Factors affecting intestinal frontier integrity in birds under thermal stress

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Pilmico Poultry Symposium, Hyatt Regency, Manila, The Philippines

Agenda of the day

01 Heat Stress (HS) in Poultry

02 Behavioral Effect of HS

03 Physiological Effect of HS

04 Hormonal Effect of HS

05 HS on Intestinal Integrity

06 HS on Molecular Biomarkers

07 HS on Intestinal Microbiota

08 Intervention Strategies on HS

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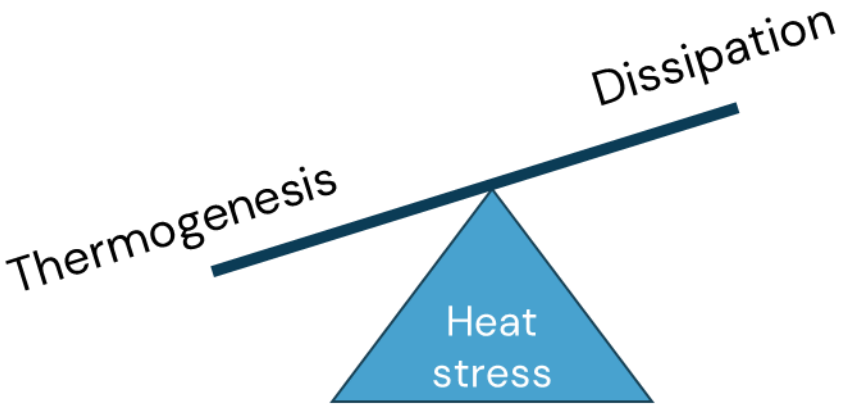
Heat Stress in poultry

Definition:

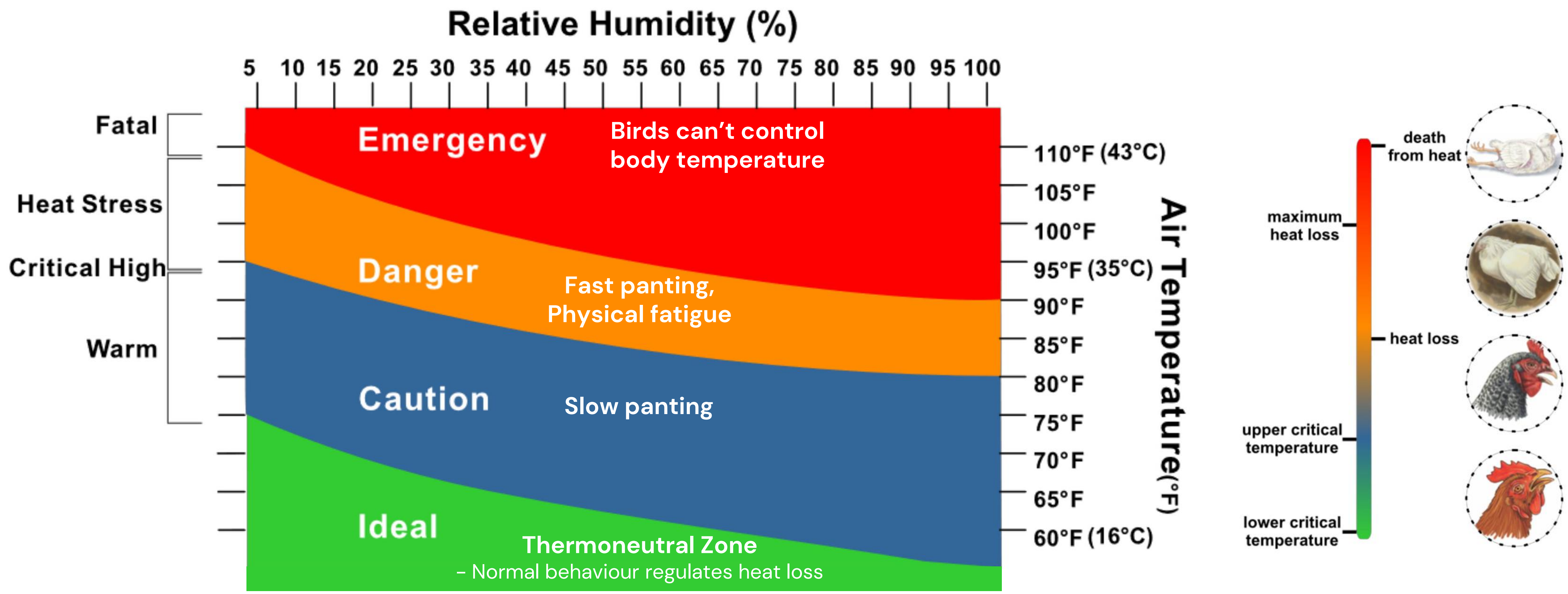
Heat stress occurs whenever the is a **negative balance** between net amount of energy flowing from the animals to the environment **(dissipation)** and the amount of heat energy produced by the animal **(thermogenesis)** *(Renaudeau et al., 2012)*.

- Imbalance caused by various factors:
  - Environmental:
    - Sunlight;
    - Thermal irradiation;
    - Air temperature, humidity and movement.
  - Host characteristic:
    - Species;
    - Metabolism rate;
    - Thermoregulatory mechanism.



Heat stress in a concern not just in summer, it also occurs during winter!

The Thermoneutral Zone and Consequences of Heat Stress



Thermoneutral zone:

- Layer – 19-22ºC
- Broiler – 18-22ºC

Adapted from Shahzad et al., 2021 (http://dx.doi.org/10.3390/su13052836)

