Muscle quality issues explained by metabolomics

Gavin Boerboom, Theo van Kempen, Alberto Navarro-Villa, Adriano Pérez-Bonilla, ICoMST 2019
Solving biological problems

- **Traditional cycle:**
  - Background knowledge → hypothesis
  - Test experimentally
  - Hypothesis is starting point

- **Inductive cycle:**
  - No real hypothesis
  - Use data to generate hypothesis
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

What is possible?

What is happening?

Completeness

Chemical diversity

Genome

Transcriptome

Proteome

Metabolome

DNA

RNA

Proteins

4 bases

4 bases

20 AA

Metabolites

Phenotype/Function

Sugars

Nucleotides

Amino acids

Lipids (Lipidome)

2 x 10^5 chemicals

Peironcely 2012
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
Metabolomics workflow

- Biological sample
- Extraction
- Chemical analysis
- Pathway analysis
- Statistical analysis
- Data extraction/Metabolite identification
Metabolomics in practice: White striping

- White stripes on breast muscles
- Various degrees
- Run parallel to direction of muscle fibers
- Unknown etiology
Poultry meat consumption increased over the years

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

Meat consumption US per person in pounds

US broiler production

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 \( \uparrow \) 12.3%
- Last 10 years:
  - Breast yield \( \uparrow \) 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

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
Petracci showed influence on meat quality

Lightness (L*)

Ultimate pH

Drip loss (%)

Cook loss (%)

Marinade uptake (%)

Purge loss (%)

Cook loss (%)

Non-marinated meat AK-shear force (kg/g)

***P≤0.001; ns=not-significant; *P<0.05

Petracci 2014, 2012
No clear etiology

- Factors linked to the occurrence of the disease

<table>
<thead>
<tr>
<th>Live production factors</th>
<th>Mode of action on white striping occurrence</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Genotype</td>
<td>High &gt; standard breast-yield</td>
<td>Petracci et al. (2013b)</td>
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<td></td>
<td></td>
<td>Lorenzi et al. (2014)</td>
</tr>
<tr>
<td>Sex</td>
<td>males &gt; females</td>
<td>Kuttappan et al. (2013a)</td>
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<td></td>
<td></td>
<td>Lorenzi et al. (2014)</td>
</tr>
<tr>
<td>Growth rate</td>
<td>higher &gt; lower</td>
<td>Kuttappan et al. (2012a; 2013b)</td>
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<td></td>
<td></td>
<td>Lorenzi et al. (2014)</td>
</tr>
<tr>
<td>Diet</td>
<td>high &gt; low energy diet</td>
<td>Kuttappan et al. (2012a)</td>
</tr>
<tr>
<td>Slaughtering weight</td>
<td>Heavier &gt; lighter</td>
<td>Kuttappan et al. (2013a)</td>
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<td></td>
<td>Lorenzi et al. (2014)</td>
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</tbody>
</table>
Aim of the study

- Identify biological pathways involved in the underlying etiology and pathogenesis of white striping
Trial at our poultry facility
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
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
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

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

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)

Data processing showed improvements

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

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

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.
Special thanks

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