Muscle quality issues explained by metabolomics

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NC STATE UNIVERSITY

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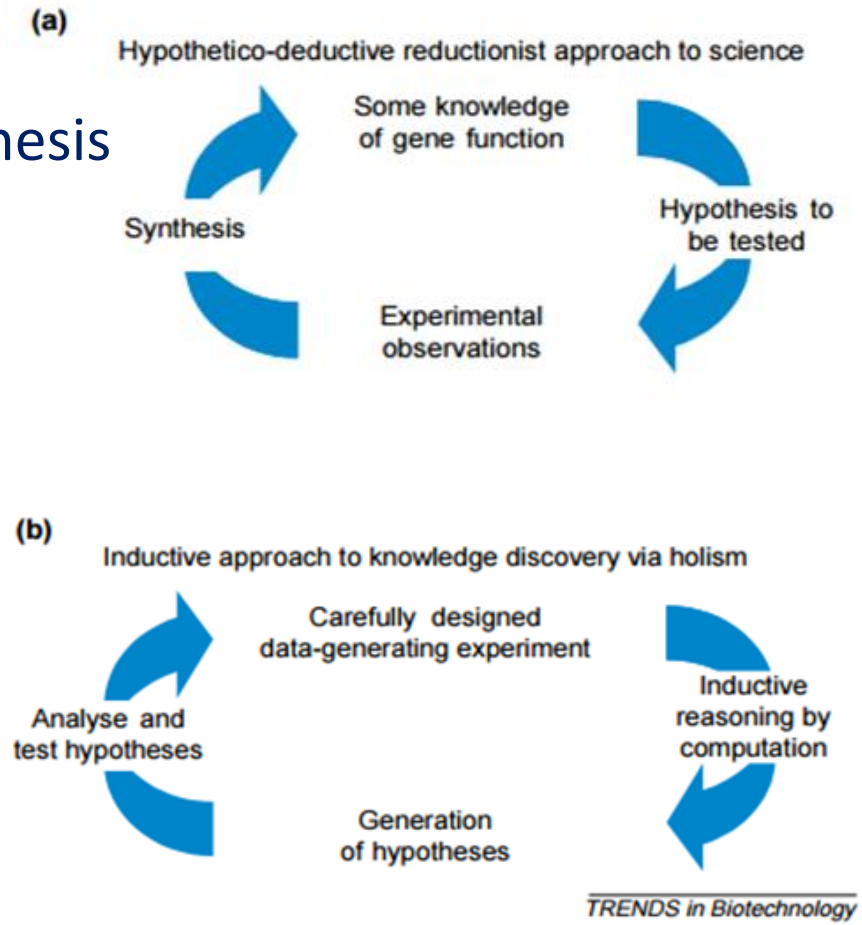
Outline

- Introduction
- Metabolomics
- White Striping
- Application of metabolomics
- Outcome of the study
- Hypothesis
- Hypothesis testing
- Conclusion

Solving biological problems

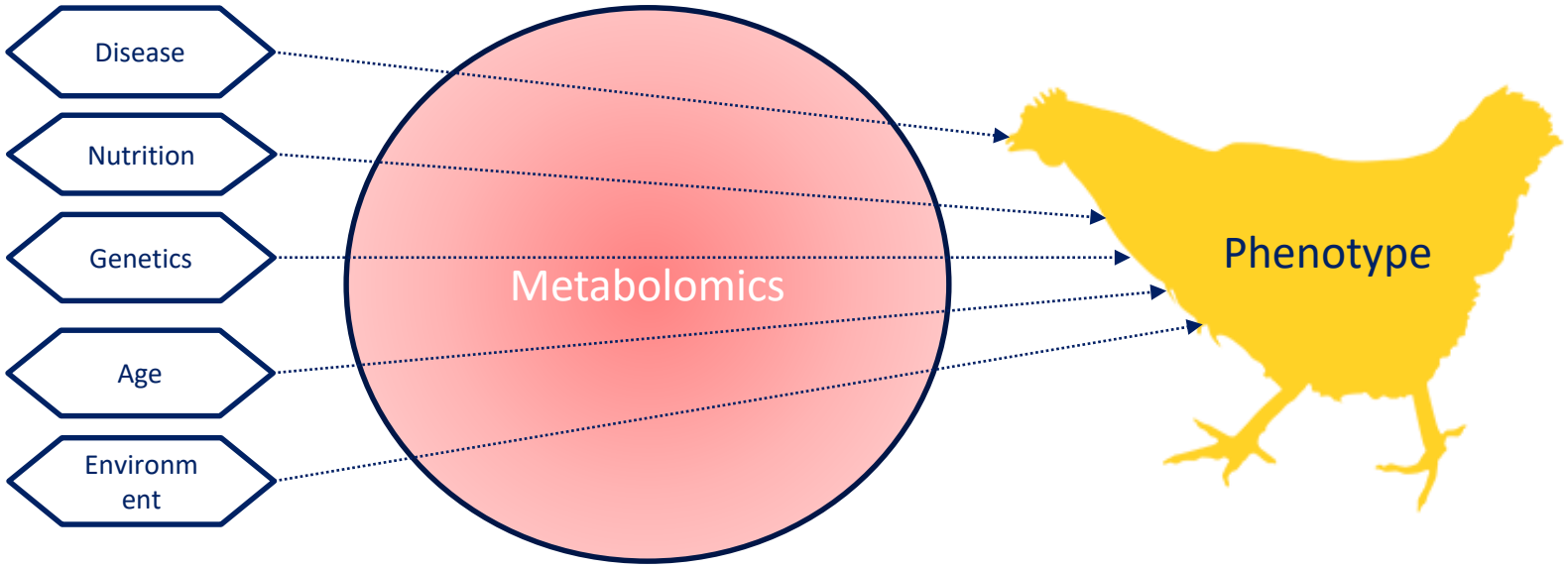
- Traditional cycle:
  - Background knowledge→hypothesis
  - Test experimentally
  - Hypothesis is starting point
- Inductive cycle:
  - No real hypothesis
  - Use data to generate hypothesis



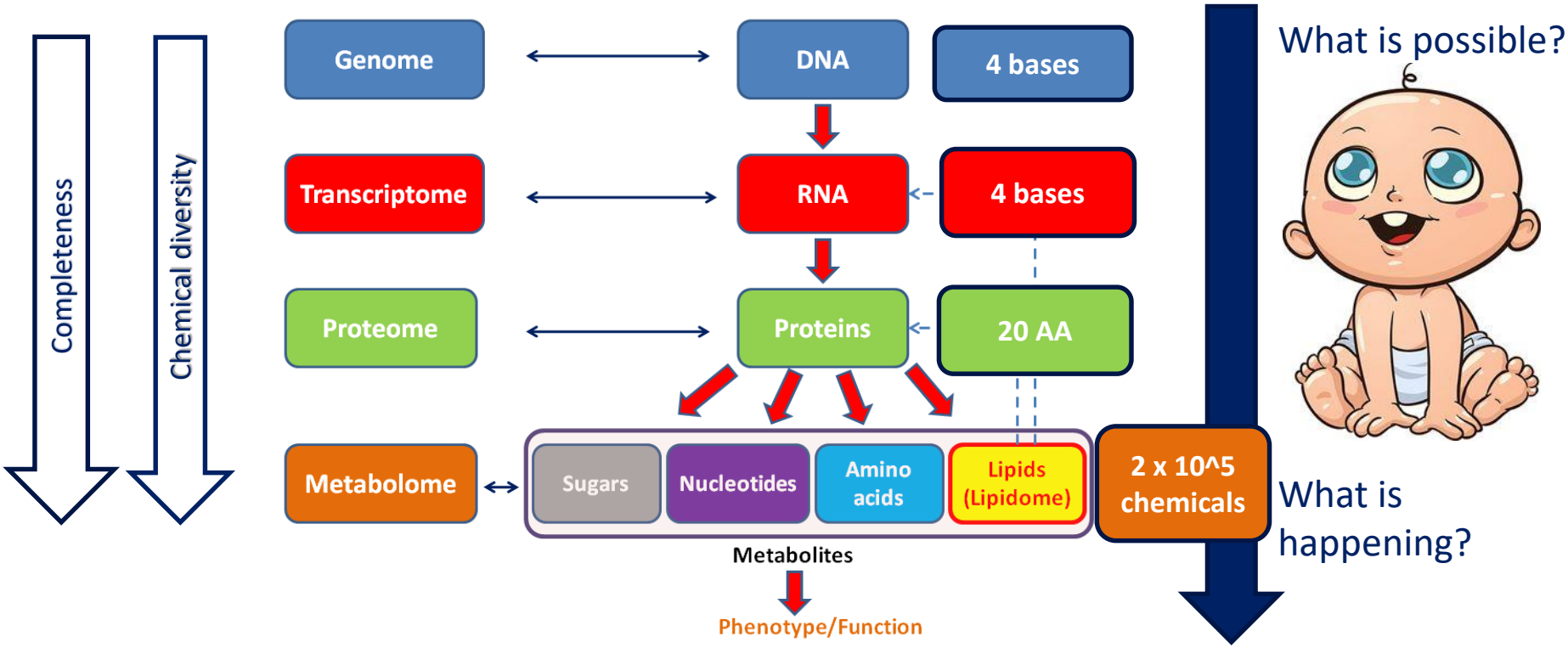
Goodacre 2004

Study as many metabolites as possible

- Ideal for an inductive approach
- Level regarded as ultimate response of system
- Time sensitive → Changes quickly