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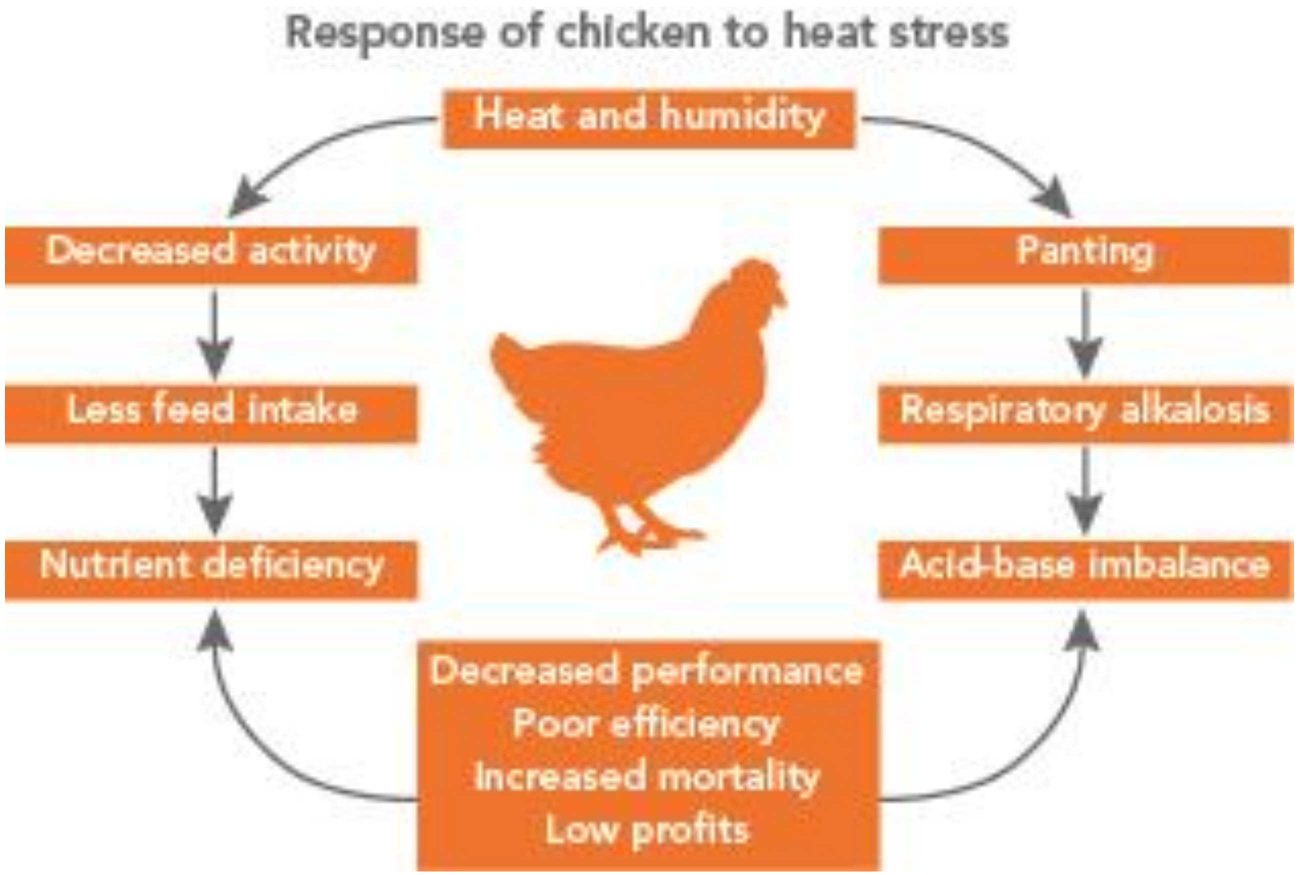
Heat stress (HS) is a severe problem in the poultry industry; it negatively affects poultry production

Negative effects of Heat Stress



Adapted from VIV articles online 2020

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Behavioral effects of Heat Stress (HS) in poultry

Negative effects of Heat Stress

Test group	Feeding (%)	Panting (%)	Walking (%)	Resting (%)	Sitting (%)	Standing (%)	Preening (%)	Aggression (%)
Control (24.3°C)	32.5 ± 1.76	NA	18.40 ± 1.52[a]	27.28 ± 1.72[b]	16.23 ± 1.86	83.32 ± 1.9	5.17 ± 0.79	0.98 ± 0.31
Hot (32.6°C)	27.84 ± 1.78	77.44 ± 3.21	9.90 ± 1.55[b]	38.68 ± 1.74[a]	11.41 ± 1.93	88.10 ± 1.9	5.22 ± 0.80	0.32 ± 0.32

Breed = DeKalb XL
Age of layers = 28 wks old, 2 wks acclimation at 23°C, duration = 9 days
Humidity = 30-40%

Source: Mack et al., 2013 (doi:10.3382/ps.2012-02589)

Treatment	Panting, %	Wing spreading, %	Squatting close the ground, %
TN (21°C)	11.76 ± 2.21[b]	6.87 ± 2.59[b]	3.89 ± 1.55[b]
HS (32°C)	**97.04 ± 3.16[a]**	**82.58 ± 3.74[a]**	**60.44 ± 6.18[a]**

Breed = Ross 708 broilers
Duration = 43 days, 3 feeding phase
Chicks maintained at 34°C on D-1, decreased 0.5°C per day to 21°C. HS group 32°C for 10h from D-15 to D-43

Source: Wang et al., 2018 (https://doi.org/10.1093/jas/sky092)

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Behavioral effects of Heat Stress (HS) in poultry

What does it mean to the producers

	Heat stress effect	Effects to Producers
Behavior	↑ respiratory rate (panting)	Dehydration → higher water requirement and consumption ⇨ **wet dropping (↑ moisture in feces)**
		↑ CO_2 loss → hypocapnia → respiratory alkalosis (acid-based imbalance) → ↓ blood calcium for eggshell mineralization ⇨ **soft egg/cracked egg, ↓ egg weight, ↓ egg production, ↓ growth performance**
		↑ energy expenditure to maintain euthermia ⇨ **↓ performance**
	Wing lifting/spreading	↑ energy expenditure to maintain euthermia ⇨ **↓ performance**
	Lethargy → ↓ feeding and walking	↓ [illegible]

Rapid increase in water and decrease in feed intake ⇨ HEAT STRESS

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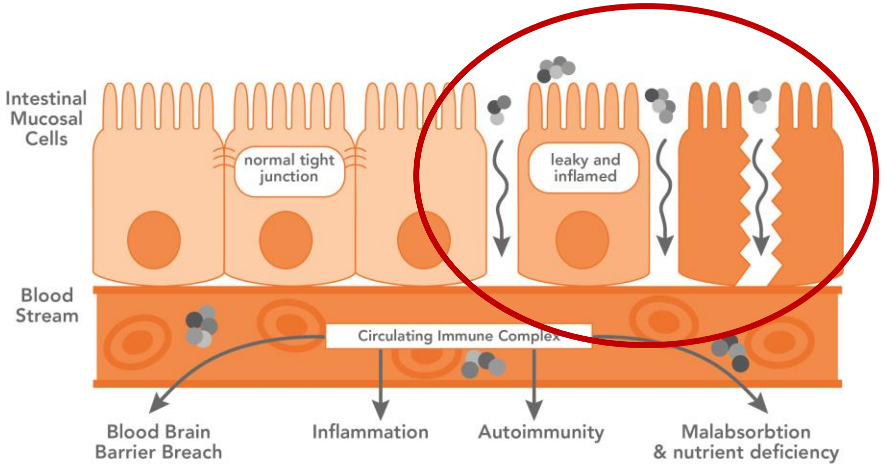
Physiological effects of Heat Stress (HS) in poultry

