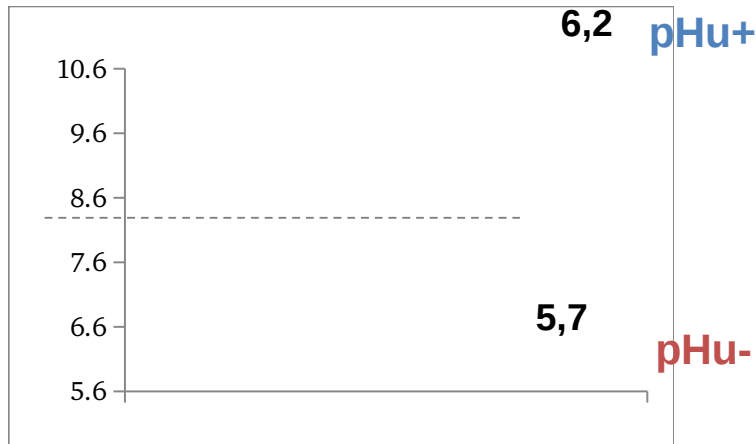
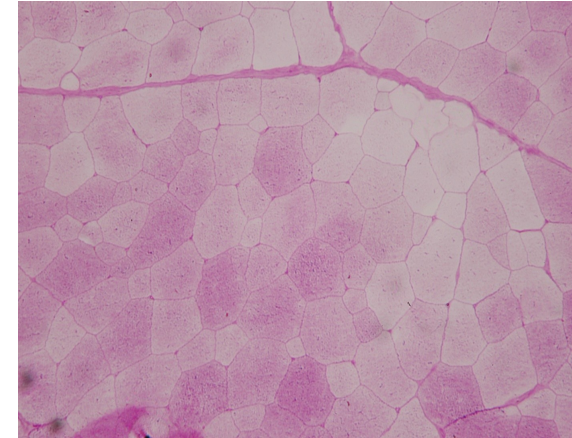


Lower blood supply



(Alnahhas et al., 2015)

Lower glycogen in muscle



**20% difference in breast muscle glycogen (P. major)**



(Alnahhas et al., 2014,2015)



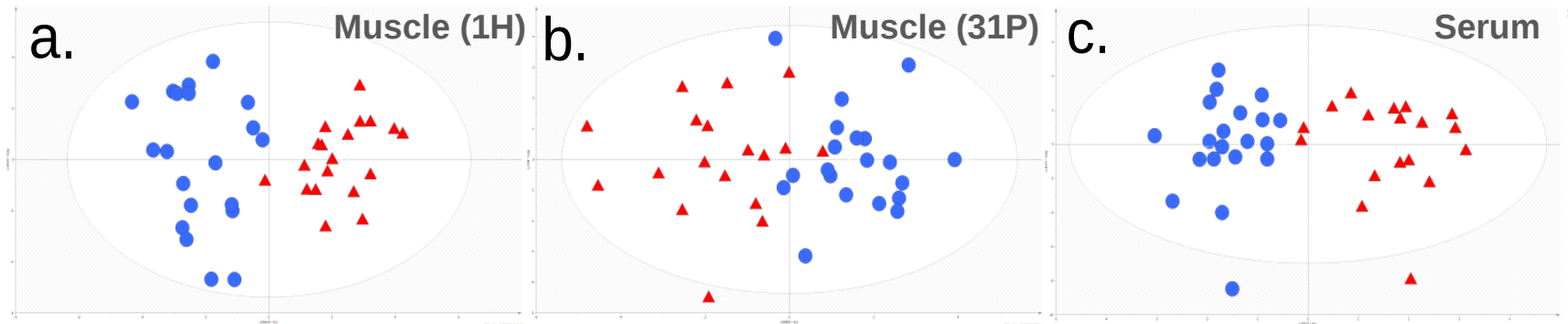
# Metabolomic profile (muscle & serum)

(Beauclercq et al., J. Proteome Res. 2016, 15, 1168–1178)

♂ **pHu-**/**pHu+** (n = 40) [6.3 vs 5.5 – glycogen 195 vs 145]



Modeling by oPLS-DA analysis



**pHu+**/**pHu-**



Polar extract of P. major muscle (1H et 31P NMR): 25 discriminant metabolites

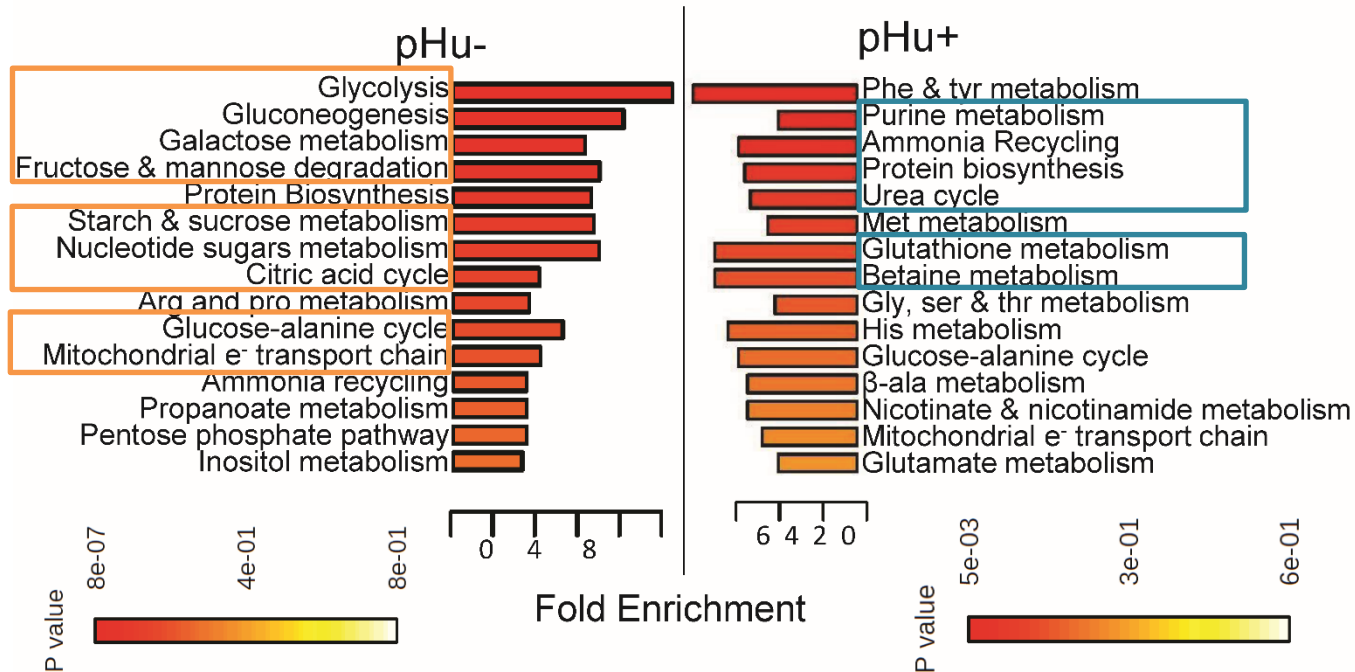
Polar extract of serum: (1H NMR): 20 discriminant metabolites





