Effects of heat stress on the transcriptomic profile of blood cells in lactating dairy goats

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Change in average surface temperature

1986-2005  
2081-2100

IPCC (2013)
Heat Stress effects in ruminants

Literature based mostly on phenotypic records
Responses of dairy goats to heat stress (HS)

- Respiratory Rate (+300%)
- Rectal Temperature (+0.58°C)
- Water consumption (+202%)
- Dry matter intake (-21%)
- Body weight (-125 g/d)
- Milk yield (-4 to -10%)
- Milk quality (-12.5% CP)
- Slower Immune response when the udder is challenged with LPS

Hamzaoui et al. (2012, 2013)
Love (2015)
Objectives

Evaluate the **transcriptomic response** and the metabolic pathways affected on **immune system** of **heat stressed** lactating dairy goats.
Experimental design

• Murciano-Granadina dairy goats in mid-lactation (n = 8)

• Climatic chamber:
  - **TN**: 15 to 20°C
  - **HS**: 37°C-12h and 30°C-12h

• TMR diet ad libitum (17% CP; 1.3 Mcal ENL) according to requirements (INRA, 2007)
Blood samples and Microarrays

- **Blood samples**: d 35 (n=8)
- **RNA extraction**: RiboPure-Blood Kit
- **RNA integrity**: Agilent Bioanalyzer 2100
- **24K Affymetrix GeneChip** Bovine Genome Array
- **GeneChip 3’IVT Express Kit**
Gene expression Data Analysis

DATA PREPARATION:
Affy package (Bioconductor)

Background correction
Normalization (log₂)
Remove absent probe sets

STATISTICS:
Student’s t-test
Benjamini and Hochberg corrected (FDR=0.05)
Fold Change threshold >/1/

PATHWAY ENRICHMENT ANALYSIS:
Pathway enrichment analysis

**DINAMIC IMPACT APPROACH (DIA):**

- DEG List + Fold Change + adj p-value
- DIA + KEGG

> = 4 genes/pathway
> 20% coverage

**Category** | **Impact** | **-Flux** | **+Flux**
---|---|---|---
1. Metabolism | | | |
0.1 Metabolic Pathways | | | |
1.1 Carbohydrate Metabolism | | | |
1.2 Energy Metabolism | | | |
1.3 Lipid Metabolism | | | |
1.4 Nucleotide Metabolism | | | |
1.5 Amino Acid Metabolism | | | |

**Impact**

- Larger →
  - Decreased/DR
  - Increased/UR

Bionaz et al. (2012)
Results

24,128 transcripts

Filtering

14,316 transcripts

Statistical analysis

143 DE genes

55 Up-Regulated

88 Down-Regulated

24K Affymetrix GeneChip Bovine Genome

31 Metabolic Pathways (immune system)
<table>
<thead>
<tr>
<th>#</th>
<th>Pathway</th>
<th>Impact</th>
<th>-Flux</th>
<th>+Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leukocyte transendothelial migration</td>
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<tr>
<td>2</td>
<td>Pyrimidine metabolism</td>
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<td>3</td>
<td>Purine metabolism</td>
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<td>4</td>
<td>Cell adhesion molecules (CAMs)</td>
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<tr>
<td>5</td>
<td>Drug metabolism - cytochrome P450</td>
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<td>6</td>
<td>Tight junction</td>
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<td>7</td>
<td>Fat digestion and absorption</td>
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<tr>
<td>8</td>
<td>RNA transport</td>
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<td>9</td>
<td>Hematopoietic cell lineage</td>
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<tr>
<td>10</td>
<td>Adipocytokine signaling pathway</td>
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<tr>
<td>11</td>
<td>PPAR signaling pathway</td>
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<td>12</td>
<td>Adherens junction</td>
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<td>13</td>
<td>ECM-receptor interaction</td>
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<tr>
<td>14</td>
<td>Calcium signaling pathway</td>
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<tr>
<td>15</td>
<td>Arginine and proline metabolism</td>
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</tbody>
</table>
1) Down-regulated pathways in HS goats

Reviewed by Strambio-De-Castillia et al. (2010)
2) Down-regulated pathways in HS goats

HEMATOPOIETIC CELL LINEAGE

- Immune cells have ≠ life time.
- Have to be constantly replaced
- Slower creation and differenciation

Seita and Weissman (2011)
3) Down-regulated pathways in HS goats

LEUKOCYTE TRANSENDOTHELIAL MIGRATION

By interaction among **Cell Adhesion Molecules** and **Extracellular Matrix**

**Step 1 Rolling**  
**Step 2 Adhesion**  

Ca\(^{2+}\) **SIGNALING**

**Step 3 Extravasation**

Reviewed by Etzioni (1996); Muller et al. (2011)
LEUKOCYTE TRANSENDOTHELIAL MIGRATION

By interaction among **Cell Adhesion Molecules** and **Extracellular Matrix**

**Step 1 Rolling** → **Selectins**
**Step 2 Adhesion** → **Integrins** and **Ig family**

**Ca²⁺ SIGNALING**

**Step 3 Extravasation**

Reviewed by Etzioni (1996); Muller et al. (2011)
4) Down-regulated pathways in HS goats

1. PPARγ SIGNALING
   \[ F(x): \] Modulates gene expression of lipid-related genes
   (Széles et al., 2007; O'Donell et al., 2014)

2. Ca²⁺ SIGNALING
   \[ F(x): \] Platelet activation and aggregation
   (Razell et al., 2013; O'Donell et al., 2014)

3. ARGININE AND PROLINE METABOLISM
   \[ F(x): \] Decrease inflammatory response
   Promote wound healing
   (Gallardo-Soler et al., 2008; Munder et al., 2009)
Up-regulated pathways in HS goats

- PYRIMIDINE METABOLISM
- PURINE METABOLISM
- CYTOCHROME p450

22.2 pg of cytosolic protein/leukocyte (humans)

\[ \text{RH} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{NADP}^+ \]

Immune cell fate for extra fuel and wound healing

Hibbs et al. (2016)
Conclusions

1. 143 Differentially expressed genes were found in healthy lactating dairy goats after 5 wk of heat stress exposure.

   • 55 of them were up-regulated in heat stress goats involved in nucleotide catabolism and oxidation.

   • 88 genes were down-regulated in heat stress goats mainly related with RNA transport, leukocyte transendothelial migration and wound healing.

2. Heat stress hinders both innate and adaptive immune responses.
Thank you for your attention