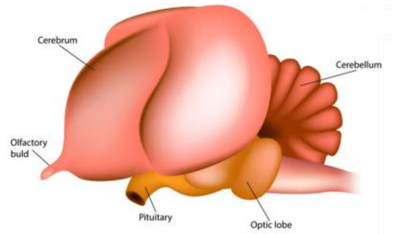
Heat Stress

Intestinal hypoxia



↓ splanchnic blood flow

Viscera vasoconstriction



Thermoregulatory response

Peripheral vasodilatation → ↑ peripheral blood flow → heat dissipation

Physiological effects of Heat Stress (HS) in poultry

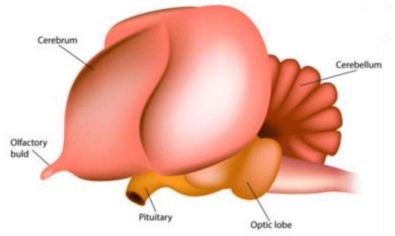
Heat Stress

Intestinal hypoxia

↓ cellular energy production & derangement of intracellular Ca^{2+} homeostasis

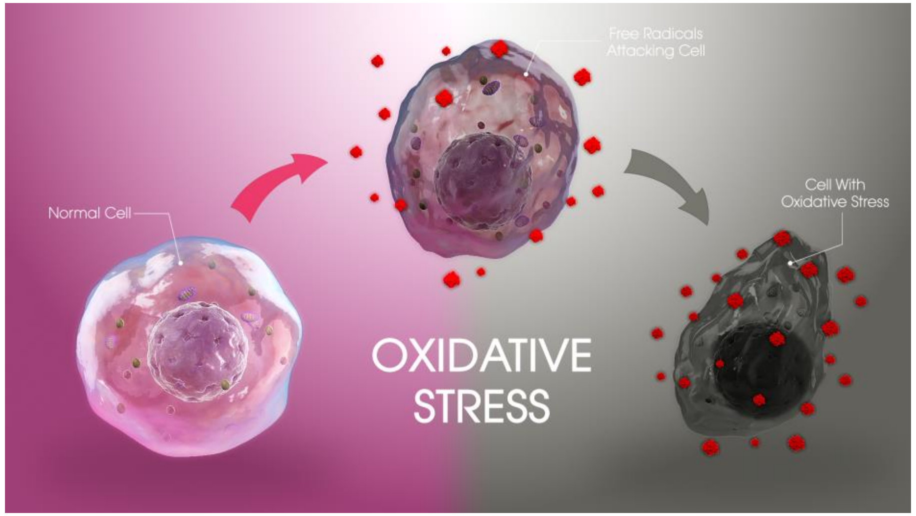
↓ splanchnic blood flow

↑ generation of reactive oxygen species (ROS) & reactive nitrogen species (RNS)



Viscera vasoconstriction

Thermoregulatory response



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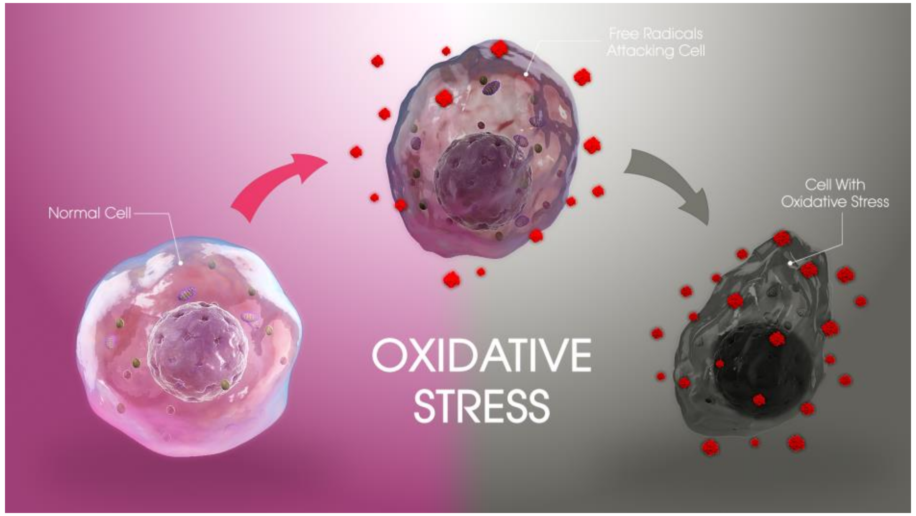
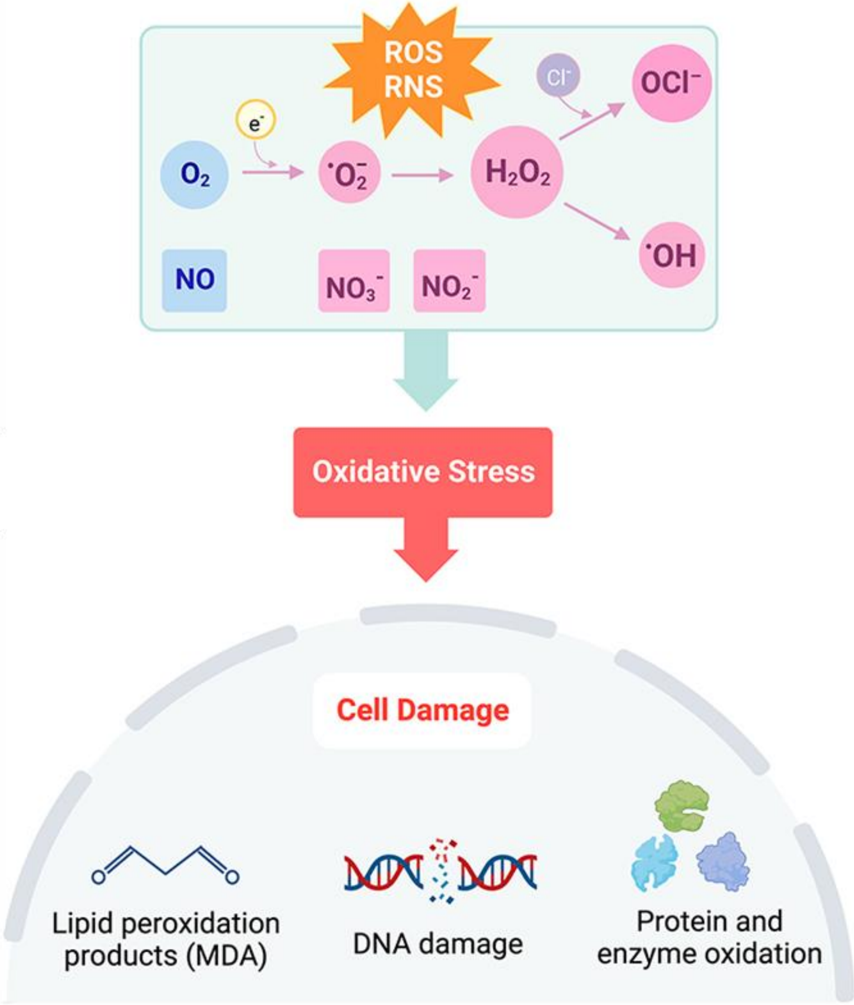
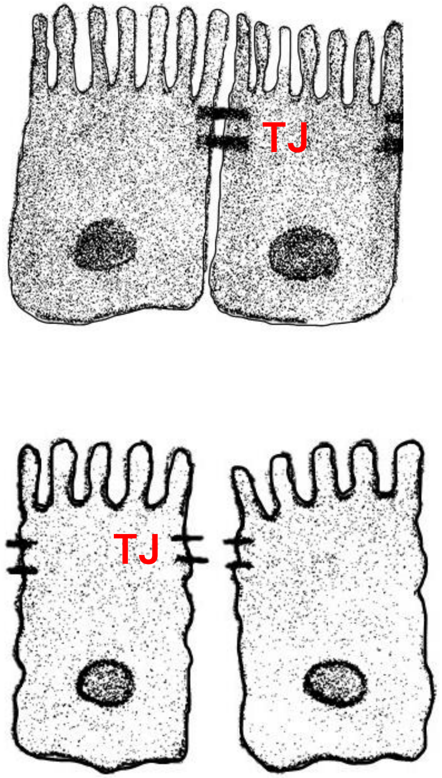
Physiological effects of Heat Stress (HS) in poultry