# Metabolomic profile (muscle& serum)

## Enrichment analysis on discriminant metabolites

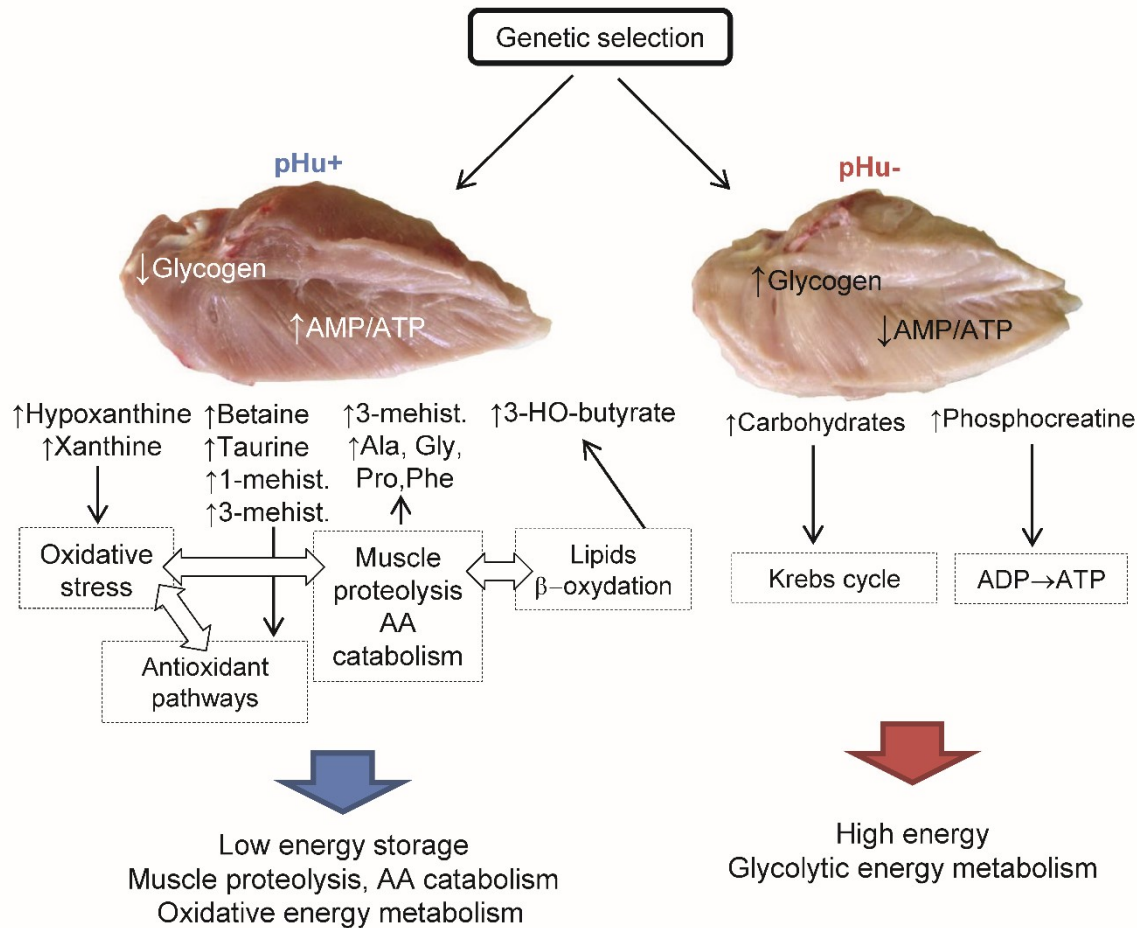


**pHu -** : Overrepresentation of metabolites related to **carbohydrate turn-over**

**pHu +**: Overrepresentation of metabolites related to **oxidation and protein catabolism**



# Metabolomic profile (muscle & serum)



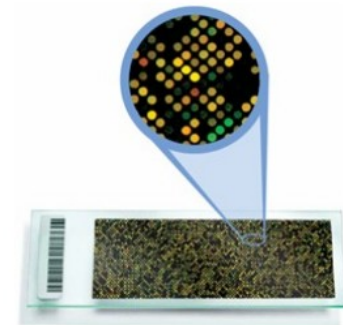
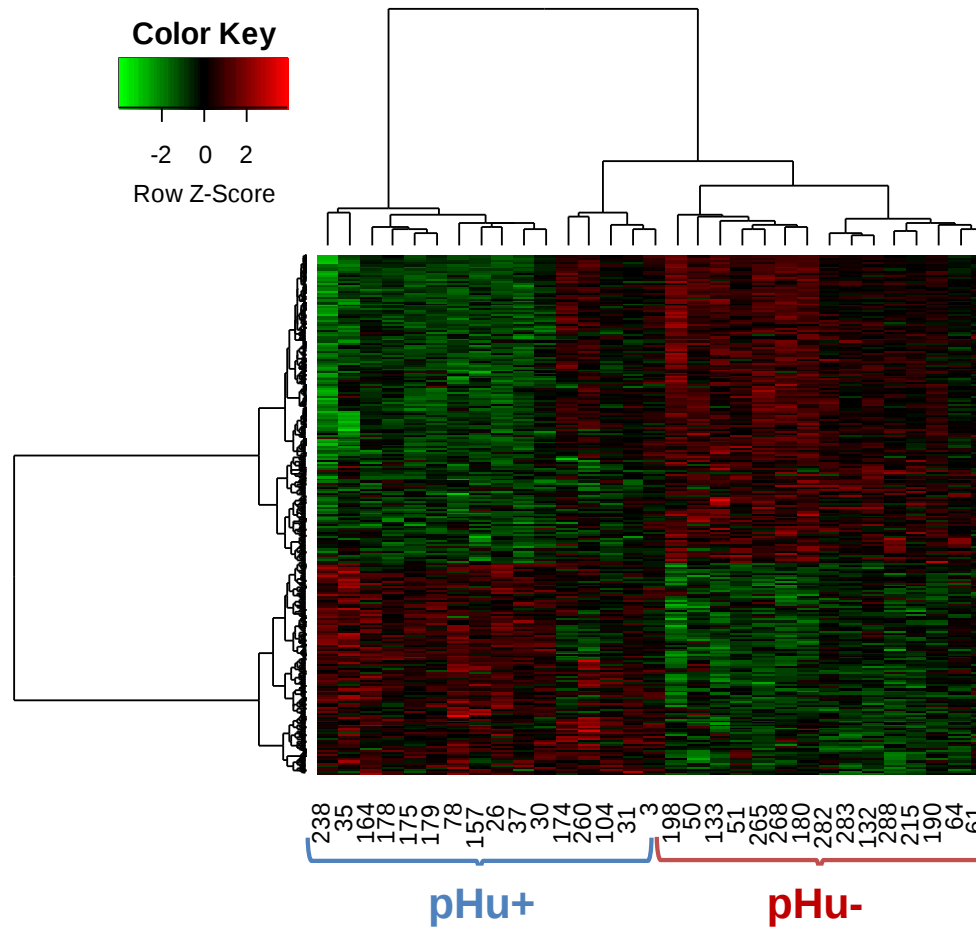
(Beauclercq et al., 2016)