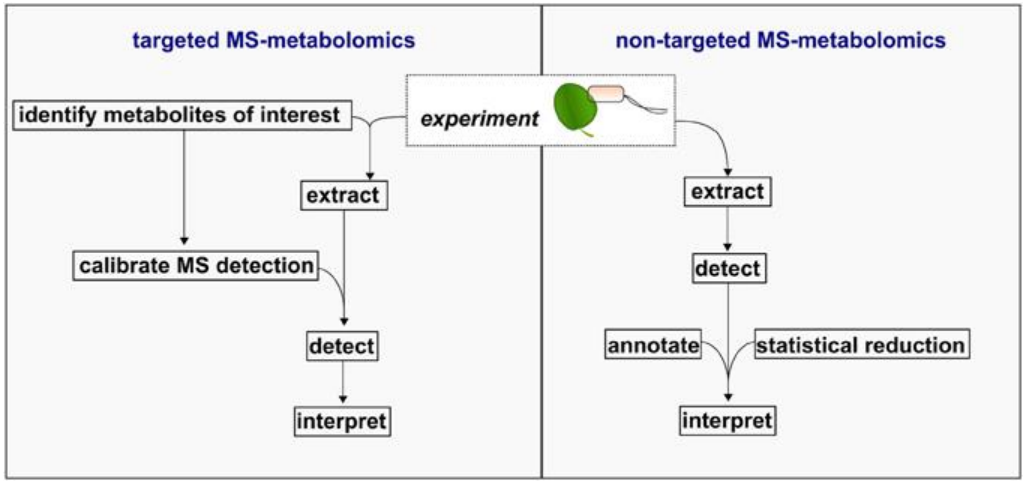


Metabolomics can give insights into biochemical origin of problem: avenue for solutions



Targeted vs Untargeted

- Two methods to perform metabolomics analysis
  - Targeted/quantitative
    - Select group of metabolites
    - Precise quantification
  - Untargeted
    - Global snapshot/fingerprinting



Johnson et al 2016, Cevallos-Cevallos 2009

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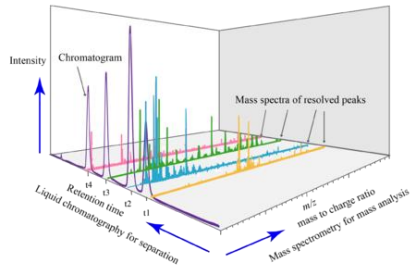
Metabolomics workflow

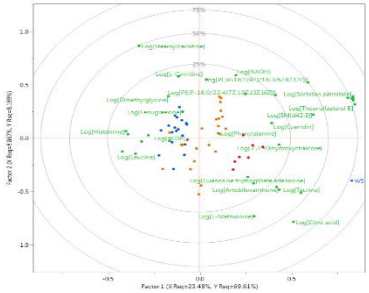
Biological sample

Extraction

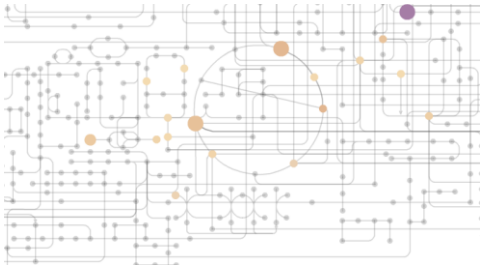
Chemical analysis



Data extraction/ Metabolite identification

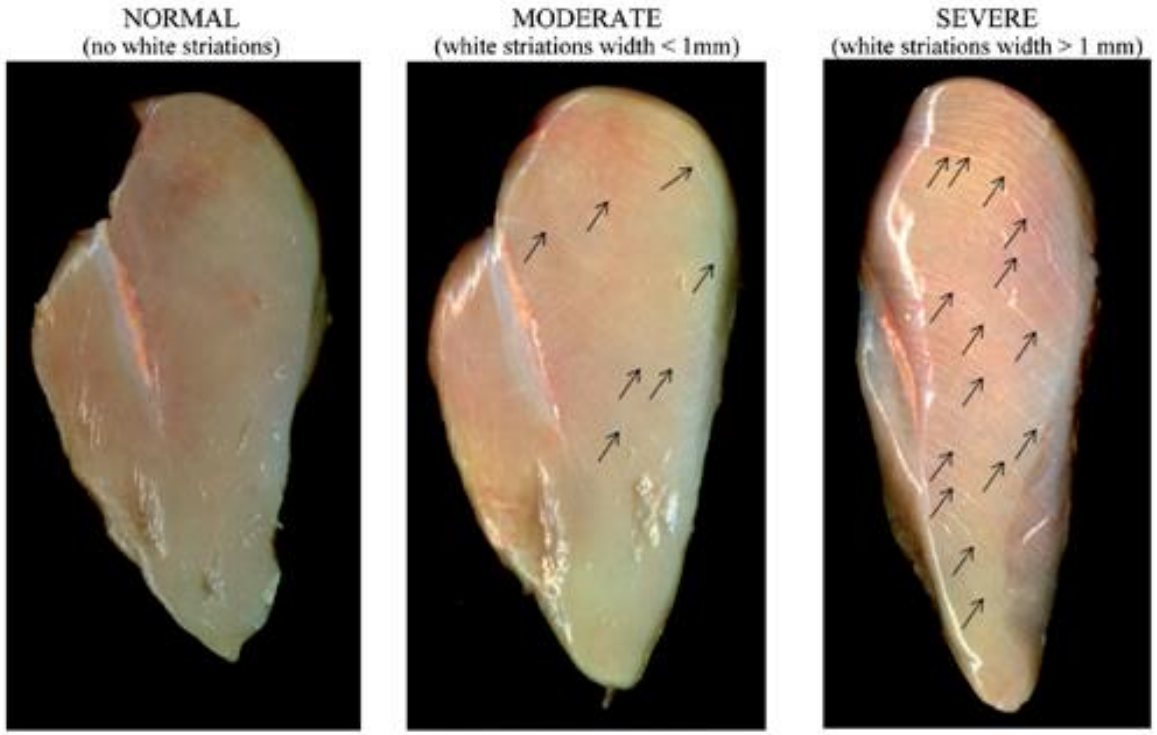


Statistical analysis



Pathway analysis

Metabolomics in practice: White striping



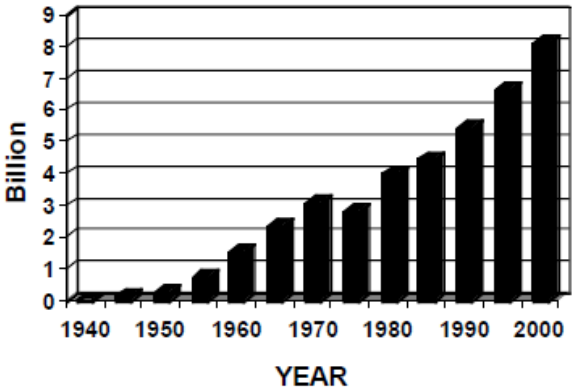
- White stripes on breast muscles
- Various degrees
- Run parallel to direction of muscle fibers
- Unknown etiology

Kuttappan 2013

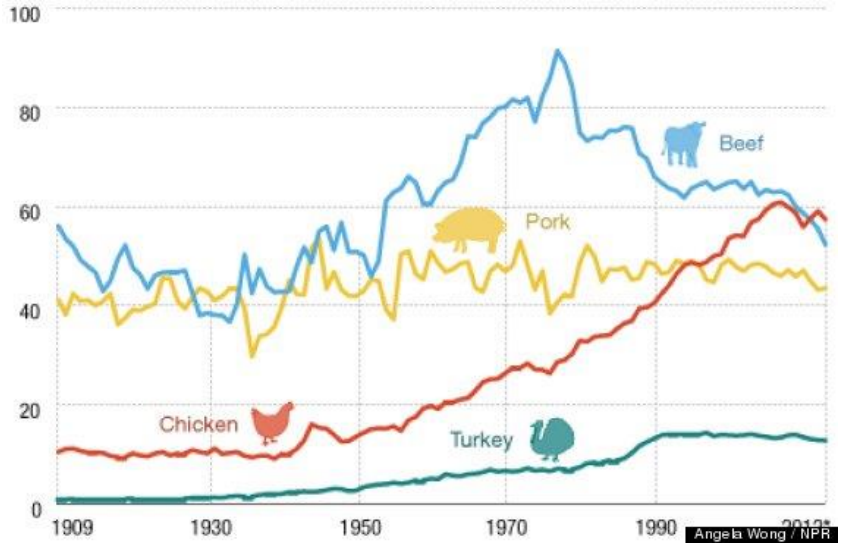
Poultry meat consumption increased over the years