Physiological effects of Heat Stress (HS) in poultry

What does it mean to the producers

	Heat stress effect	Effects to Producers
Cardiovascular system	Peripheral vasodilatation & viscera vasoconstriction	GIT hypoperfusion → ↓ nutrient supply to GIT → ↓ GI barrier & functionality ⇨ ↓ **performance, ↑ disease susceptibility**
		GIT hypoxia → oxidative stress → ↓ GI barrier & functionality ⇨ ↓ **performance, ↑ disease susceptibility**

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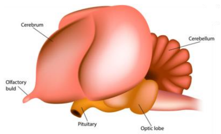
Hormonal effects of Heat Stress (HS) in poultry

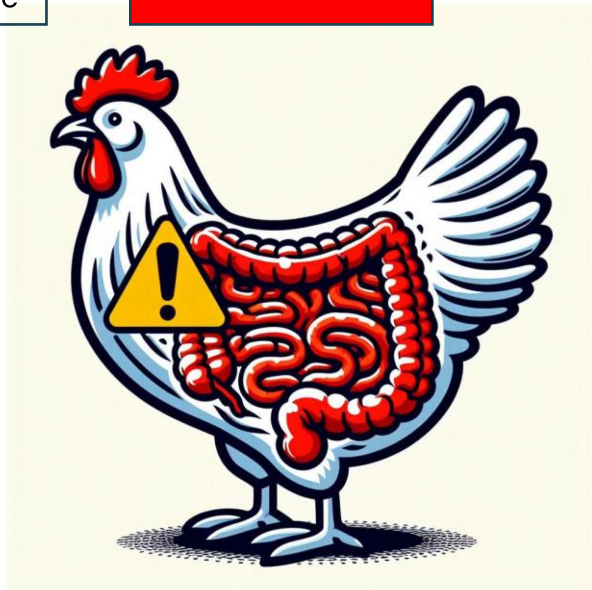
Heat Stress

Hypothalamic-pituitary-adrenal axis activation



Immunosuppression ← ▲ Adrenaline ▲ Corticosterone → Oxidative stress → Lipoperoxidation Cell Membrane Mitochondrial Membrane → Irreversible Cell Damage

▲ Adrenaline ▲ Corticosterone ↓ Inflammation ↓ ▲ TNF ▲ INF 8 ▲ IL-8 etc. ↓ Disruption of binding protein in epithelia → ▲ Hepatic bacterial translocation (Leaky Gut) → Oxidative stress



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Hormonal effects of Heat Stress (HS) in poultry

Negative effects of Heat Stress

Parameter	Group		*P*-value
	Control (22°C)	HS (32°C)	
Initial BW (g)	1,246.67 ± 7.14	1,268.54 ± 11.40	0.313
Final BW (g)	1,833.33 ± 12.73[a]	**1,663.54 ± 40.82[b]**	0.001
ADFI (g/bird/d)	171.88 ± 3.09[a]	138.42 ± 3.03[b]	<0.001
ADG (g/bird/d)	83.81 ± 0.95[a]	**56.43 ± 2.60[b]**	<0.001
F/G (g/g)	2.05 ± 0.02[b]	**2.46 ± 0.05[a]**	<0.001
CORT (ng/ml)	75.84 ± 3.18[b]	**90.27 ± 3.23[a]**	0.002

▲ corticosterone
▼ growth performance

[a,b] Differ according to one-way ANOVA followed by Duncan's test ($P < 0.05$).