# Transcriptomic profile (muscle)

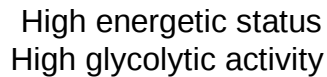
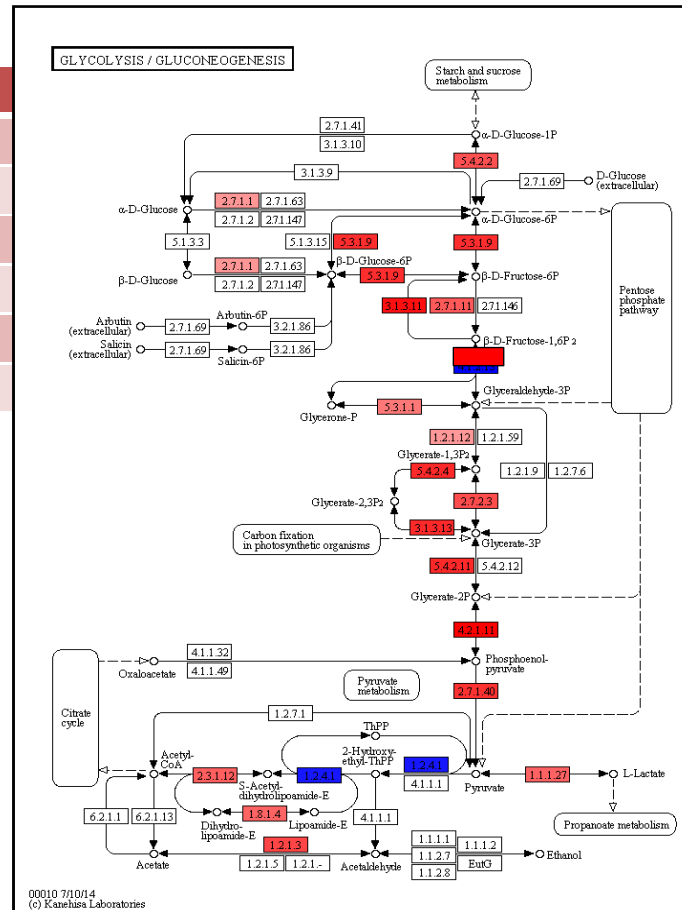
♂ pHu-/pHu+ (n = 31)

Agilent microarray (monocolor 8 × 60K)



# Function

<b>pHu-</b>
Glycolysis/neoglucogenesis
ATP Concentration $\square$
Amino-Acid Métabolism
Muscle cell organisation
Purine metabolism
Triglyceride Synthesis

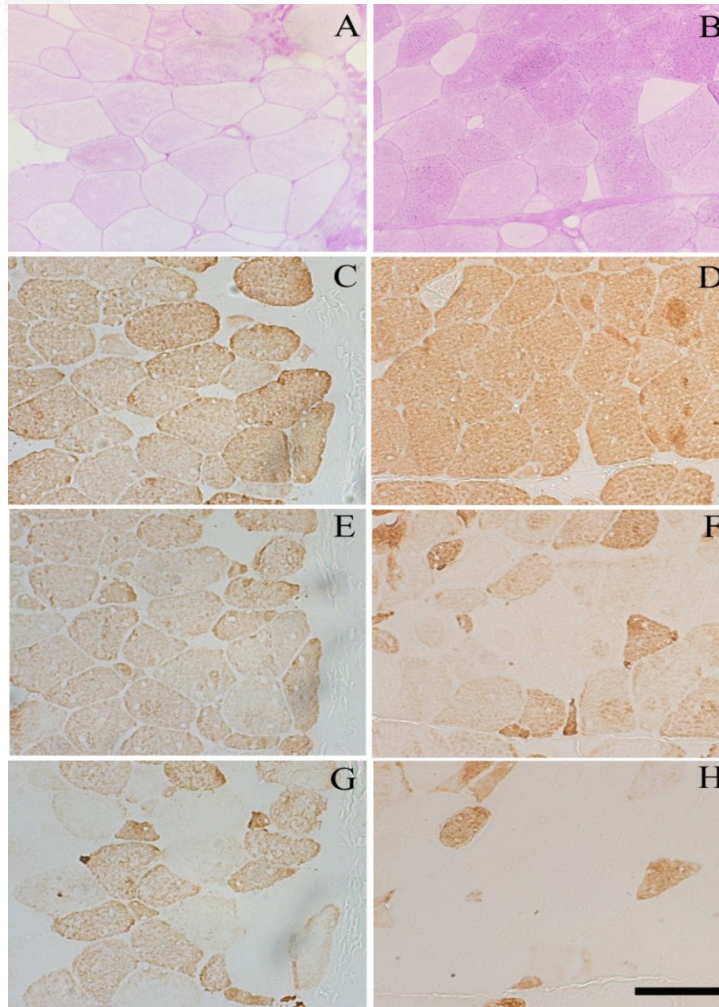
SCIENTIFIC  
REPORTS

# Histological profile

pHu+

pHu-

Glycogen



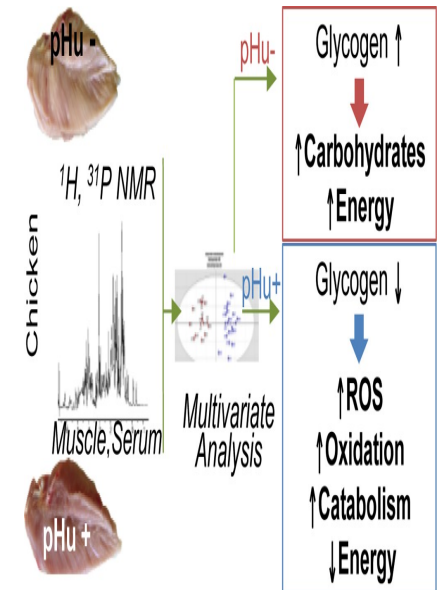
Adult fast-myosin

Embryonic and neonatal fast-myosin

Neonatal fast-myosin

100 μm

**pHu+**: Higher ratio of cells expressing embryonic and neonatal developmental fast-myosins, which could be the sign of higher regeneration process



# Summary : consequences of increased pHu

- Low blood supply → low cell oxygenation, low nutrient supply
- Intensive use of in situ glycogen
- Elevated protein and muscle degradation
- Increased oxidative stress and production of reactive oxidative species (ROS) → altered redox homeostasis
- Muscle dysfunction? Fiber alterations? Link with emergent defects?



# What is the link between metabolic and structural meat-defects ?



?