- Low cost
- Good nutritional profile
- Suitable for further processing
- Absence cultural/religious aversions

US broiler production



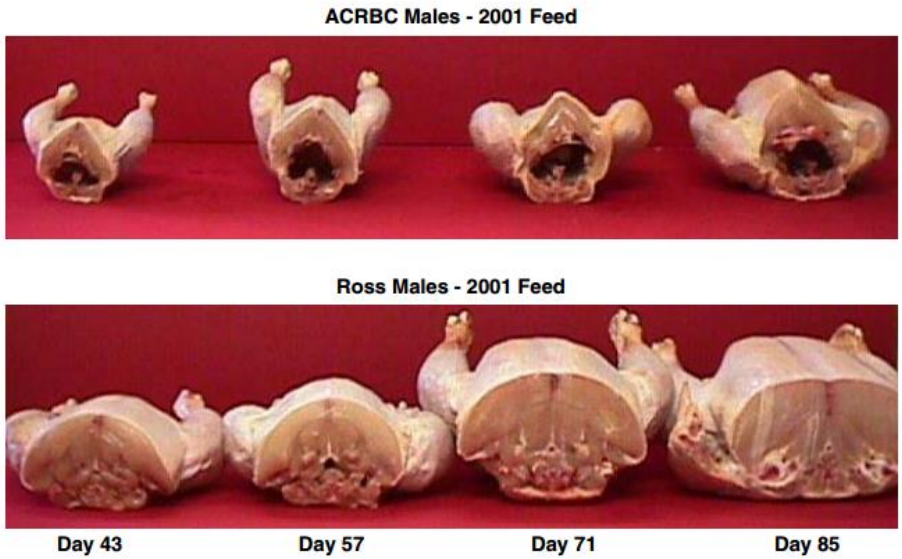
Meat consumption US per person in pounds



Earth policy Institute, Petracci 2015, 2013

Genetic selection made growth possible

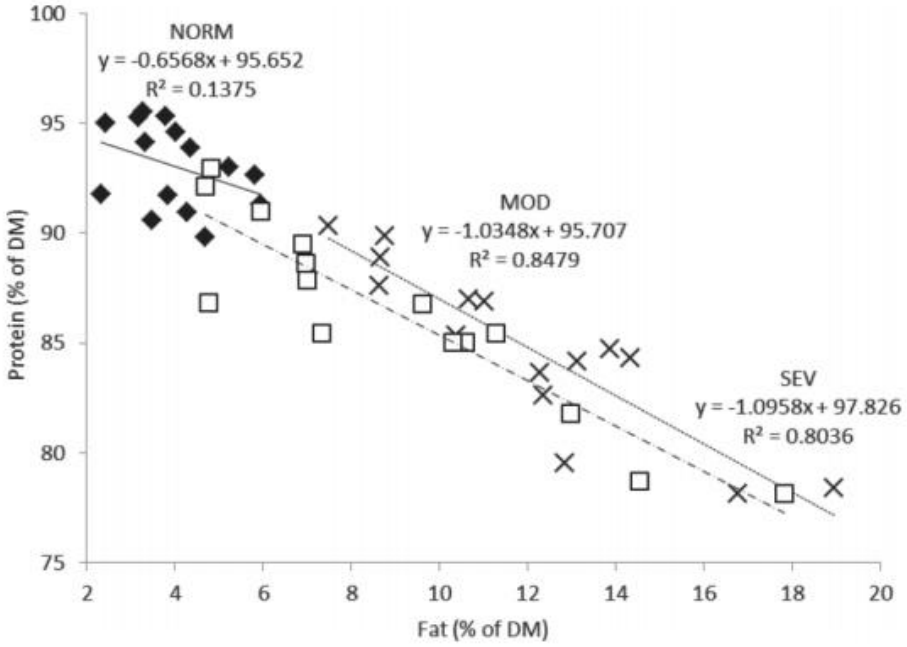
- 85-90% due to genetic selection
  - 10-15% due to nutrition
- In 50 years:
  - Broilers 5 times larger
  - Edible carcass ⬆ 12.3%
- Last 10 years:
  - Breast yield ⬆ 5%



Havenstein 2003

White striping affects meat composition and consumer acceptance

- Pathological changes
  - Increase in fat and collagen
  - Decrease in protein
- Affects consumer acceptance
  - Perceived high fat
  - >50% would not buy



Kuttappan et al 2013, 2012

Why Your Chicken Breast Has White Stripes + What It Means for You

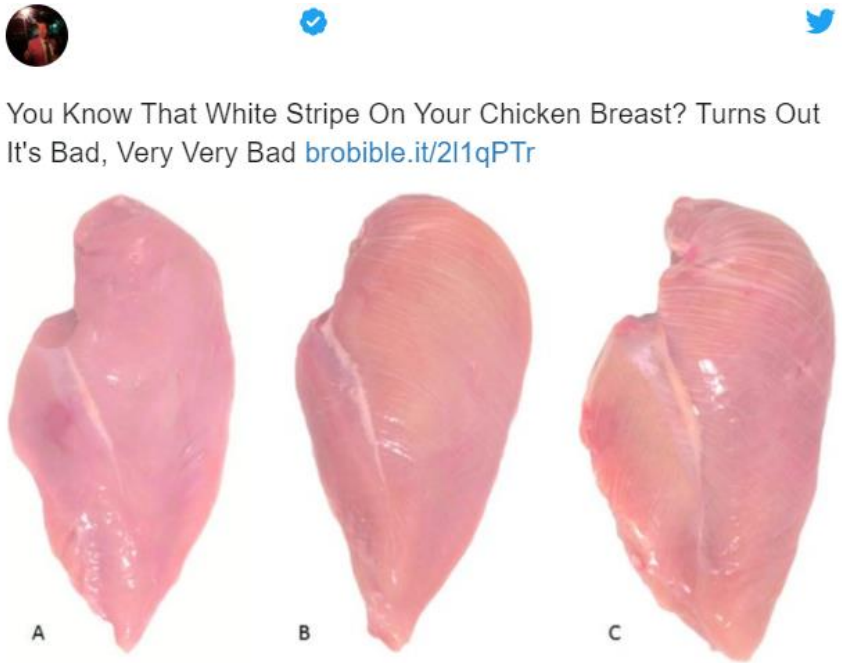
LYNDSAY BURGINGER | POSTED APRIL 19, 2019

HEALTH

What Those White Stripes on Chicken Breasts Mean for Your Health

CLUCKING HELL This is what the white stripes in your chicken really are... and why you need to look out for them

Most people who regularly eat the nation's favourite meat will have noticed pale stripes cropping up in raw cuts of chicken breasts without really knowing what they are



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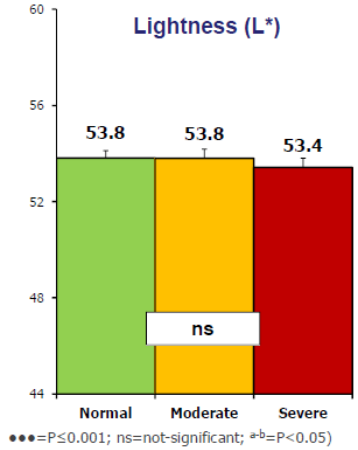
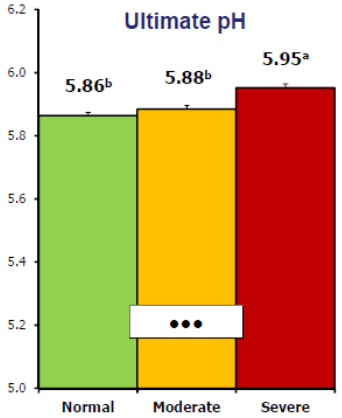
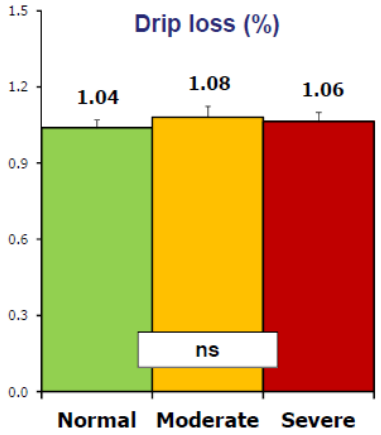
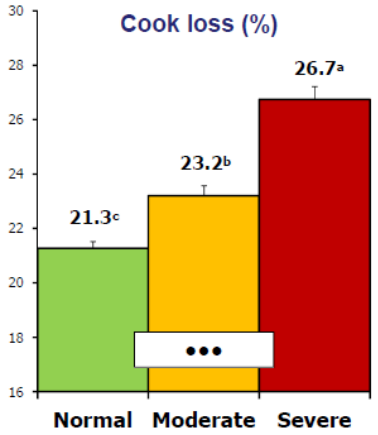
Petracci showed influence on meat quality





