Heat Stress in Pigs- What are the Effects on Muscle Metabolism and Pork Quality?

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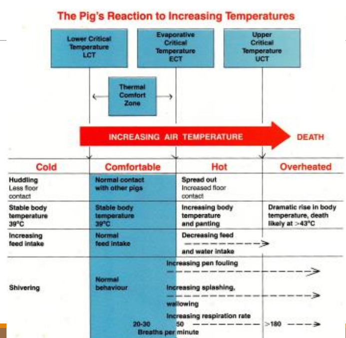
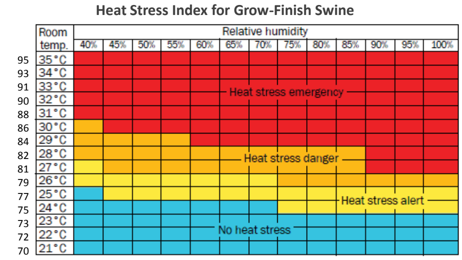
Smithfield

What is Heat Stress?



What is Heat Stress?



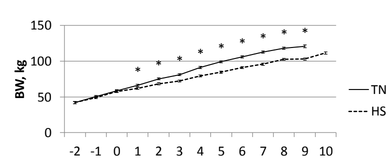
Introduction

- **Heat stress costs livestock industry more than $2 billion annually**
  - **Swine industry - $300 million**
  - **Subcutaneous adipose tissue**
  - **Lack of functional sweat glands**
- **Reduced efficiency**
- **Slowed growth**
- **Increased morbidity/mortality**
- **Decreased carcass value**

St. Pierre et al., 2003. J Dairy Sci. 86, E52-E57
Baumgard & Rhoads, 2013. Annu Rev Anim Biosci. 1, 311-377

Effects on Production

- Finishing can take 1-4 weeks longer!
  - Barrows kept at 32° C (~90° F) from 14 wk of age until slaughter at ~110kg



Cruzen et al. 2015. J Anim Sci. 93, 2587-2596

Effects on Production

- Pigs heat stressed (32 °C) for 5 weeks starting at 14wk of age

Item	Finishing TN ($n = 24$)	HS ($n = 24$)	SEM[2]	Finishing
Wk 0				
Loin eye area, cm^2	29.2	28.7	0.7	0.63
Back fat depth, cm	1.20	1.21	0.04	0.87
Wk 5				
Loin eye area, cm^2	46.5	40.1	0.9	<0.0001
Back fat depth, cm	1.82	1.53	0.07	<0.0001

Cruzen et al. 2015. J Anim Sci. 93, 2587-2596

Chronic Heat Stress and Carcass Composition

- Pigs heat stressed (32 °C) for for 7-10 wk until slaughter wt

Item	Finishing Effects TN ($n = 24$)	HS ($n = 24$)	SEM[2]	Finishing
Lean tissue, kg	25.9	28.1	0.3	<0.0001
Lean tissue, % side	61.4	67.1	0.8	<0.0001
Separable fat, kg	5.7	4.5	0.3	0.008
Separable fat, % side	14.9	9.7	0.9	0.0002
Bone, kg	4.88	5.08	0.08	0.11
Bone, % side	11.60	12.13	0.19	0.08
Skin, kg	3.11	2.86	0.08	0.08
Skin, % side	7.39	6.84	0.19	0.10
Back fat, cm	2.48	2.17	0.11	0.06
Loin eye area, cm^2	47.6	49.7	1.5	0.38

Cruzen et al. 2015. J Anim Sci. 93, 2587-2596

Chronic Heat Stress and Carcass Composition - Proximates

- LD proximate analysis
- Pigs heat stressed (32 °C) for for 7-10 wk until slaughter wt

Item	Finishing TN ($n = 24$)	HS ($n = 24$)	SEM[2]	Finishing
Moisture, %	73.19	73.98	0.16	0.003
Protein, %	23.42	23.19	0.12	0.17
Lipid, %	1.85	1.65	0.23	0.48