White striping



Wooden breast



Spongy muscle

# White striping

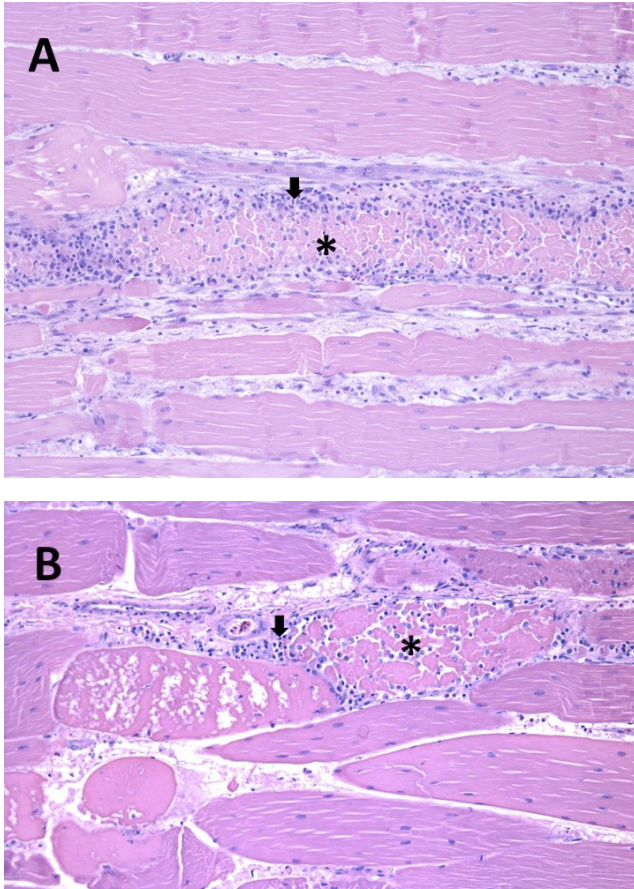


(from: Kuttappan *et al.*, 2012)



# White striping

Longitudinal cross-sections

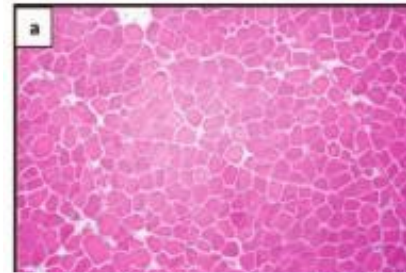


\* = muscle fiber necrosis

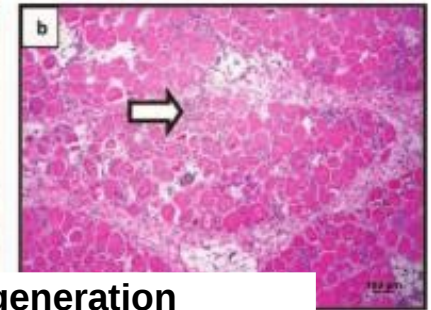
↑ Neutrophil and macrophage infiltration

Transversal cross-section

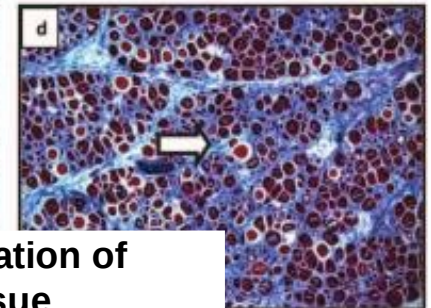
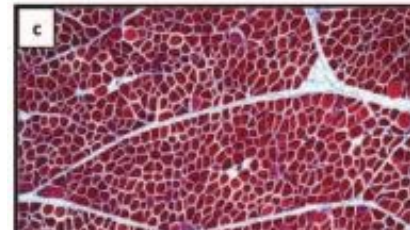
**NORMAL**



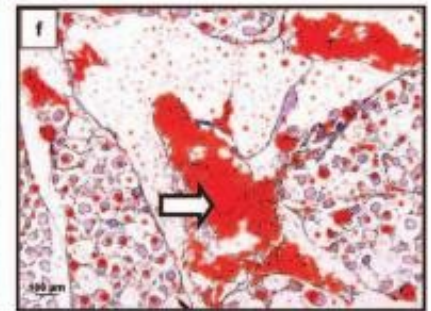
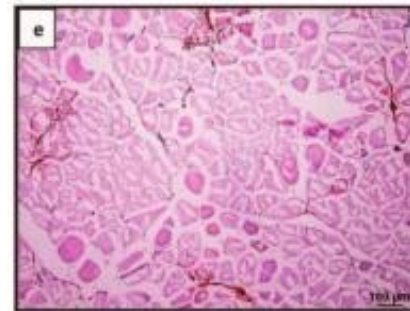
**WHITE STRIPING**



**Muscle fiber degeneration**



**Fibrosis: accumulation of connective tissue**

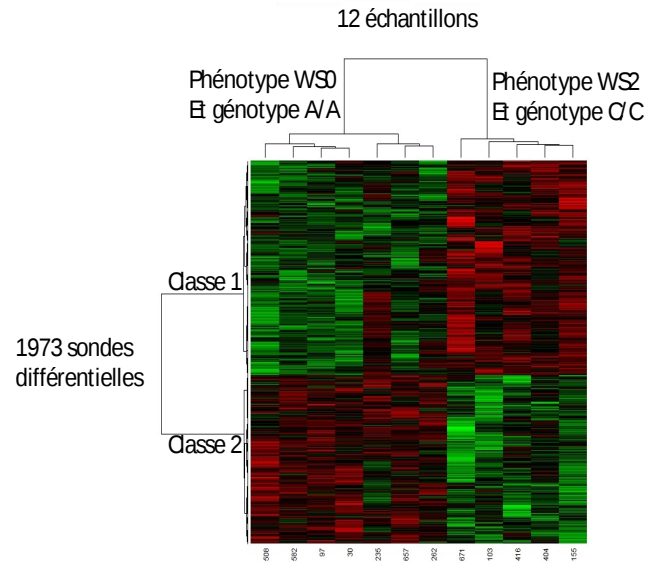


**Lipidose: accumulation of lipids**

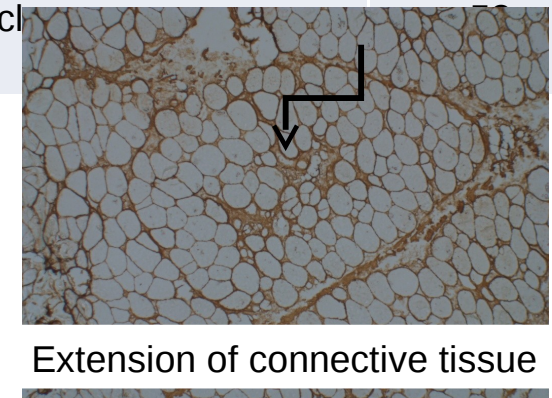
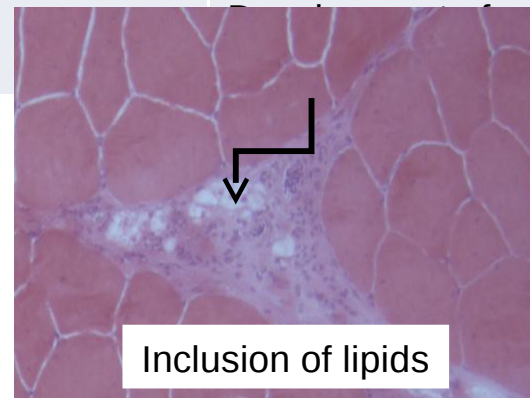
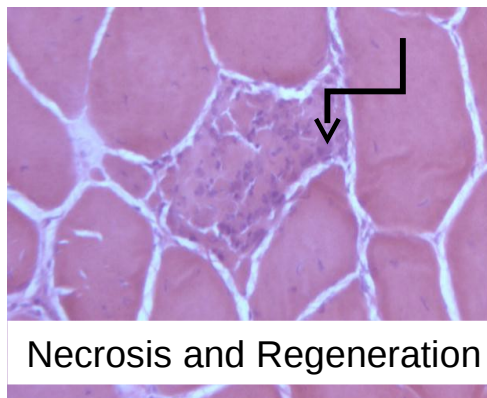
# White striping