●●●=P≤0.001; ns=not-significant; [a-b]=P<0.05)





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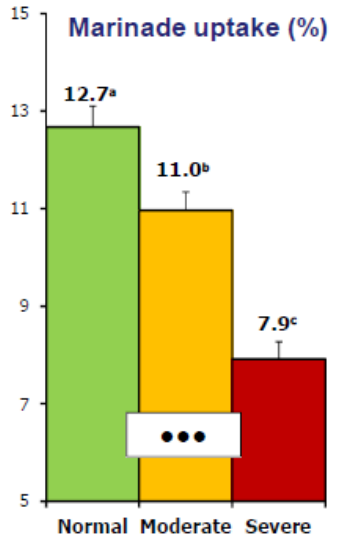

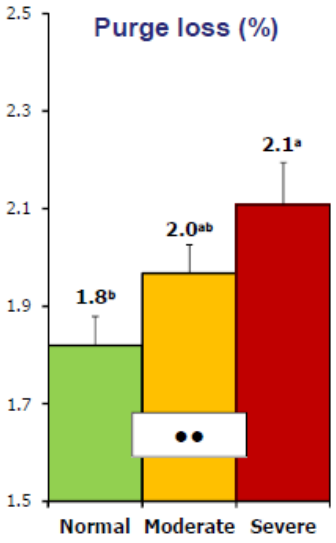

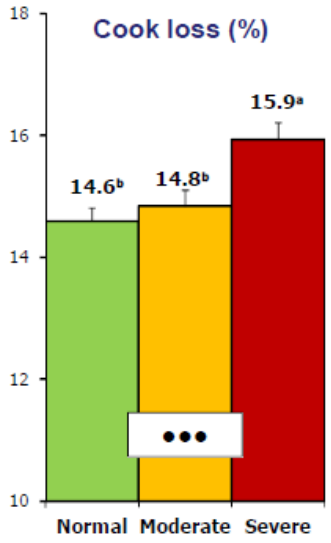

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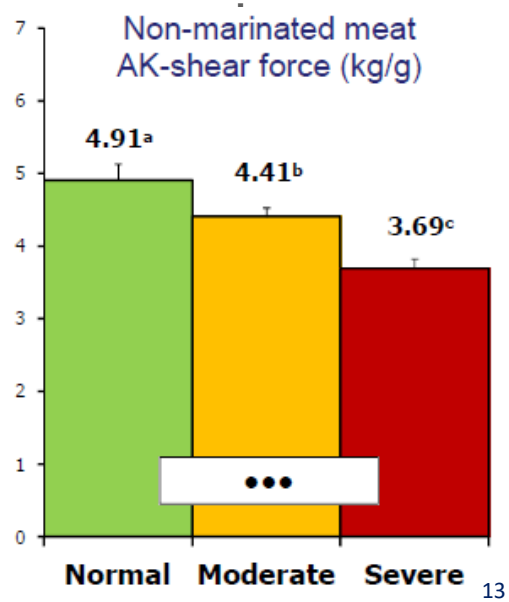

●●●=P≤0.001; [a-c]=P<0.05

Petracci 2014, 2012

No clear etiology

- Factors linked to the occurrence of the disease

Live production factors	Mode of action on white striping occurrence	References
Genotype	High > standard breast-yield	Petracci et al. (2013b)
		Lorenzi et al. (2014)
Sex	males > females	Kuttappan et al. (2013a)
		Lorenzi et al. (2014)
Growth rate	higher > lower	Kuttappan et al. (2012a; 2013b)
		Lorenzi et al. (2014)
Diet	high > low energy diet	Kuttappan et al. (2012a)
Slaughtering weight	Heavier > lighter	Kuttappan et al. (2013a)
		Lorenzi et al. (2014)

Aim of the study

- Identify biological pathways involved in the underlying etiology and pathogenesis of white striping

Trial at our poultry facility

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Sampling most important step

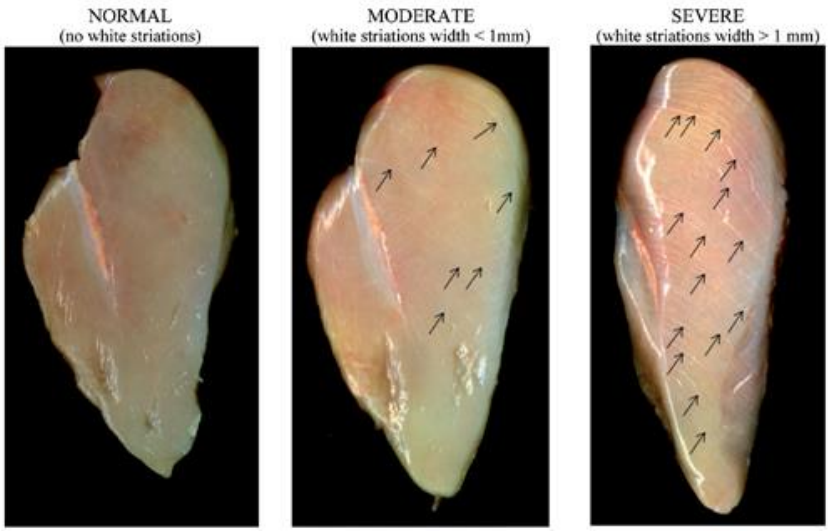
- Sample collection most important step in metabolomics
- Make sure to minimize variance introduction
  - Collect without bias
  - Metabolome responds to almost all outside influences
  - Sampling and handling procedures must be identical between groups
  - Tissue is still active
    - Rigorous collection + handling protocols

Lin et al 2007

Pectoralis major samples collected at necropsy

- ROSS 308 males
- Raised to 35 days
- 3-phase feeding using commercial feed (wheat-based)
- Final BW: ≈2300 gr
- Pectoralis major samples (N=51) collected at slaughter
- WS scoring based on Kuttappan et al 2012

	Total incidence (%)
• Normal (N=19)	32
• Moderate (N=24)	59
• Severe (N=8)	9



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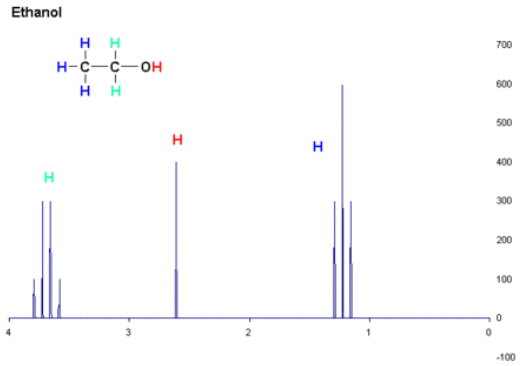
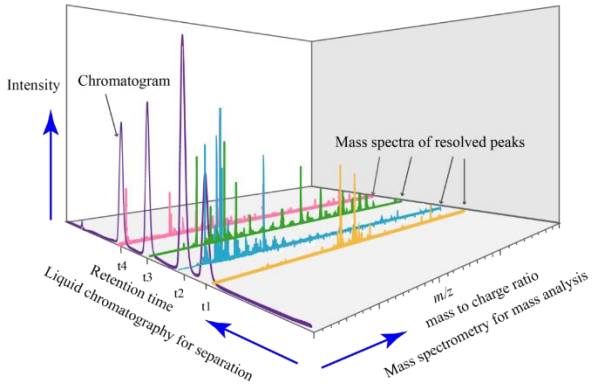
NMR and MS to analyze samples

- NMR:
  - Highly quantitative
  - Reproducible/non-destructive
  - Requires no pre-treatment
  - Low sensitivity



- MS:
  - High sensitivity
  - Molecular specificity
  - Low sample requirements
  - Need for separation/purification



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Wishart 2007, Beltran 2012

Dataset

- GC-MS LC-QTOF/MS used
- Dataset contained 599 metabolites
- Retention time and molecular mass also available
- Some false annotations were removed
  - Components that could not be there
    - Alpha-tocopherol succinate

Data processing to improve biological interpretability

- Extracting relevant biological knowledge a major challenge
  - Unknown metabolites
  - False annotations
  - Difference in order of magnitudes
    - Signal molecules vs sugars

- Best method depends on:
  - Biological question
  - Properties of the dataset
  - Data analysis method

Van den Berg 2006, Bais 2011,Gromski 2014

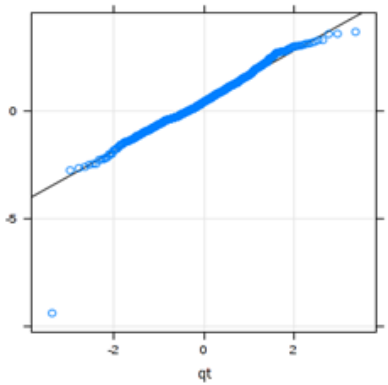
3 common steps applied in data processing