Cruzen et al. 2015. J Anim Sci. 93, 2587-2596

Effects on Pork Quality

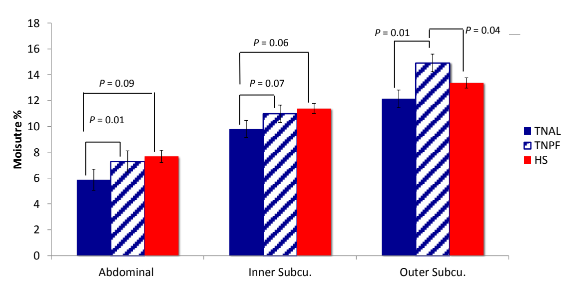
- Summer vs Winter– Brazil
  - No difference in pH at 45 min or 24 hr
  - Summer pork (LD and semimembranosus) had greater light reflectance ($P < 0.0001$)
  - Summer carcasses had less bruising
- Constant Heat Stress - 30°C, 3 weeks – LD
  - Lighter (45 min and 24 hr) vs thermal neutral
  - Lower 24 hr ($P = 0.02$), but not 48 hr pH ($P = 0.08$) vs thermal neutral
  - Increased 48 hr drip loss and shear force ($P < 0.01$)
  - Greater malondialdehyde content ($P < 0.01$)

dalla Costa et al. 2006. Livestock Sci. 107, 29-36
Yang et al. 2014. Asian Australas J Anim Sci. 27, 1763-1772

Fat Quality

- Flimsy Fat issues – Fat is softer, leading to problems
  - Especially Bellies
- Current work by **Seibert et al.** to determine cause of flimsy fat in HS pigs (data presented at Midwest ASAS meeting this year)
  - ~114 kg pigs – 21 d of treatment (8 pigs/trt)
  - TNAL: Thermoneutral ad libitum
  - TNPF: Thermoneutral pair-fed
  - HSAL: Heat stress ad libitum

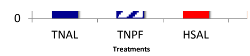
Adipose Tissue Moisture Content

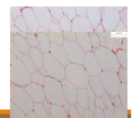


Adipocyte Size

Abdominal Adipocyte Area

HS induced flimsy fat not due to smaller adipocyte size

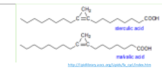




- **No differences at inner and outer subcutaneous depots**

Sterculic Oil

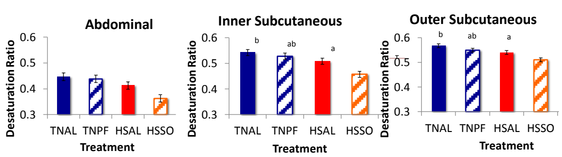
- Sterculic Oil:
  - Derived from seeds of the *Sterculia foetida*
  - Contains cyclopropenoic FAs
  - Bind to active site of SCD rendering it inactive
  - **Prevent insulin-stimulated SCD activity** (should decrease FA desaturation)



 Corl *et al.* 2001; Davis and Vincent 1997

Adipose Tissue Fatty Acid Composition

HS does not affect overall FA composition



Fat Quality

- It is POSSIBLE that "flimsy fat" in heat stressed pigs is primarily due to reduced nutrient intake
  - Increased moisture content
  - No difference in fatty acid profile
  - No difference in adipocyte size

- Dietary strategies are a potential solution to harden fat in heat stressed pigs

Cellular/Protein Level and ACUTE Heat Stress

Objective: To identify the effects of acute heat stress on the skeletal muscle protein profile in gilts

Methods – Experiment 1

- 32 crossbred gilts
- Heat Stress: 37 °C, 40% humidity
- 0, 2, 4, or 6 hr
- Red and white *semitendinosus* collected, frozen in liquid nitrogen, and stored at -80 °C until analysis of sarcoplasmic extracts

Methods – Experiment 2

- 24 crossbred gilts
- Heat Stress: 37 °C, 40% humidity
- Thermal Neutral: 20 °C, 40% humidity
- Pair-Fed Thermal Neutral: 20 °C, 40% humidity
- Euthanized at 12 hr
- Red and white *semitendinosus* collected, frozen in liquid nitrogen, and stored at -80 °C until analysis of sarcoplasmic extracts

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Materials and Methods

- 2D DIGE used to compare sarcoplasmic extracts
  - 2-Dimensional Difference In Gel Electrophoresis
  - Method of comparing two 2D samples against a reference in the same gel
- Determine the identity of significant spots
  - (P < 0.10) via mass spectrometry