(unpublished results)

- Global gene expression analysis (microarrays) → functional analysis



	Fonctions identifiées par IPA®	Differential genes
Pathways	Wnt/Ca <sup>+</sup> : regulation of cellular Ca <sup>++</sup> , involved in embryonic tissue development	43
	HGF (hepatocyte Growth Factor) : activation of satellite cells during muscle regeneration	85
Disorders	Pathology of connective tissue: hyperplasia of connective tissue and lipodystrophy	28



# Wooden Breast



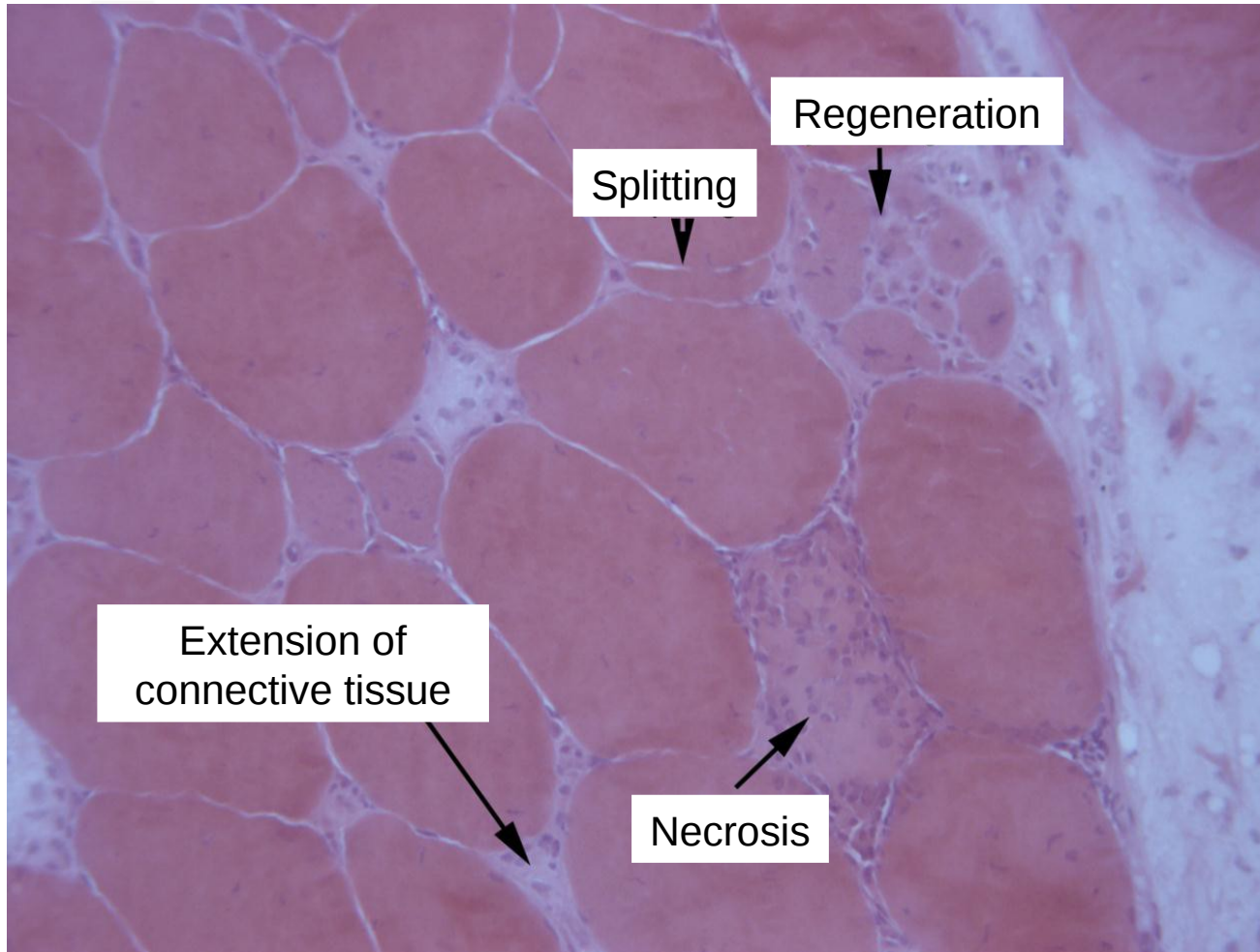
Decreased blood supply?

Decreased glycogen store?

	Shear force	Ultimate pH	Lightness	redness
Normal	8.39	5.88	48.74	0.19
Wooden breast	<b>16.24</b>	<b>6.09</b>	50.18	<b>-1.46</b>