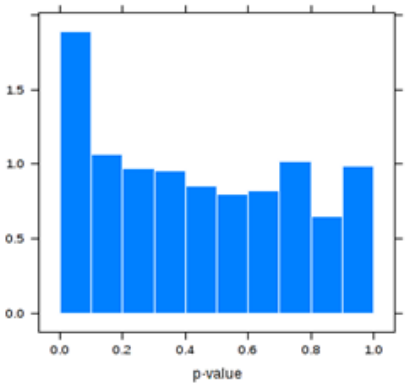
- Sample normalization
  - Reduce non-biological variation
    - Internal standard, reference sample, etc
- Data transformation
  - Remove heteroscedasticity (variability differences)
    - Log transformation, power transformation, etc
- Data scaling
  - Make variables comparable
    - Scaling by sd (auto-scaling)
    - Scaling by biological range (range-scaling)

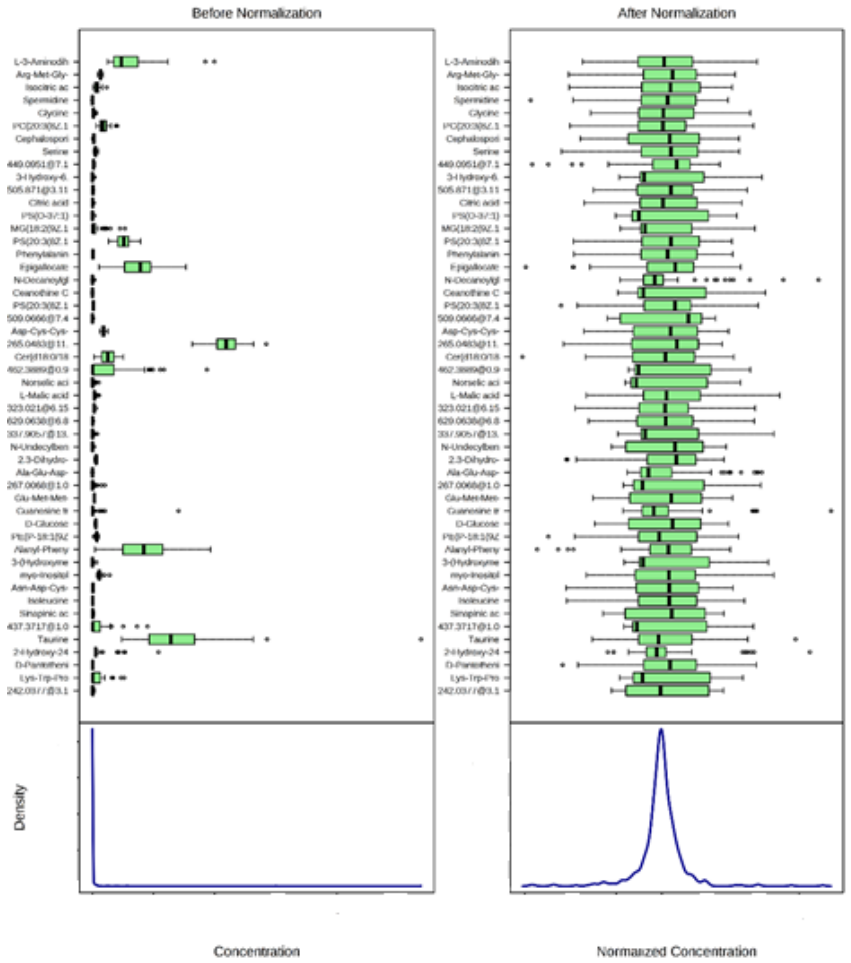
Van den Berg 2006, Bais 2011, Gromski 2014

Data processing showed improvements

- Normalized on internal standard
- Auto-scaling
- Log-transformation







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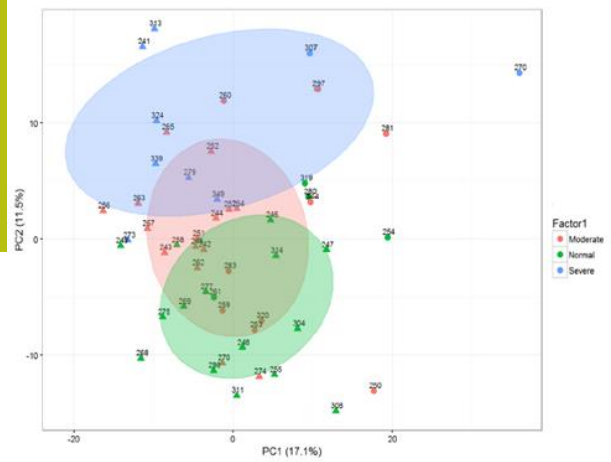
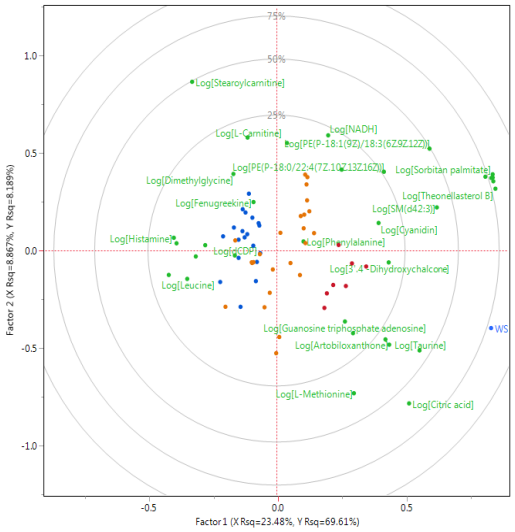
Visualization of data

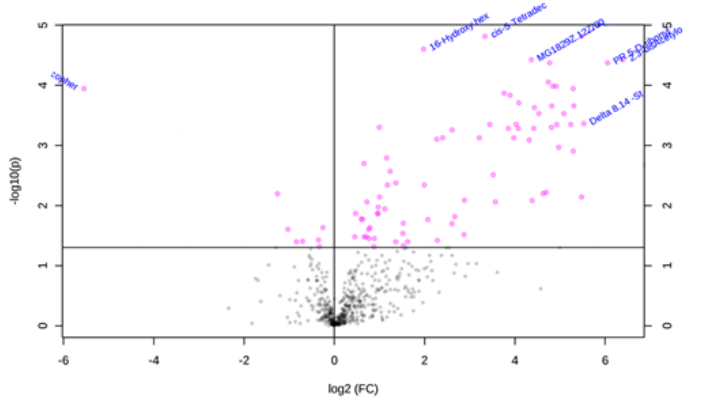


- Visual representation of data + reduce dimensionality
- Principal component analysis
  - Create “super metabolites” that explain max variance



- Partial least square analysis
  - Based on max correlation

- Volcano plot
  - Significance vs fold-change



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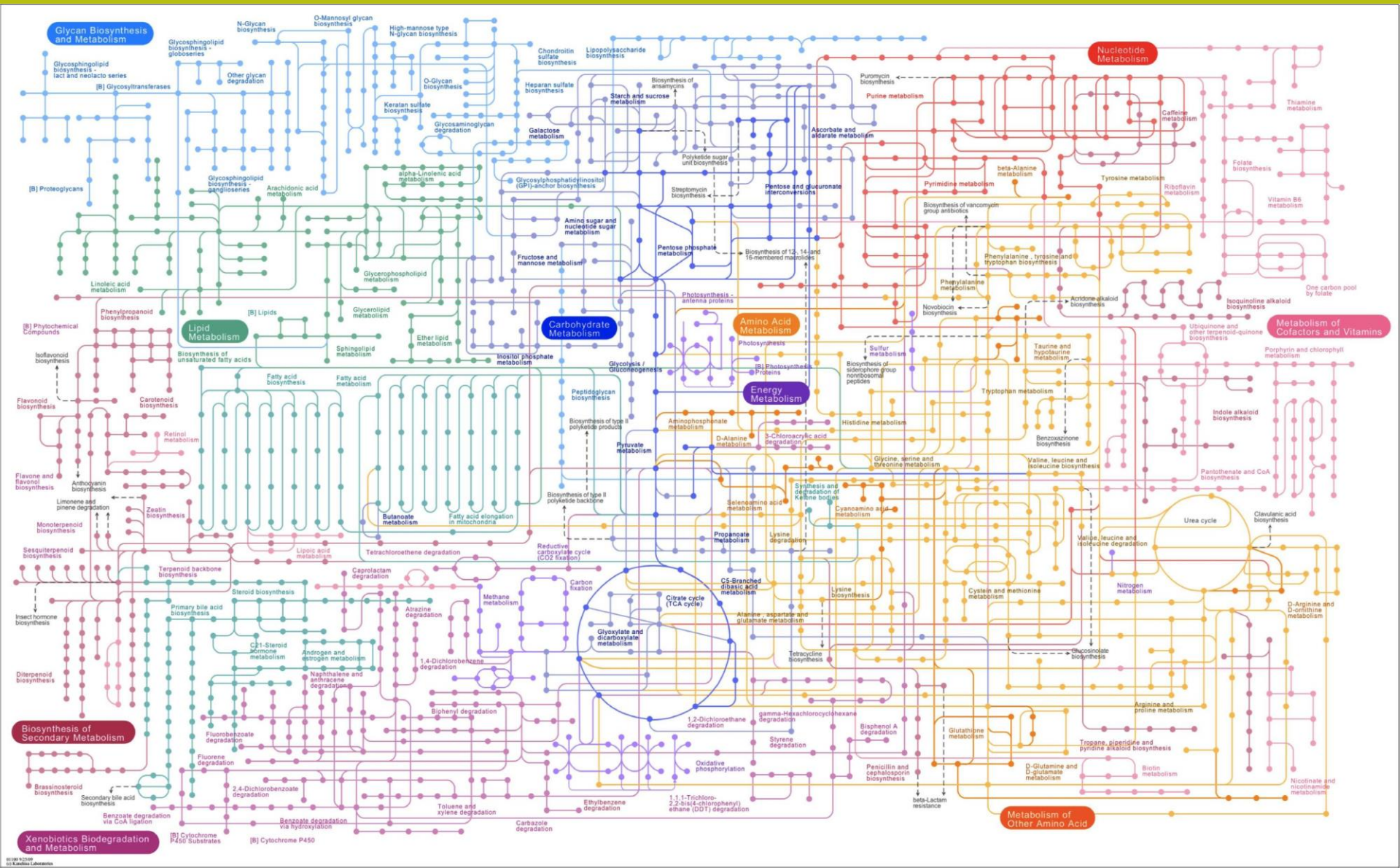
Xi et al 2014, Kemsley et al 2007

