Molecular mechanisms associated with methionine deficiency in meat-type chickens

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Methionine

- Supplemented in diets by including either DL-MET or MHA-FA (Analogue)
- Affects
  - growth
  - feathering
  - Body composition
  - feed efficiency,
  - immunity
- Several studies with varying results

Objective

To study the molecular mechanisms in the liver and Pectoralis major (P. major) muscle in growing chickens when dietary MET is restricted.

Experimental Design

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONTROL</th>
<th>DEFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine (%)</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.56</td>
<td>0.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basal Diet</th>
<th>CP (%)</th>
<th>ME (Kcal/g)</th>
<th>L-MET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter (0-3 wk)</td>
<td>23</td>
<td>3.03</td>
<td>0.23</td>
</tr>
<tr>
<td>Grower (3-5 wk)</td>
<td>21</td>
<td>3.15</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Starter diet: supplemented with 0.30% L-Met
Growth and FCR during methionine restriction phase

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWG3.4</td>
<td>601*</td>
<td>545*</td>
</tr>
<tr>
<td>BWG4.5</td>
<td>640*</td>
<td>612*</td>
</tr>
<tr>
<td>FCR3.4</td>
<td>1.67*</td>
<td>1.89*</td>
</tr>
<tr>
<td>FCR4.5</td>
<td>1.78</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Transcriptome Analysis

- **Tissues**
  - *Pectoralis major*
  - Liver
- Animals per tissue: 5 males
- Sex: Males

Sequence Mapping

- Reads were inspected and trimmed for adapter sequences
- Mapping >> Galgal4 using Tophat
- Genes and transcripts were detected using Cufflinks v2.1.1
- Gene models: known and predicted genes
- Transcript abundance was measured in fragment per kb of exon per million of map reads (FPKM)

Differential Expression

- **Within tissue Analysis**
- Control vs Treatment (Restricted Met)
- Gene-level differential analysis-Edge R
- DE at FDR P<0.05
- RT-PCR on 15 DE genes selected randomly between liver and *P. major* using StepOnePlus

Results

- 90-93% of reads were mapped
- About 12,596 (15,508) annotated mapped

- Liver: 64 DE
- *P. major*: 919 DE

NGS vs RT-PCR

\[ R^2 = 0.9246 \]
Downregulated genes

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0006955 Immune response</td>
<td>2.06E-11</td>
</tr>
<tr>
<td>GO:0006952 Defense response</td>
<td>4.64E-09</td>
</tr>
<tr>
<td>GO:0045321 Leucocyte activation</td>
<td>7.17E-05</td>
</tr>
<tr>
<td>GO:0006954 Inflammation response</td>
<td>8.02E-05</td>
</tr>
<tr>
<td>GO:0050853 B cell receptor signaling</td>
<td>2.40E-04</td>
</tr>
</tbody>
</table>

Upregulated genes

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0016651 Oxidoreductase activity</td>
<td>0.015</td>
</tr>
<tr>
<td>GO:0004908 Interleukin 1 receptor activity</td>
<td>0.015</td>
</tr>
<tr>
<td>GO:0016491 Oxidoreductase activity</td>
<td>0.015</td>
</tr>
<tr>
<td>GO:0003779 Actin binding</td>
<td>0.015</td>
</tr>
<tr>
<td>GO:0003954 NADH dehydrogenase activity</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Gene Ontology-P. major

| GO:0071944 Cell periphery          | 2.80E-07 |
| GO:0005886 Plasma membrane         | 8.10E-07 |
| GO:0000323 Lytic vacuole           | 0.002   |
| GO:0005764 Lysosome                | 0.002   |
| GO:0002102 Podosome                | 0.002   |

Signaling Pathway Impact Analysis

- Classical probabilistic component
- Magnitude of expression change
- Position of DEG
- Topography of pathway-gene interaction
- Type of signaling between them
- SPIA does not provide false positives beyond the expected values
What is going on during methionine deficiency?

- Reduced skeletal and cardiac muscle contraction
  - MYH13 - MYH6
  - MYH1 - ACTN2
  - MYH3 - MYL1
  - MYH2 - MM - CK
  - CKM2
  - May affect walking ability

Major response to inflammation
- Fc gamma R-mediated phagocytosis pathway
  - NCF1
  - SYK
  - PLCG2
  - VAV3
  - INPP5
  - CD32a
- B-cell signaling
- Inflammation mediators
  - IL1R1, 5HTR and TRPA1

Osteoclast differentiation
- Bone homeostasis
  - Bone forming osteoblast and bone resorbing osteoclast
- Methionine deficiency > osteoclast differentiation
  - TF nuclear factor of activated T cells (NFATC1) is induced
- NF-kB
  - Induction of osteoclast differentiation genes
  - Prolong survival of osteoclast
  - Increased resorption of bones
Osteoclast differentiation

- Supportive mechanisms and/or genes
  - Calcium signaling pathway (FDR=0.027)
  - SYK, BTK, BLNK and PLC-gamma
- Osteoclastogenesis genes (DE)
  - CTSK
  - MMP9
  - MMP7

What is going on during methionine deficiency?

- Changes cellular levels of SAM, SAH and Hcy?
- Hcy >> CYS (Transsulfuration)
- CYS >> precursor of Glutathione (GSH)
- Changes in cellular GSH levels?
- Changes in ROS levels?

News Flash!!!

Dietary Methionine Deficiency

Excess ROS or GSH depletion?
Conclusion
Dietary methionine deficiency
• Negatively affect growth and feed efficiency
• Possibly reduce skeletal and cardiac muscle contraction
• Potentially increase cellular stress
• Associated with muscular inflammation ????
• Compromised immunity?
• Reduced bone quality?

Acknowledgement
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• Ms. Lindsey Rachett

Thank You