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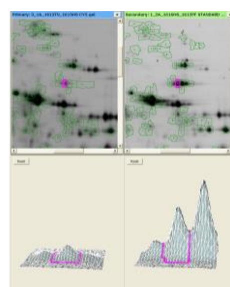
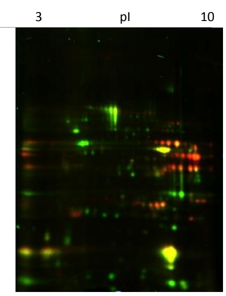
2D-DIGE





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Results

- Heat Shock Proteins
- Metabolic Enzymes
- Antioxidant Enzymes
- Structural Proteins

- Generally increased in abundance
  - Hsp70
  - Grp75
  - Hsp27/Beta 1
  - Hsp20/Beta 6
  - Hsp 60

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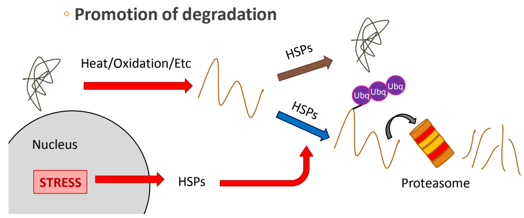
Heat Shock Proteins

Increase in heat shock protein activity
- **Chaperones which repair/prevent damage**
  - **Refolding**
  - **Stabilization / Prevention of aggregation**
  - **Promotion of degradation**



Heat Shock Proteins – Implications for Meat Quality

- Active heat shock proteins may protect the myofibril
  - Downregulated gene expression of Hsp27 and α-B Crystallin associated with beef tenderness
  - Increased abundance of Hsp70 associated with tough beef
  - α-B Crystallin protects bovine desmin and titin from degradation by calpain-1

Bernard et al. J Ag Food Chem. 2007;55:5229-37
Jia et al. J Anim Sci. 2009;87:2391-9
Lomiwes et al. Meat Sci. 2014;97:548-57

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Results

- Heat Shock Proteins
- Metabolic Enzymes
- Antioxidant Enzymes
- Structural Proteins

- Indications of greater glycolytic capacity
  - Phosphofructokinase
  - Phosphoglycerate kinase
  - Glycogen phosphorylase

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Results

- Heat Shock Proteins
- **Metabolic Enzymes**
- Antioxidant Enzymes
- Structural Proteins

- UDP-Glucose Pyrophosphorylase
  - Glycogenesis

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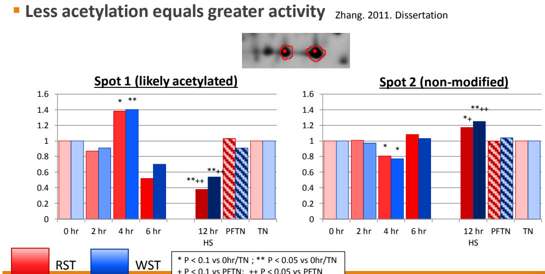
UGP2 – Enzyme in Glycogenesis

- **Less acetylation equals greater activity** Zhang. 2011. Dissertation



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Results

- Heat Shock Proteins
- **Metabolic Enzymes**
- Antioxidant Enzymes
- Structural Proteins

- Fiber Type Dependent
  - RST more affected than WST
  - RST also had increases in TCA cycle components

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Metabolism

More glycolytic metabolism w/ heat stress

- Greater lactate production, pyruvate kinase activity in muscle from chronically heat stressed broilers (Zhang et al., 2012)
- Heat stress increases insulin sensitivity in rodents and basal insulin concentrations in several species (Rhoads et al., 2013)
- Changes in gene expression due to heat stress in rats are muscle specific (Sanders et al. 2009)

Zhang et al., 2012. Poultry Sci. 91:2931-2937
Rhoads et al., 2013. J Anim Sci. 91:2492-2503
Sanders et al., 2009. FASEB J. 23:598.7

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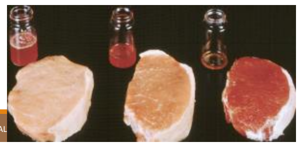
Metabolism – Implications for Meat Quality

- Increased glycolytic capacity does not bode well!
- UGP2 indicates acute heat stress would reduce glycogen production, whereas longer term stress could increase it
  - May be compensation for reduced nutrient intake COMBINED with increased glycolysis
- PSE concerns
  - RATE of pH decline