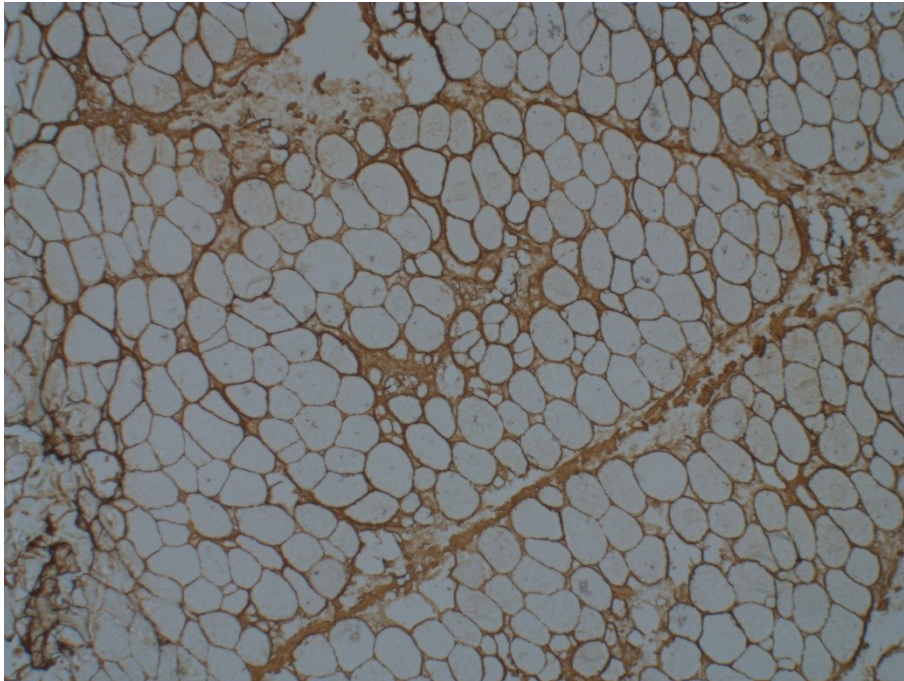
# Wooden breast



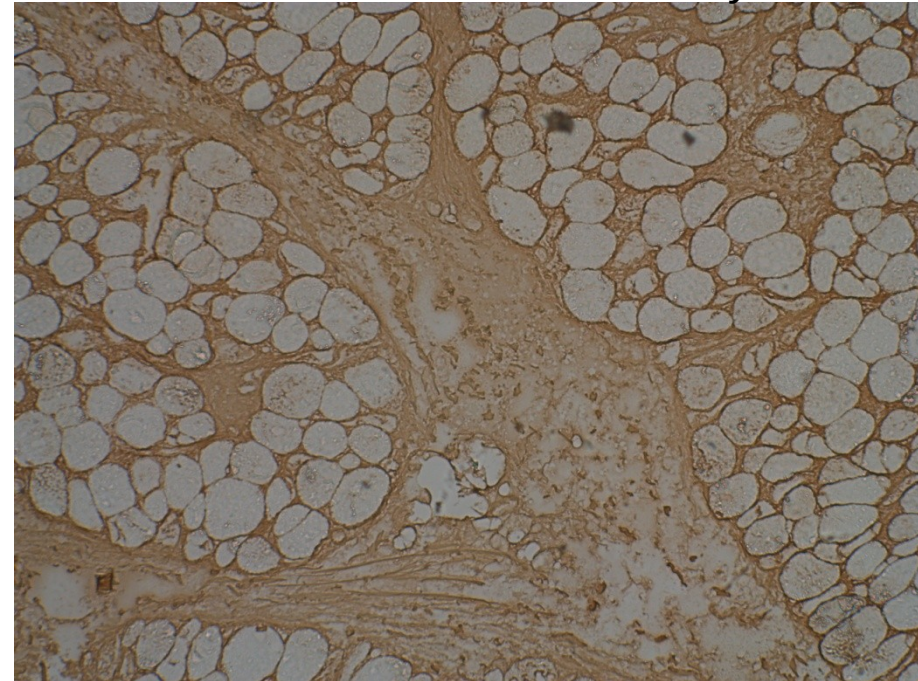
# Wooden breast

- Very extended fibrosis (collagen III immunolabelling)

Normal muscle

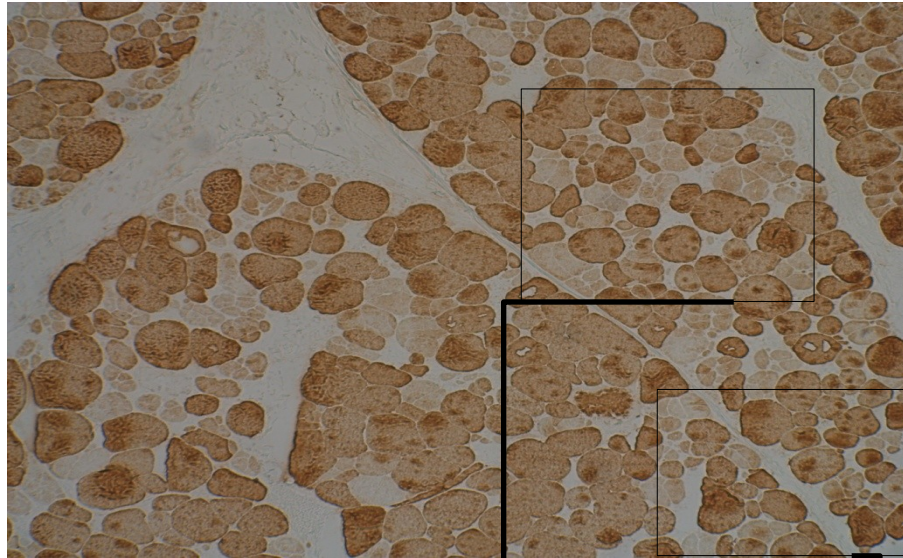


Woody muscle



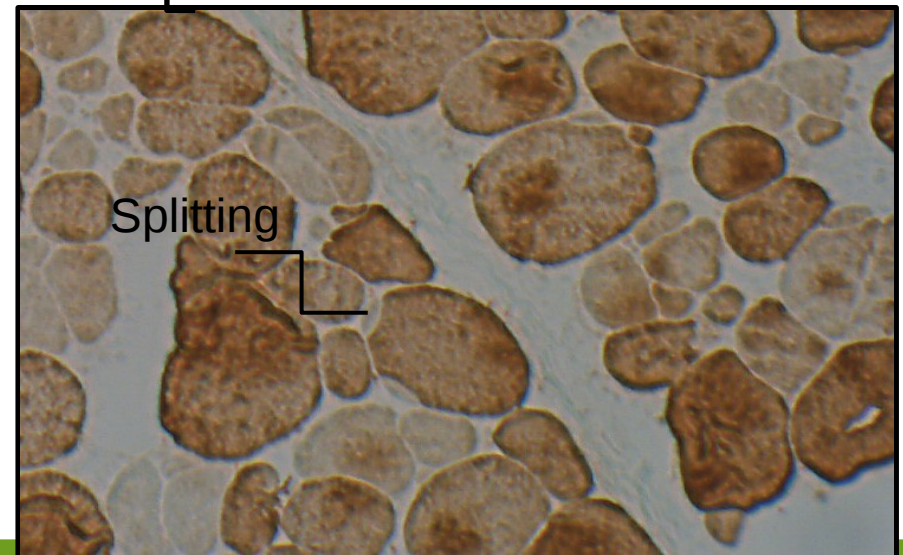
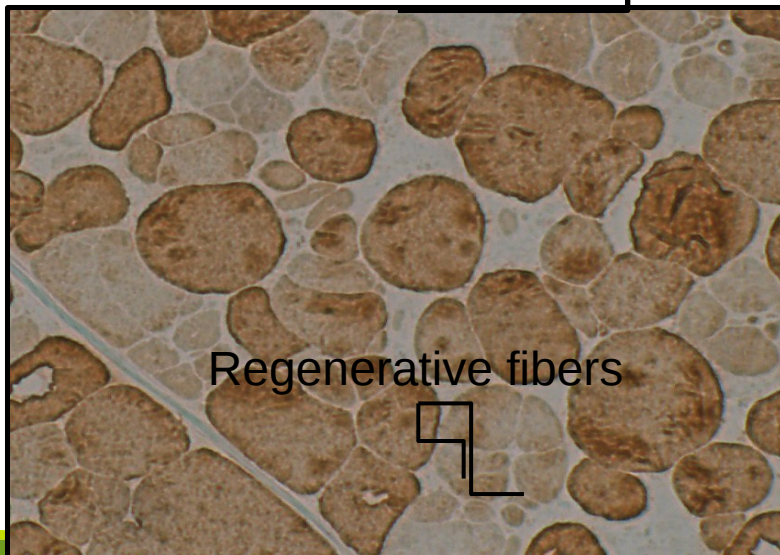