Statistical analysis

- Analysis based on design and data distribution

- Large datasets→large amount of comparisons
  - Increased chance of false positives
- False discovery rate or Bonferroni correction
  - Reduces number of false positives

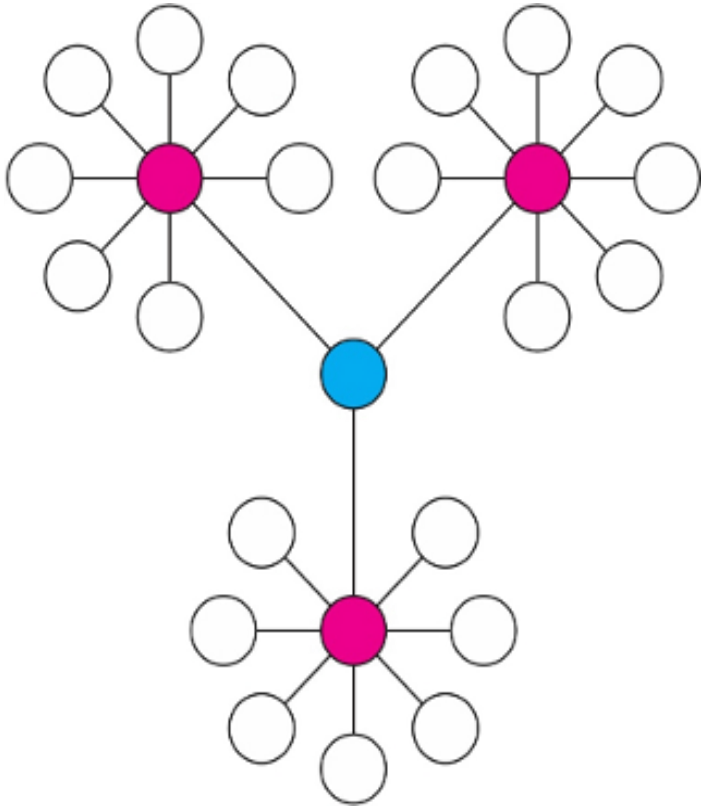
Metabolic pathways



Pathway visualization

- Visualization of changes in pathways
- Pathway enrichment analysis
  - Rank lists metabolites
  - Shows subtle changes

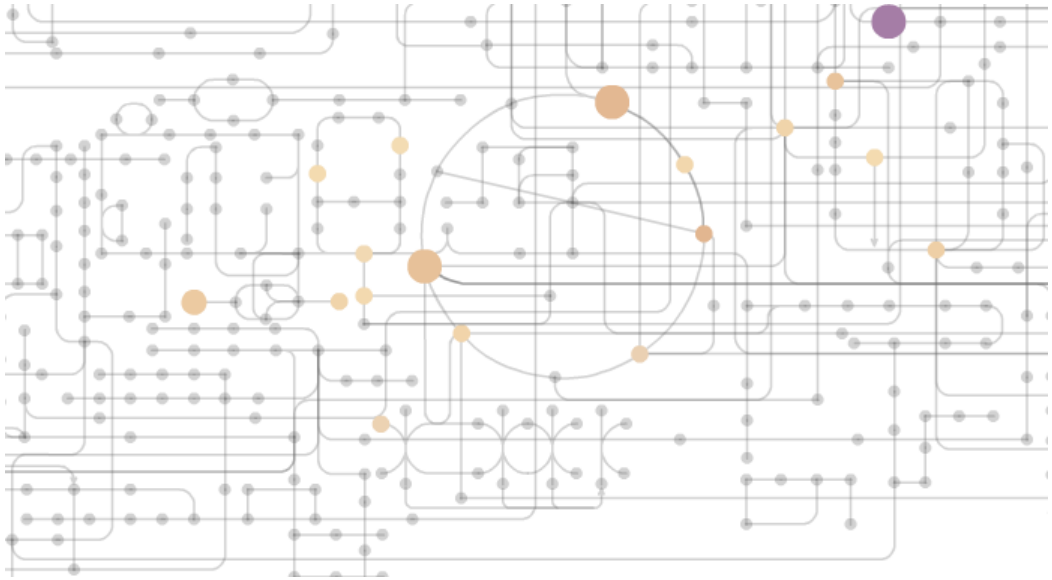
- Pathway analysis
  - Ranks pathways based on metabolite location within a pathway
  - Betweenness centrality (blue)
  - Degree centrality (red)



Ipath

- Gives overview of all changes
- Shows significance and fold change

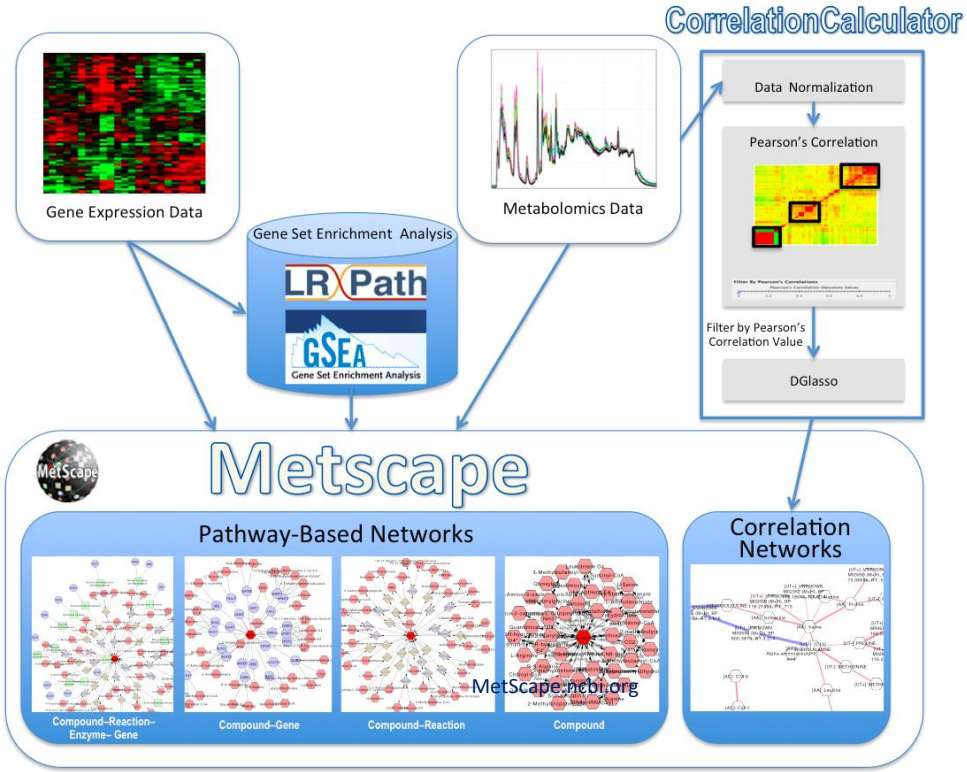
- Cons:
  - Overwhelming



Metscape

- Build pathway based on data
- Multiple build options
- Cons:
  - Only 3 databases

    (human, mouse and rat)