Breed = Arbor Acres
Age of broilers = 28 days old, duration = 7 days
Humidity = 55±5%

Source: Ma et al., 2021 (https://doi.org/10.1016/j.psj.2020.09.090)

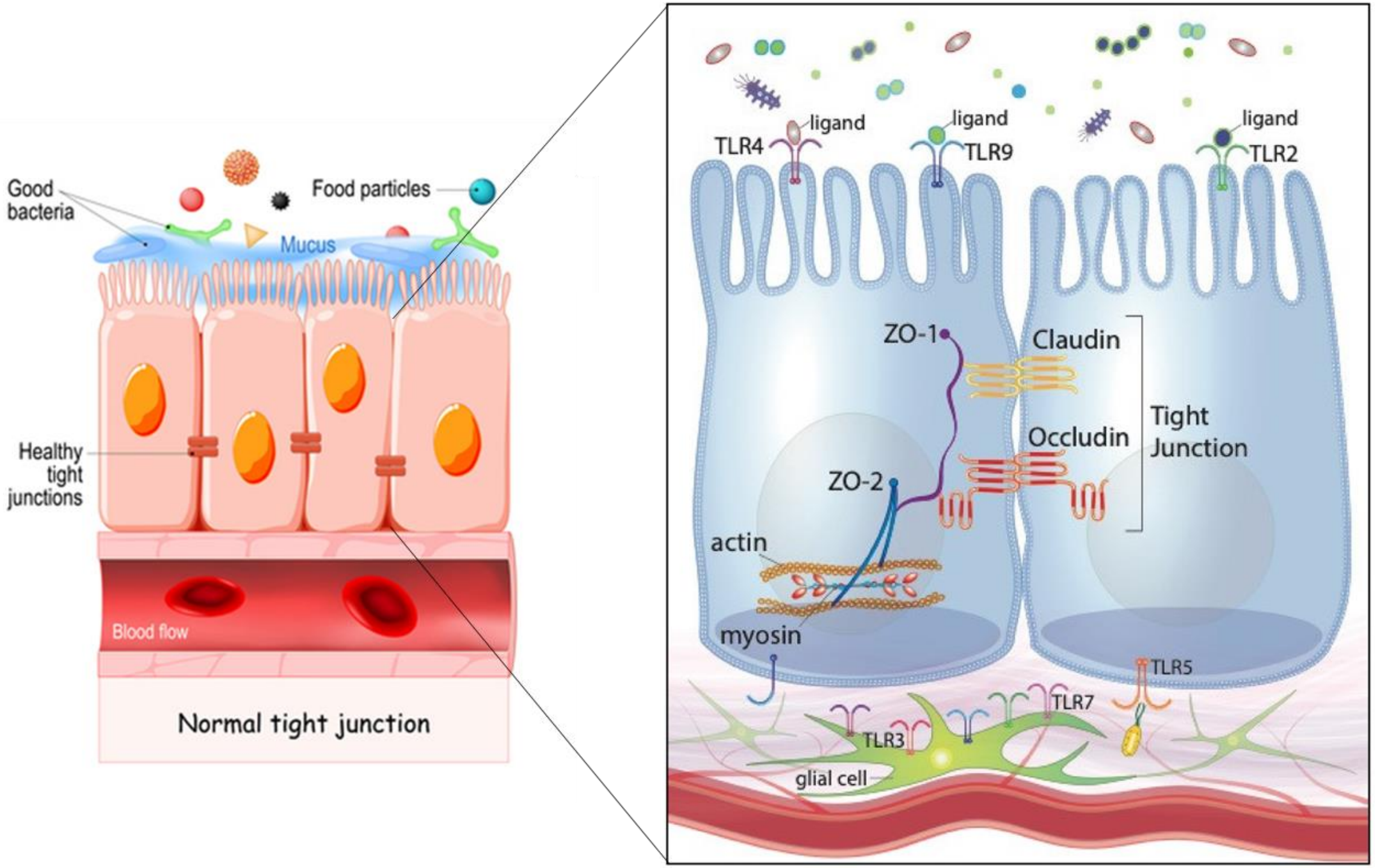
Hormonal effects of Heat Stress (HS) in poultry

What does it mean to the producers

	Heat stress effect	Effects to Producers
Hormonal	Hypothalamic-pituitary-adrenal axis activation – ↑ circulating glucocorticoids (e.g., corticosterone)	↓ **growth potential**
		↓ protein synthesis and ↑ protein breakdown in skeletal muscles ⇨ ↓ **lean tissue yield**
		↑ **fat deposition**
		↓ immunocompetence ⇨ ↑ **infectious susceptibility and medical cost**
		↓ **GI barrier**

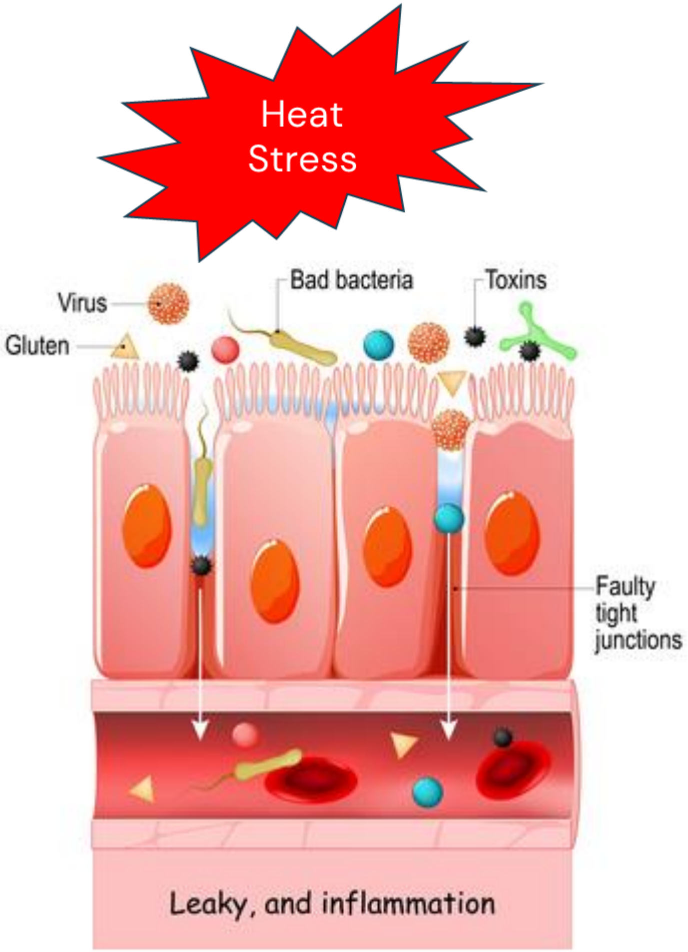
Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

The role of tight junction



- Border protectors and gate guards
  - Control the passage of molecules through selective paracellular pores
  - Avoid the entry of endotoxins, pathogenic bacteria

Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

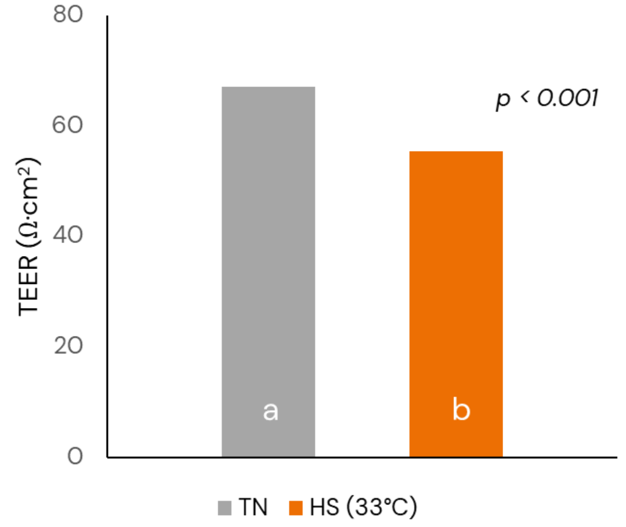


Tight junction protein expression

Item	Occludin	Zonula occludens-1
Thermoneutral zone	1.12[a]	1.06[a]
Heat stress (33°C)	0.66[b]	0.58[b]
p-value	0.001	0.002

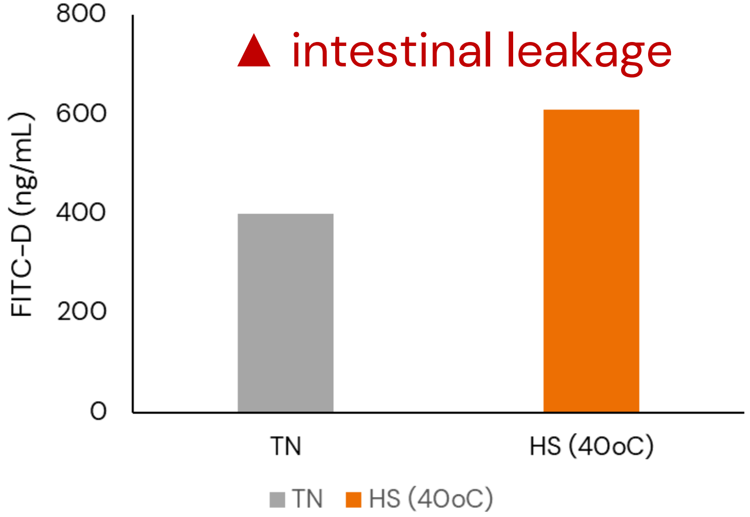
Source: Song et al., 2014

▼ intestinal integrity



Source: Song et al, 2014)

▲ intestinal leakage



Source: Tabler et al., 2020

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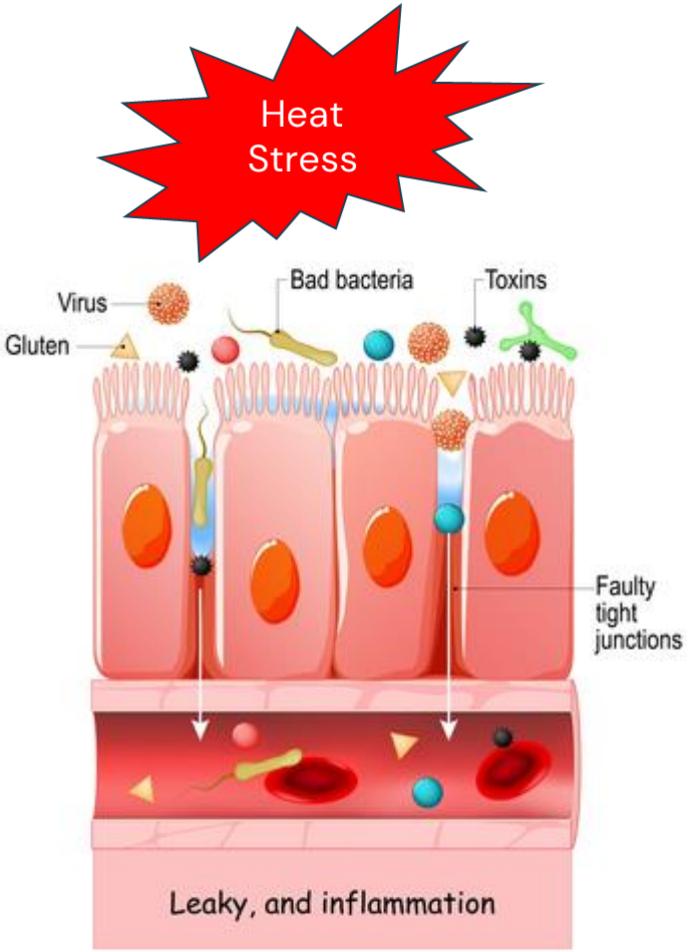
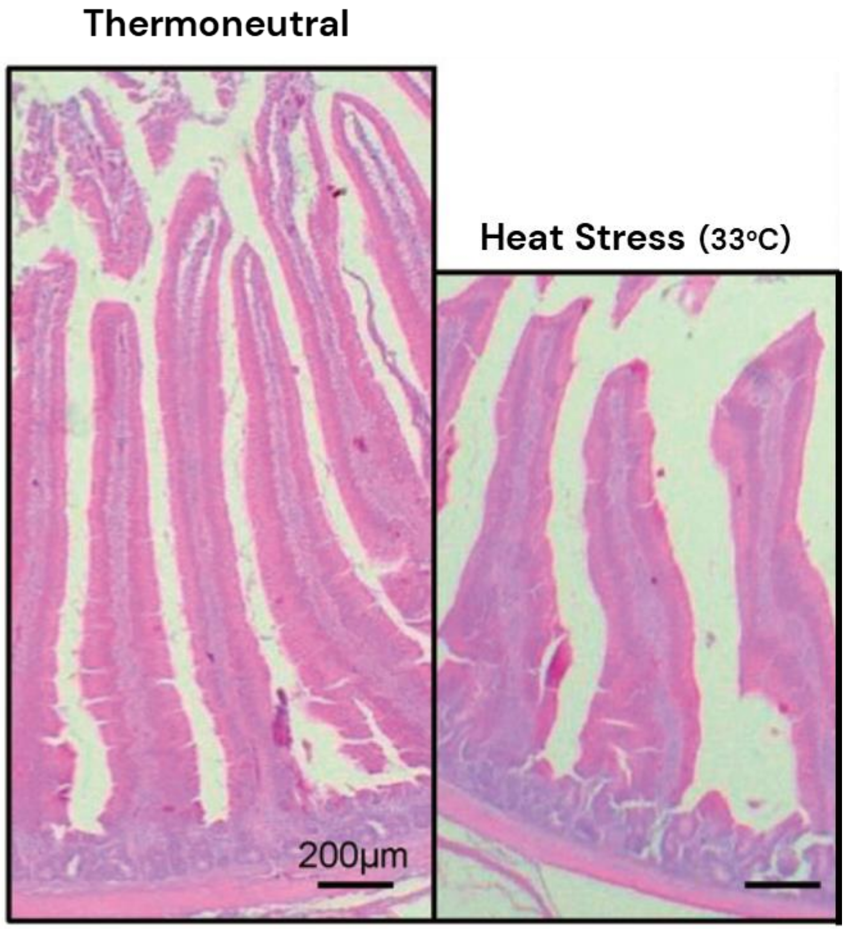
Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

