Molecular response to heat stress and lipopolysaccharide in chicken macrophage-like cell line

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Heat Stress in Changing Climates

- **Ambient temperatures > 26°C**
- **Relative humidity > 40%**
  - Problem of tropical climates – „modern” vs. indigenous breeds
  - Climate change – more frequent heat waves in moderate climates

Fig. 1 Geographical pattern of surface warming for late 21st century (2090-2099) relative to 1986-2005. Source: IPCC Fourth Assessment Report: Climate Change 2014
LIVING WITH A HOSTILE “HEAT” ENVIRONMENT

GENETIC POTENTIAL

100%

Appetite declines

Panting

Increased thirst

Control of body fluids is lost

Increased urine output and loss of Electrolytes

Acid/Base balance upset

Production machinery, enzyme systems and the internal environment are so depressed that production within cells is switched off

Wet droppings and further loss of electrolytes

No growth

Smaller and fewer eggs, weak shells

Sexual disturbance in breeders

Bone abnormalities

Growth stops

Viral and bacterial diseases thrive

Ascites

Viral nephritis

I.B.

Other viral diseases (Malabsorption syndrome)

Cell recovery is lost

Immuno-suppression resistance to disease and toxins is lost (aflatoxin)

Sudden deaths

Evolution Formula \((Na^+ + K^+)-(Cl^- + S^-)\) PROGRESSIVE DECLINE IN ACID-BASE AND ELECTROLYTE BALANCE (Proved by research)
Cost of Heat Stress in Poultry

Productivity losses
- Increased mortality
- Decreased growth rate
- Depressed appetite
- Lower shell quality
- Lower meat quality
- Loss of electrolites

Investments
- Ventilation
- Shades
- Cooling systems (e.g. cooler pads)

Fertility losses
- Lower egg production
- Decreased fertility of males

Health decline
- Metabolic disorders
- Decreased disease resistance
- Systemic inflammation

COST OF HEAT STRESS

$125-165 million per year in USA

(St-Pierre et al., 2003)
Whole-genome molecular responses to heat stress in chickens

- **Brain**
  - Li et al., 2011

- **Liver**
  - Coble et al., 2014
  - Luo et al., 2014

- **Muscle**
  - Li et al., 2011
  - Luo et al., 2014

- **Testes**
  - Wang et al., 2014

- **LMH cell line**
  - Sun et al., 2015

EAAP, Warsaw, Aug 31 2015
Heat stress and endotoxemia in chicken immune cells (HD11)

**HEAT STRESS**

- Lipopolysaccharide (LPS)

**EXPERIMENTAL OUTLINE**

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<thead>
<tr>
<th>HS, LPS</th>
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<th>4h</th>
<th>6h</th>
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<tr>
<td>45°C</td>
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* The experiment was replicated three times
Gene expression study

Heat shock proteins & factors

HSP25, HSPA2, HSPA14, HSPB8, HSP90AA1, HSPH1, HSF2, HSF4, HSF5, DNAJA4

Immune response

IL1B, IL8, CD40, IL12B, IL18, LITAF, IFNB, IFNG, iNOS, CCL4, CCL5

Stress response & apoptosis

SERPINH1, BAG3, RB1CC1, UBB, CIRBP, TP53, CASP1, CASP3, CASP7, CASP9, CASP8

Signaling

MAPK9, MAPK8IP3, TGFB2, TGFB3, SMAD6, NLRC5, TLR4, MyD88, TRAF6, JUN, IRAK4

44 target genes + 2 housekeeping genes
Log$_2$ Fold Change Cluster Analysis

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Heat shock proteins, stress response

**HSPH1** (105/110kDa)

**HSPA2** (70kDa)

**DNAJA4**

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**HSP25**

**BAG3**

**UBB**

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Heat stressed cells

Heat stress
Oxidative stress

Mature protein → Misfolded protein → Proteasome → Degradation

HSP → P-substrate complex

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Adapted from Benarroch (2011)
Immune-related genes

CCL4

CCL5

IL8

IL1B

iNOS

Fold induction

2h 4h 6h 10h

Fold induction

2h 4h 6h 10h

Fold induction

2h 4h 6h 10h

Fold induction

2h 4h 6h 10h

HS-LPS

HS-NO LPS

NO HS-LPS
Immune response to LPS was reinforced by heat stress

HEAT STRESS

Lipopolisaccharide (LPS)

DAMP
Danger-associated molecular pattern

PAMP
Pathogen-associated molecular pattern

Chemokines

Inflammation

Nitric Oxide

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Spearman Correlation Network: Heat Stress

Time point: 2h
Spearman Correlation Network ($r > 0.8$)

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Spearman Correlation Network Network (r > 0.8)

Time point: 2h
Spearman Correlation Network (r > 0.8)
Spearman Correlation Network: Heat Stress & LPS

Time point: 2h
Spearman Correlation Network ($r > 0.8$)
Conclusions

1. The chaperones responsible for thermoregulation of the macrophages are: HSPH1, HSPA2 and HSP25
2. HSPA14 seems to have regulatory role in the gene network
3. Heat stress reinforced expression of LPS-activated chemokines: CCL4, CCL5, IL8, pro-inflammatory IL1B and inducible nitric oxide synthase (iNOS)
4. Synergistic effects of HS&LPS indicate the molecular response to the Danger-Associated Molecular Pattern (DAMP) provided by heat stress
5. There was similar regulation of the genes upon heat stress *in vivo* (literature) and *in vitro* (this study)
Acknowledgements

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Thank you!!!