Results

- Heat Shock Proteins
- Metabolic Enzymes
- Antioxidant Enzymes
- Structural Proteins

- Increased MnSOD & Peroxiredoxin 6
- Decreased Peroxiredoxin 1 & 2

Antioxidant Proteins – Implications for Meat Quality

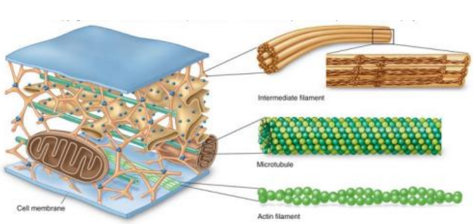
- There is clearly an oxidative response to Heat Stress
- Increase of some antioxidant proteins is a benefit
- But loss of others is a concern

Results

- Heat Shock Proteins
- Metabolic Enzymes
- Antioxidant Enzymes
- Structural Proteins

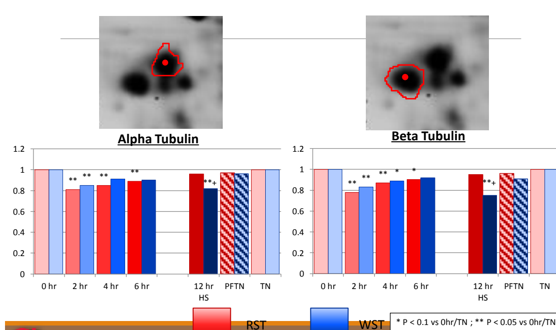
- Alterations in microtubules and microfilaments

Cytoskeleton



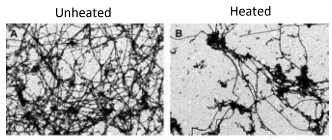
http://www.yellowtang.org/images/three_protein_fi_be_c_la_784.jpg

Tubulin



Microtubule Response to Heat Stress

- Tubulin from *Artemia* embryos heated to 35°C for 8 hours assembles poorly (Day et al., 2003)

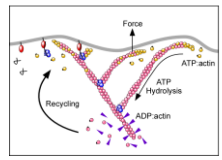


- Hyperthermic treatment (45°C, 30 min) of CHO AA8 cells results in complete collapse of microfilaments/ microtubules (Grzanka et al. 2008)

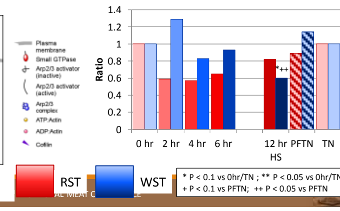
Day et al. 2003. Cell Stress & Chaperones. 8(2), 183
Grzanka et al. 2008. Neoplasma. 55(5), 409

Actin

- Any actin in the sarcoplasmic fraction should be soluble G-actin

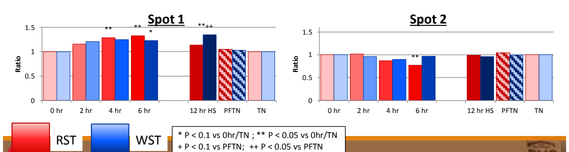


http://www.dayel.com/research/arp23-complex/



Cofilin

- Cofilin (unphosphorylated form) responsible for disassembly of actin filaments
- Phosphorylated cofilin → less actin disassembly → greater cytoskeletal stability



Cytoskeletal Structure

- Microtubules are negatively affected by heat stress
- Conditions are more favorable for microfilament (actin) assembly
- Function to preserve cytoskeletal structure?

Cytoskeletal Structure – Implications for Meat Quality

- Tenderness?
  - More stable actin
  - Less stable tubulin
- Water Holding Capacity
  - IF there is PSE already, might less stable cytoskeletal structure contribute to water loss?

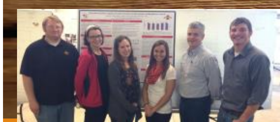
Discussion

- What can be done to reduce the effects or prevent heat stress?
  - Diet
  - Lairage
  - Transport strategies
  - Production strategies
  - ??

Thanks!

USDA United States Department of Agriculture
National Institute of Food and Agriculture

Agriculture and Food Research Initiative Competitive Grant 2011-67003-30007



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