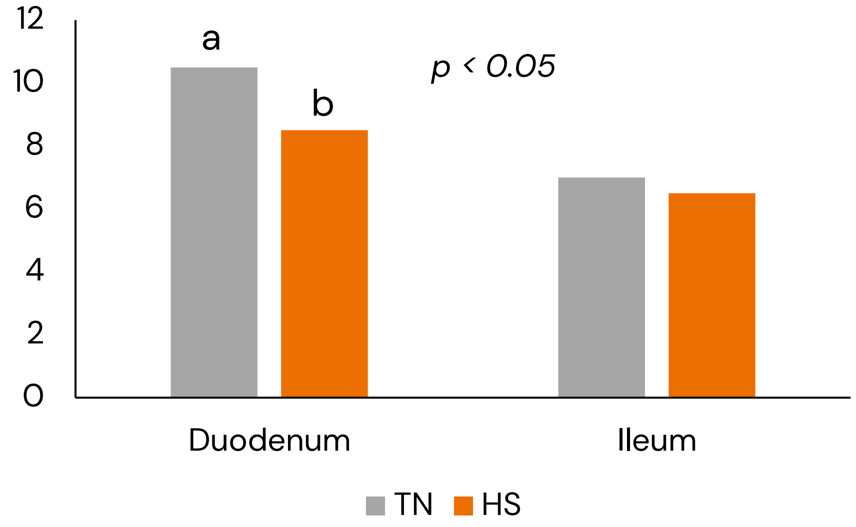




Morphology of duodenum

▼ intestinal absorptive surface

Villus height: crypt depth



Source: Nanto-Hara et al., 2020

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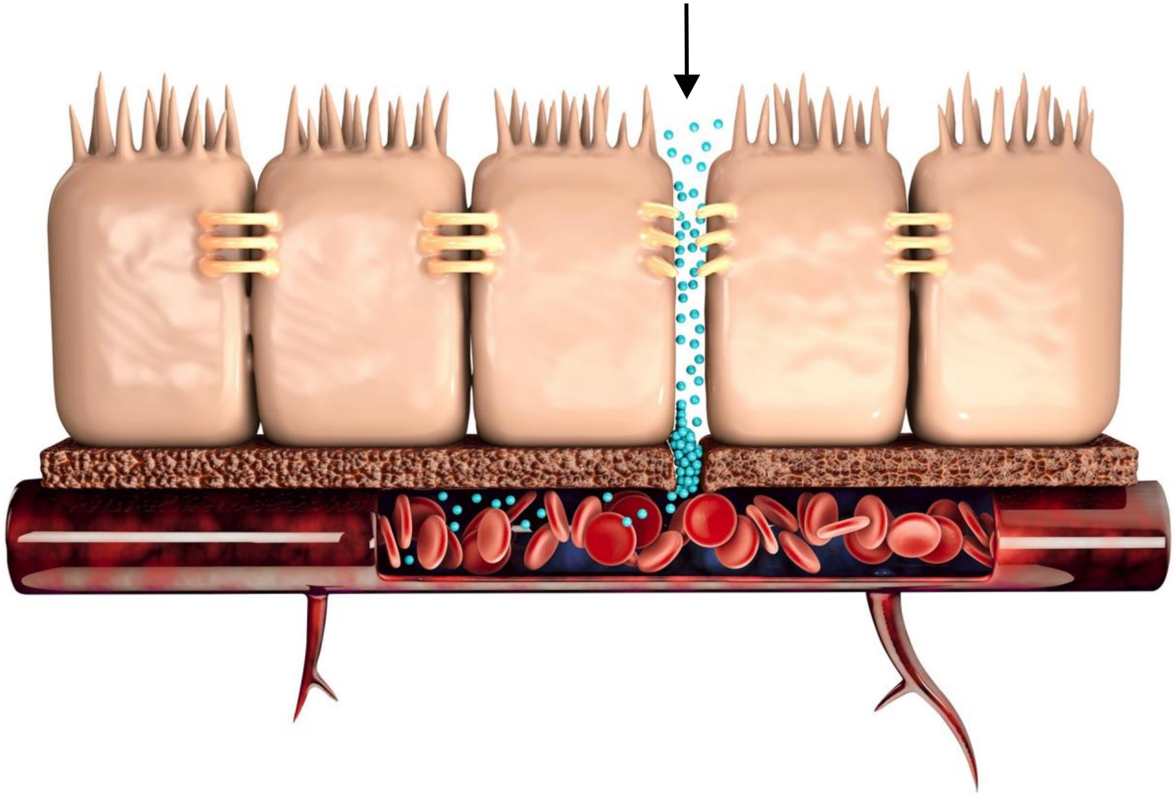
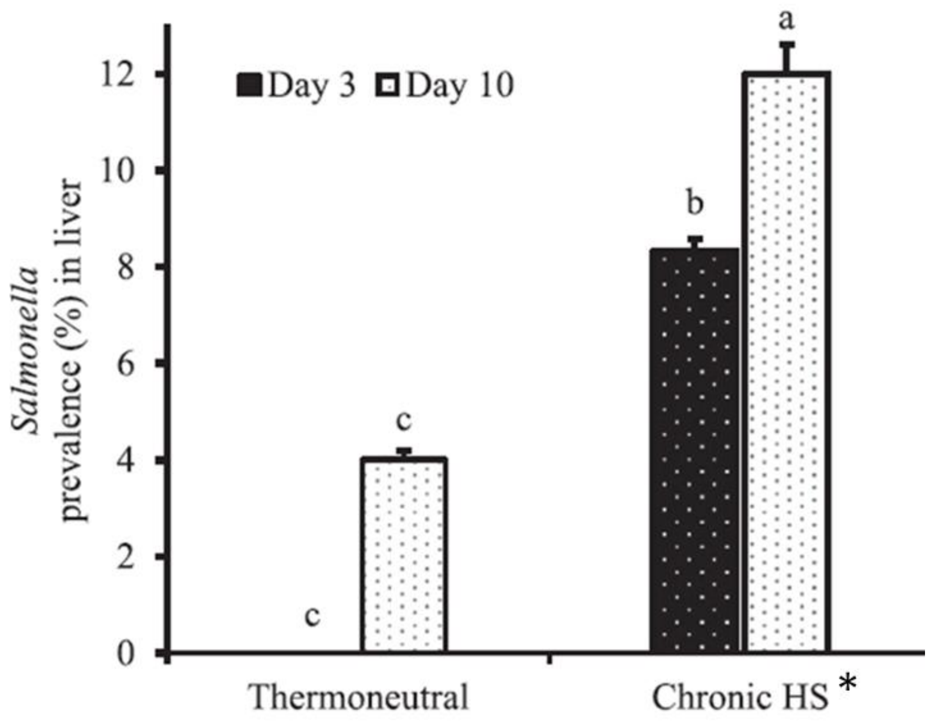
Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