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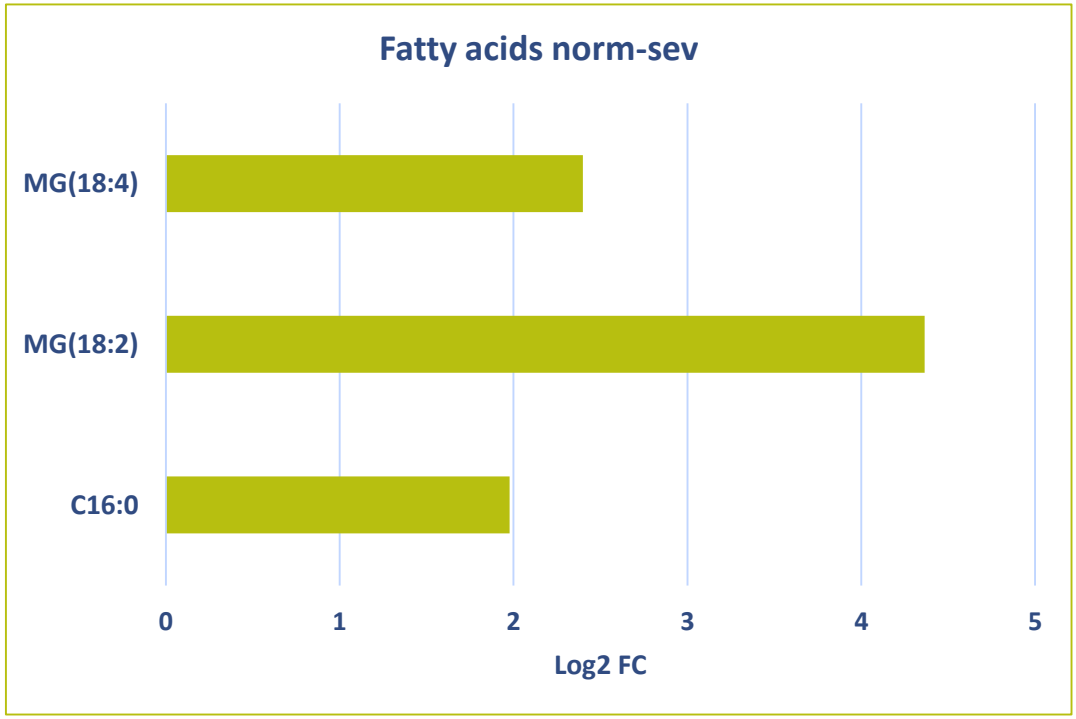
Changes in lipids & carnitine in WS-affected broilers



- Increase in fatty acids
- Increase in cis-5-C14:1 carnitine (marker for β-oxidation disturbances)
- Low levels of C16 + C18 carnitine
  - transfer LCFA from cytoplasm to mitochondria

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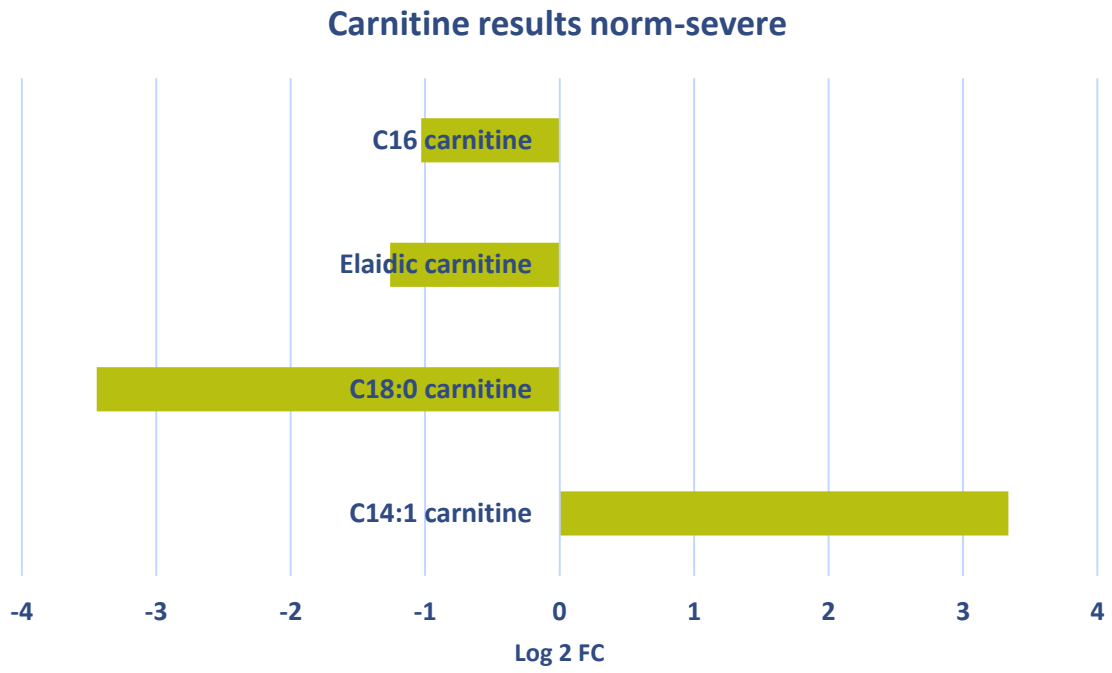
McCoin et al. 2016, Huang et al 1992, Listenberger et al. 2003, Van der Vusse et al. 1992, Gonzalez 2008, Papah et al. 2017, Polak et al. 2009

Changes in lipids & carnitine in WS-affected broilers



- Increase in fatty acids
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Hypoxia can explain changes in beta-oxidation

- Oxygen deprivation→mitochondrial architectural changes

  →diminish mitochondrial beta-oxidation,

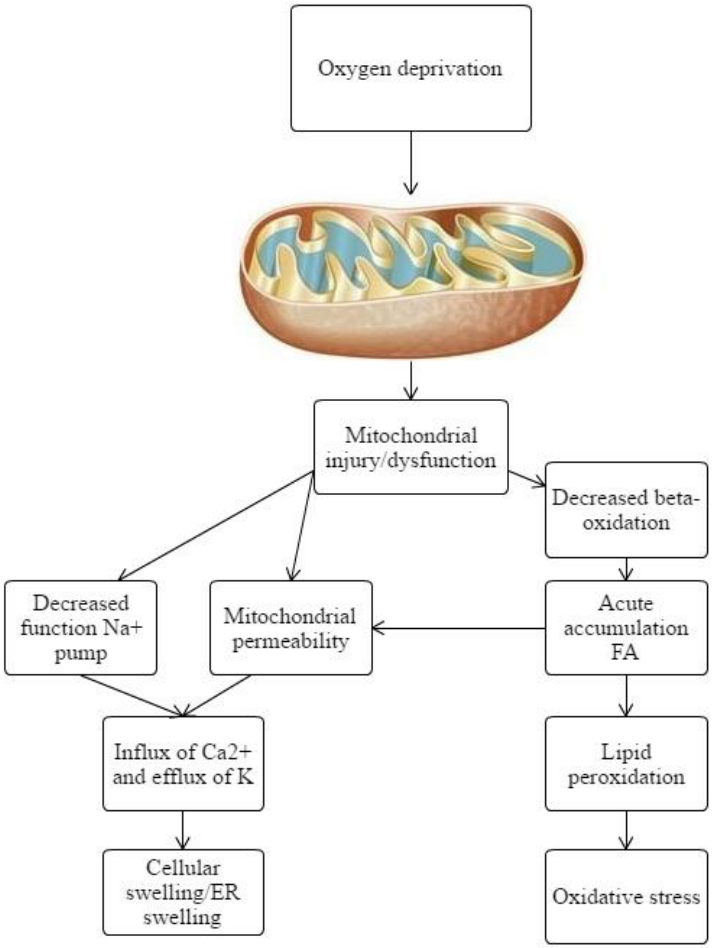
  TCA and respiratory chain→acute accumulation of FA

- Peroxisomes continue → no transporter needed→

  Accumulation of fatty acids intermediates

  - Such as monoacylglycerides→detergents!

- White lesions→attempt to minimize cellular damage from ectotopic extracellular lipids



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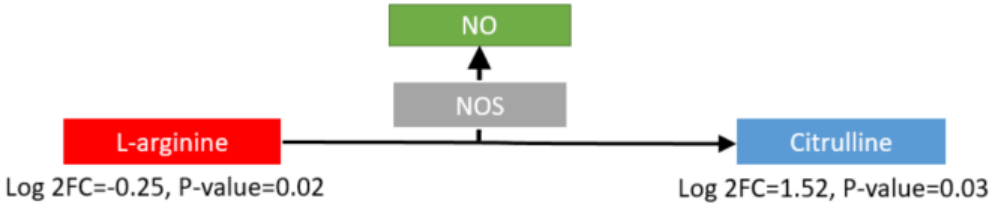
Arginine metabolism increased for NO

- Arginine levels lowered
- Citrulline levels increased
- Increased conversion to create NO
- NO needed for enhanced blood flow
- Limited system
  - broilers unable to synthesize arginine
  - Already maxed out?!