# Wooden breast



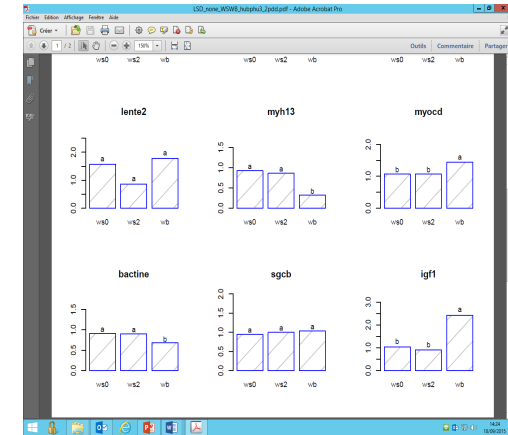
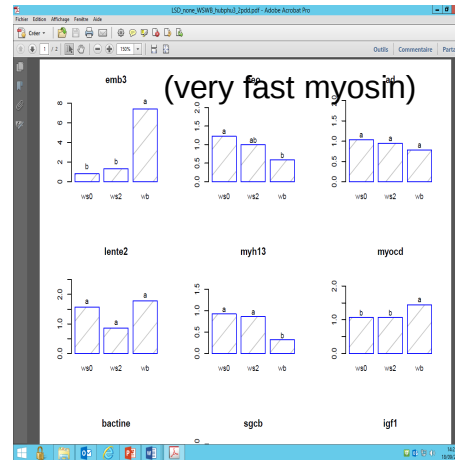
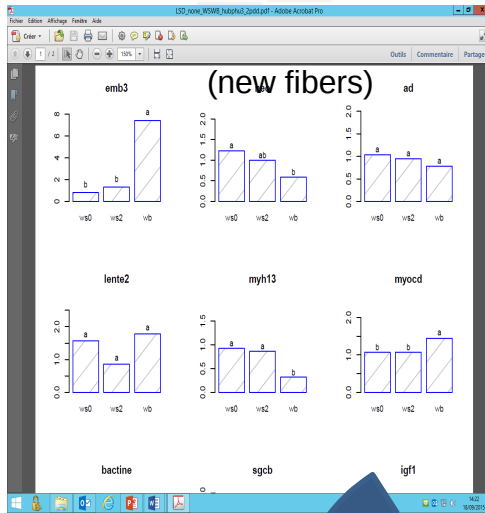
- Muscle fibers variable in size
- Regenerative fibers
- Splitting

(Adult Myosin Heavy Chain labelling)

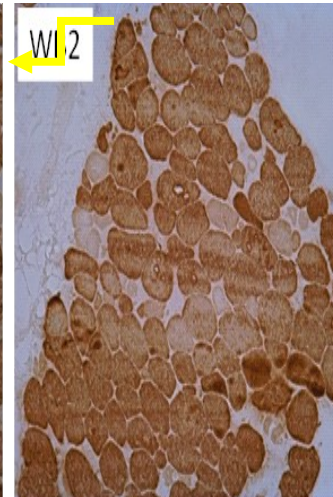
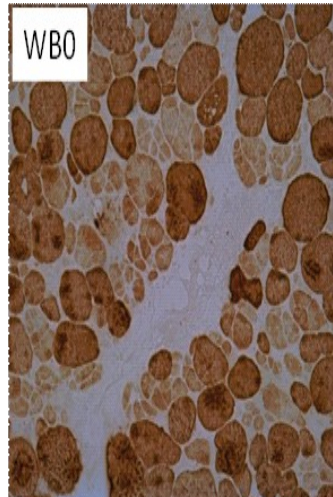


# Wooden breast

- Change in muscle typing
- Muscle growth factor



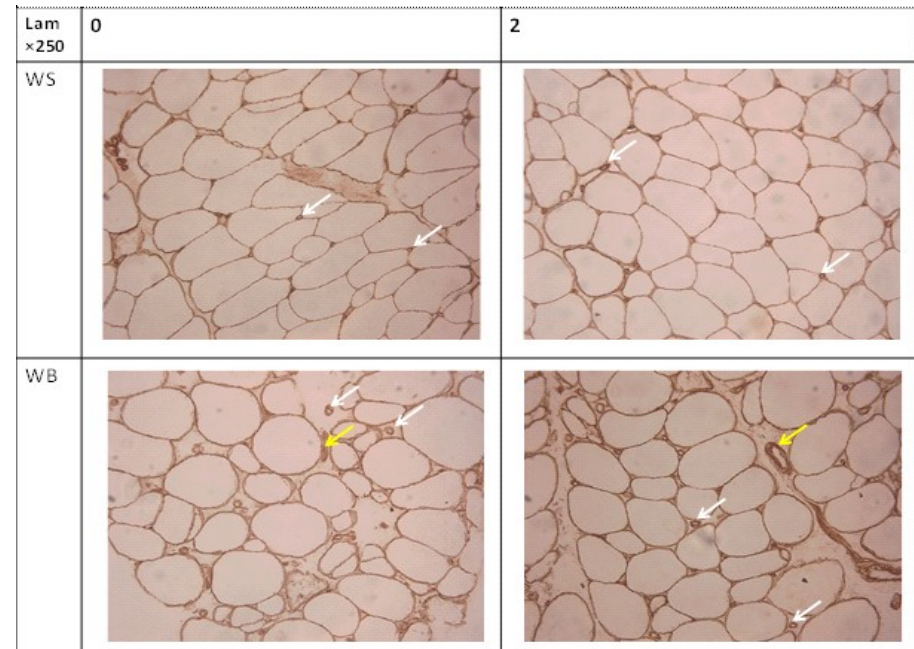
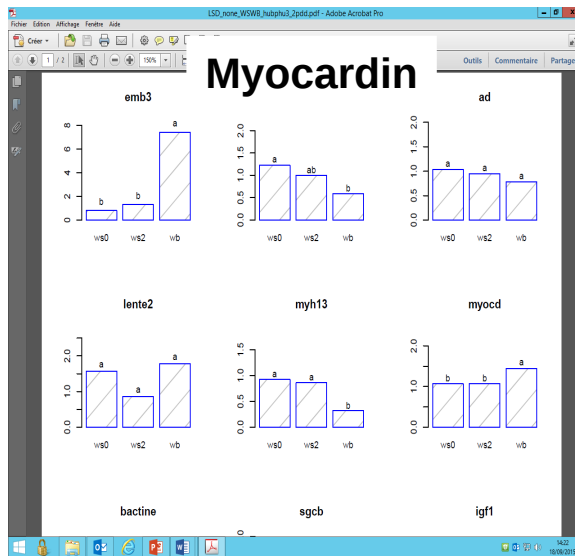
↑ Marker of early stages of development



↑ proliferation and differentiation of muscle fibers

# Wooden breast

- o Smooth muscle formation
- o Angiogenesis



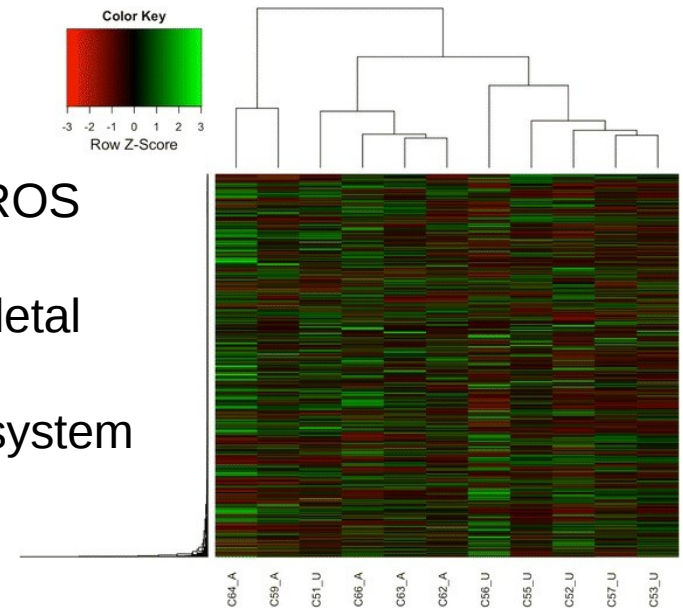
- o Capillaries disconnection: poorer oxygenation, and lower nutrient intake of fiber



# Wooden breast

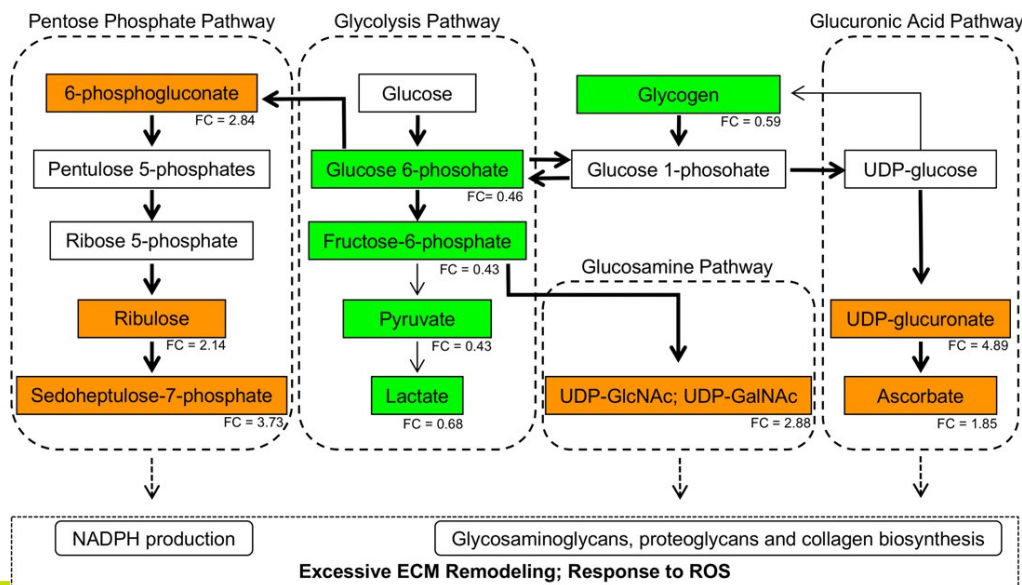
(Mutryn et al., 2015)

- Global gene expression analysis by RNA-sequencing:
  - Abnormal accumulation of intracellular calcium
  - Hypoxia
  - Increased oxidative stress and accumulation of ROS
  - Fiber-type switching (fast to slow)
  - Increased cellular repair and regeneration in skeletal muscle
  - Downregulation of genes related to coagulation system



# Wooden breast

- Metabolite profiling (GC/MS; LC-MS/MS) (Abasht et al., 2016)
- Much lower glycogen content (1.7-fold)
- Increased oxidative stress,
- Elevated protein levels,
- Muscle degradation,
- Altered glucose utilization.
- Altered redox homeostasis (↑ hypoxanthine, xanthine)



**WB**



**Common regulations between WB and pHu+ muscles**



**pHu+**

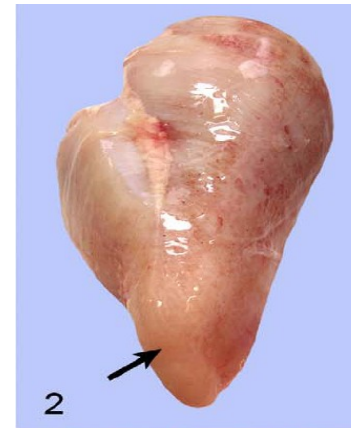
# Major meat quality defects

## pH-related defects



## Muscle 'dystrophic' syndromes

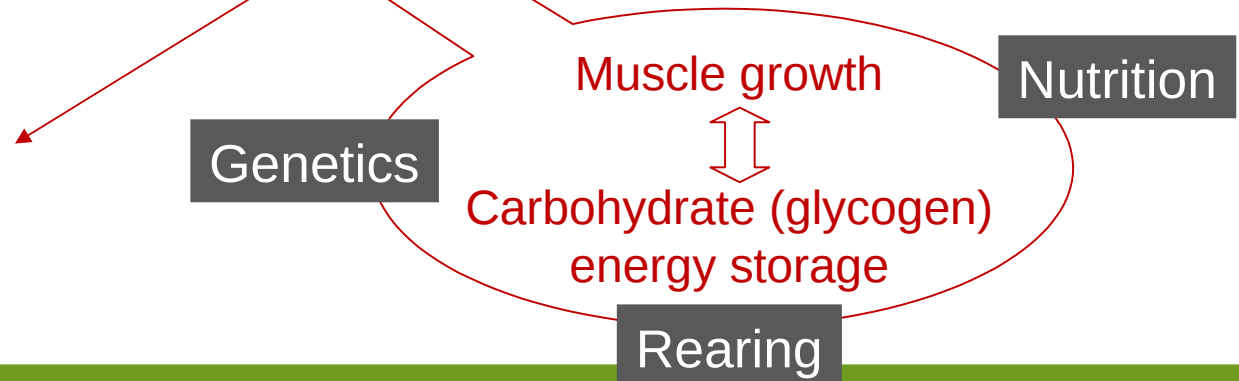
Wooden breast



White stripping



## Oxidative damages

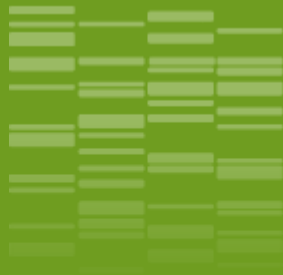


# Conclusions

- Genomics and metabolomics are very helpful to **unravel meat quality determinism** and identify **pertinent biomarkers for diagnosis purpose**
- It highlights some **common regulatory pathways** underlying meat defects related to muscle growth and glycogen depletion...
  - Poor muscle fiber vascularity → poor oxygenation of the muscle, anaerobic glycolysis, oxidative stress
  - Production of ROS (↓ redox homeostasis)
  - Increased protein and amino acid catabolism
  - Change in muscle fiber typing
  - Increased regeneration process
- But also **specific processes** related to wooden breast and white striping
  - Muscle fiber necrosis
  - Cell repair processes (fibrosis, Lipidosis)  
➡ **Pseudo-hypertrophy of muscle (dystrophic condition)**

# Perspectives

- **Developing non invasive methods to identify breeders prone to develop meat defects:**
  - Genetic markers useful for selection (genomics)
  - Biomarkers such as blood metabolite, enzyme activity, or gene expression
- **Ensuring an optimal and harmonious development of broilers with positive impact on animal robustness and meat quality:**
  - Livestock management, especially during early life
    - Preserve glycogen store in muscle,
    - Delay the differentiation processes to produce more fibers of smaller size
    - Increase the pool of available satellite cells to insure proper cell growth and renewal (limit fibrosis and lipidosis)
- **Considering the use of more robust strains with more harmonious body development:**
  - Gains expected in term of mortality, meat waste at the slaughter plant, increased processing yield



# Thanks for your attention

## Special thanks to

- S. BEAUCLERCQ, PhD student
- E. LE BIHAN-DUVAL, senior scientist (genetics)
- Many others at
  - URA – INRA Nouzilly
  - PEAT – INRA Nouzilly