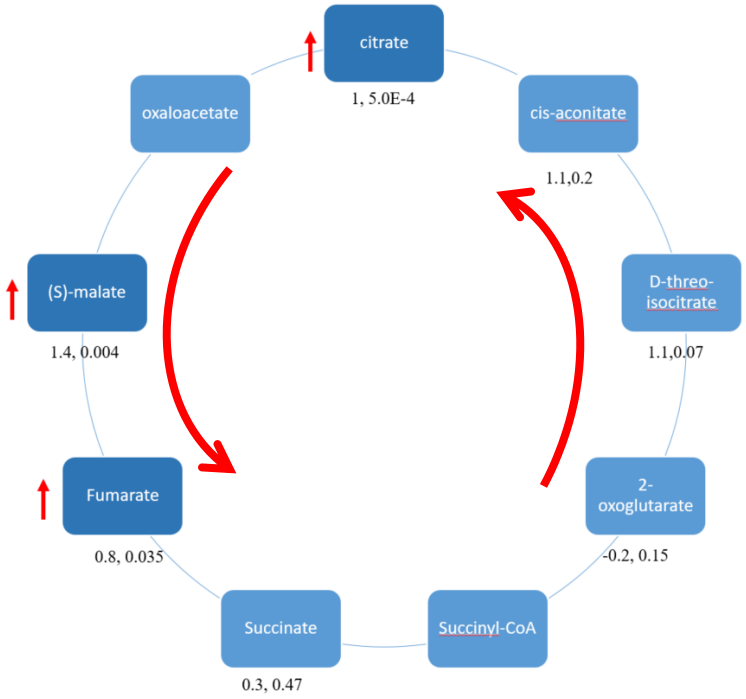
- NO mediator of hypoxia/re-oxygenation-induced damage



Lundberg 2008, Fernandes 2010

Krebs cycle disturbed

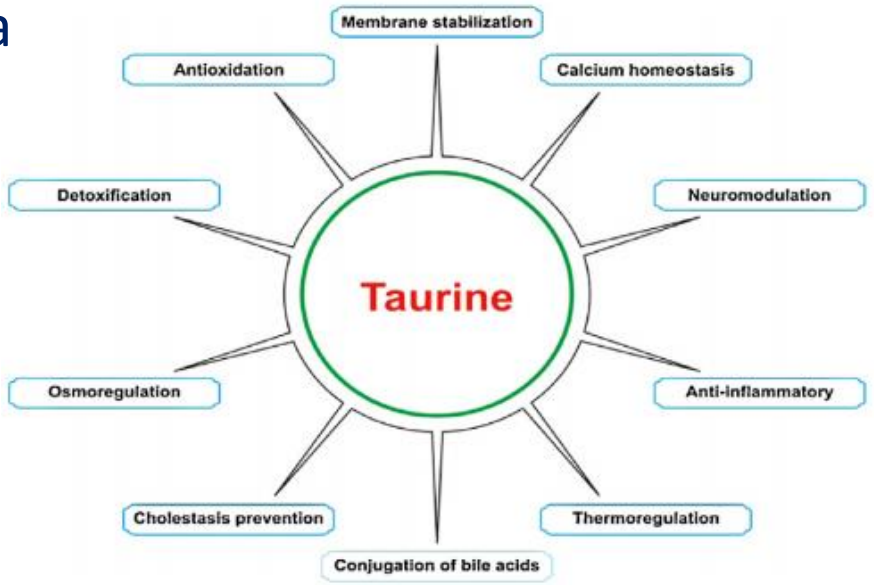
- Increases in citrate, fumurate and malate
- Parts of TCA cycle increases
- Oxaloacetate-fumurate flux/backflux
  - Anaerobic conversion:
  - Oxaloacetate→malate→fumurate →succinate
- Citrate formed from glutamine during hypoxia
  - Glutamine→2-oxoglutarate
  - Accompanied by increase 2-hydroxyglutarate (P:0.04)



Wise et al. 2011, Brekke et al. 2012,

Increase in the osmolyte taurine to prevent damage

- Taurine levels increased 2 fold (P<0.01)
  - Stabilization of the sarcolemma
  - Modulates potassium channels
  - Controls intracellular calcium
  - Released to prevent damage
    - especially calcium-related
  - Higher levels also upon reperfusion



Conte Camerino et al. 2004, Hoving-Bolink et al. 2000, Mankovskaya et al. 2000 , Sirdah 2014

Hypothesis: blood supply is bottleneck

Hypothesis

- Rapid tissue growth
- Blood vessel development borderline adequate
- Animal tries to compensate hypoxia by producing nitric oxide
  - Nitric oxide also leads to oxidative stress

- White striping seems to be caused by hypoxic conditions resulting from inadequate blood flow

Conclusion: metabolomics is a great tool for meat sciences

- White striping had no clear etiology
- Metabolomics great to increase understanding
- Solution found based on metabolomics work

- Metabolomics does have unique challenges
  - Data collection needs to be well controlled
  - Targeted/Untargeted
  - Data analysis
  - Data interpretation

  - Advances in the field of metabolomics are still made and hence will lower the threshold

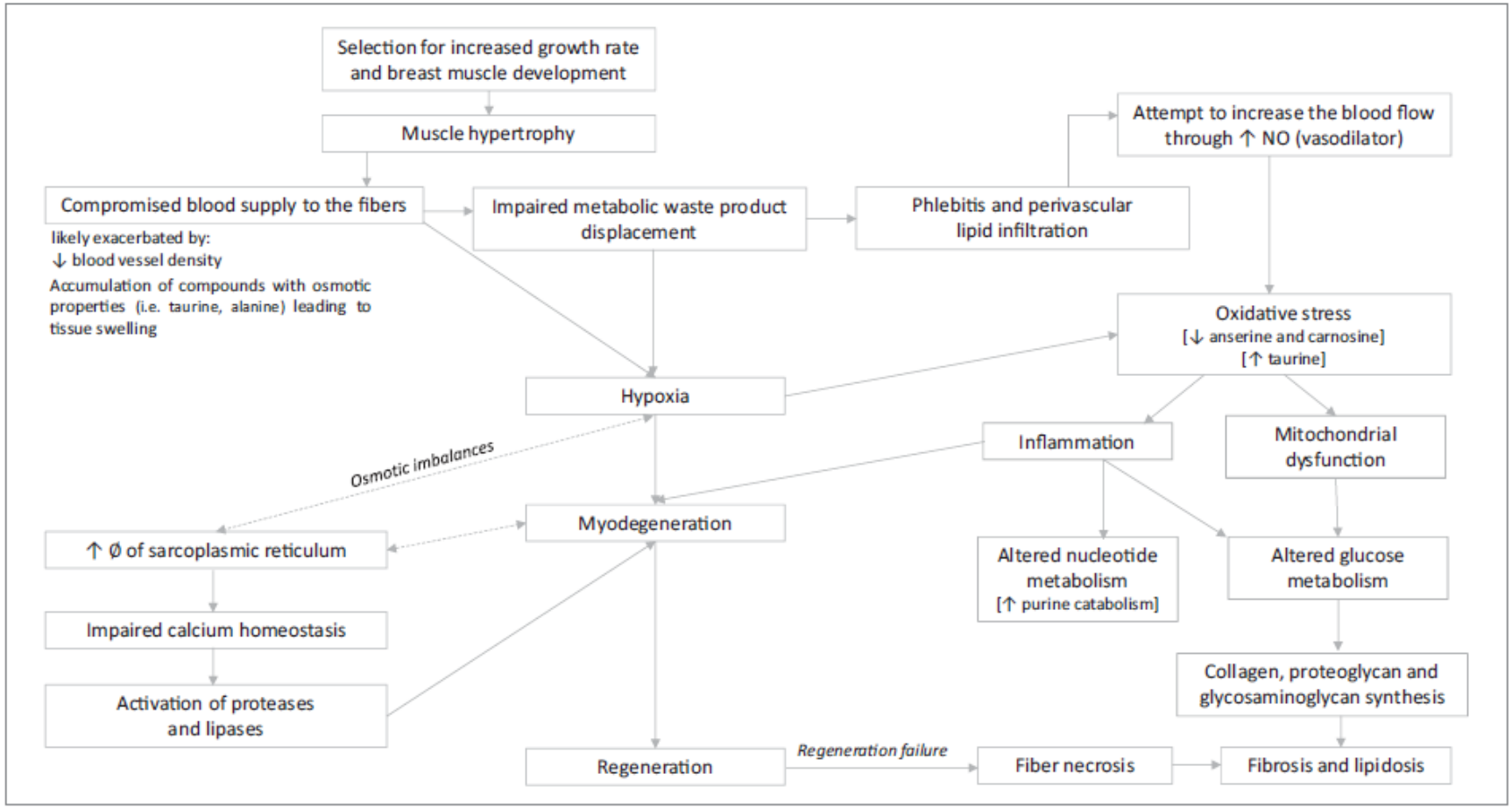


Figure 2–Schematic representation of the possible etiologies and mechanisms leading to the development of white striping (WS), wooden breast (WB), and spaghetti meat (SM) abnormalities.

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METABOLOMIC DISCOVERIES

METABOLON

Thank you

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