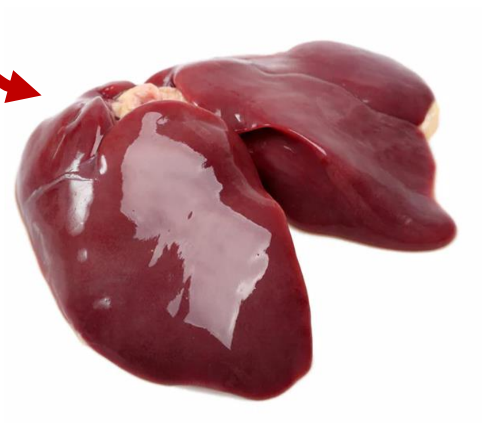
Bacterial translocation

Salmonella



▲ Salmonella translocation to liver





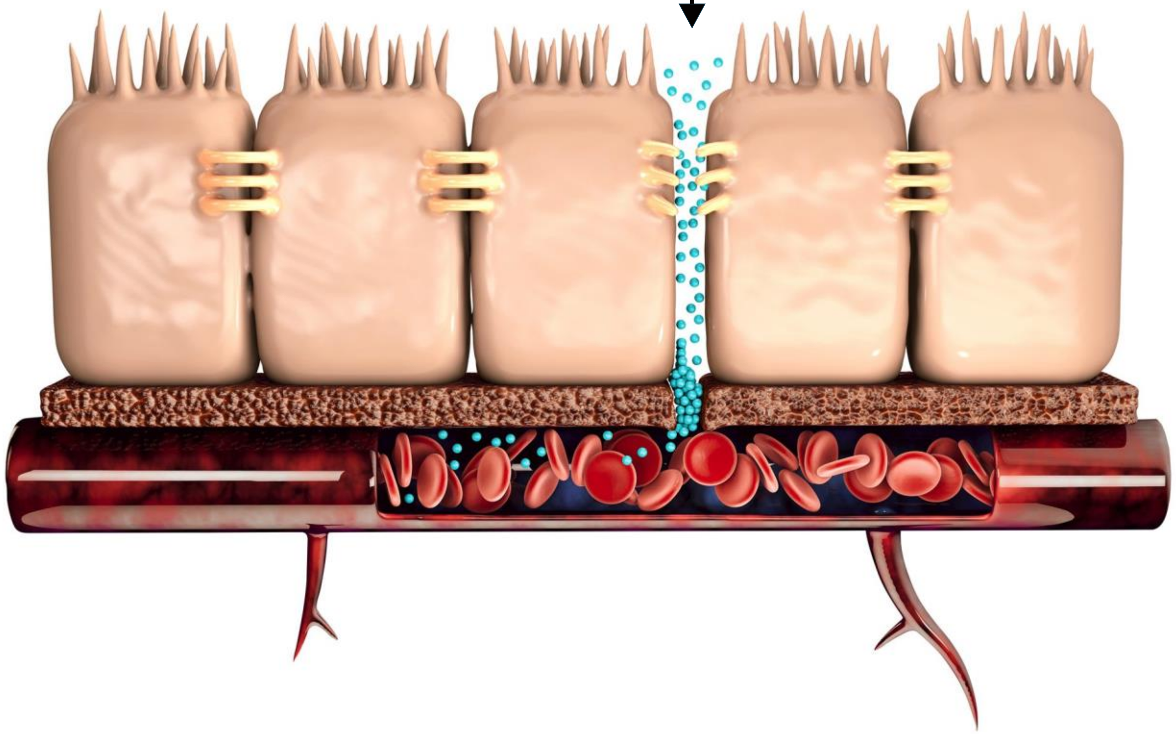
* T° 30±2°C, 33-53% RH from D-26 to D-35, breed – Hubbard

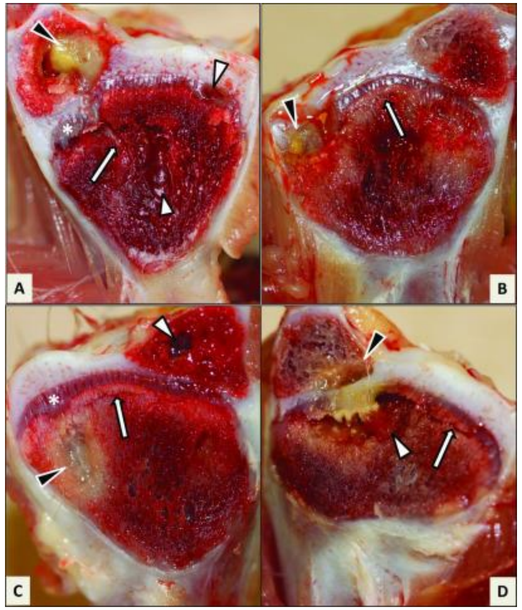
Source: Alhenaky et al., 2017

Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

Bacterial translocation

Bacteria (e.g., *Enterococcus cecorum*)

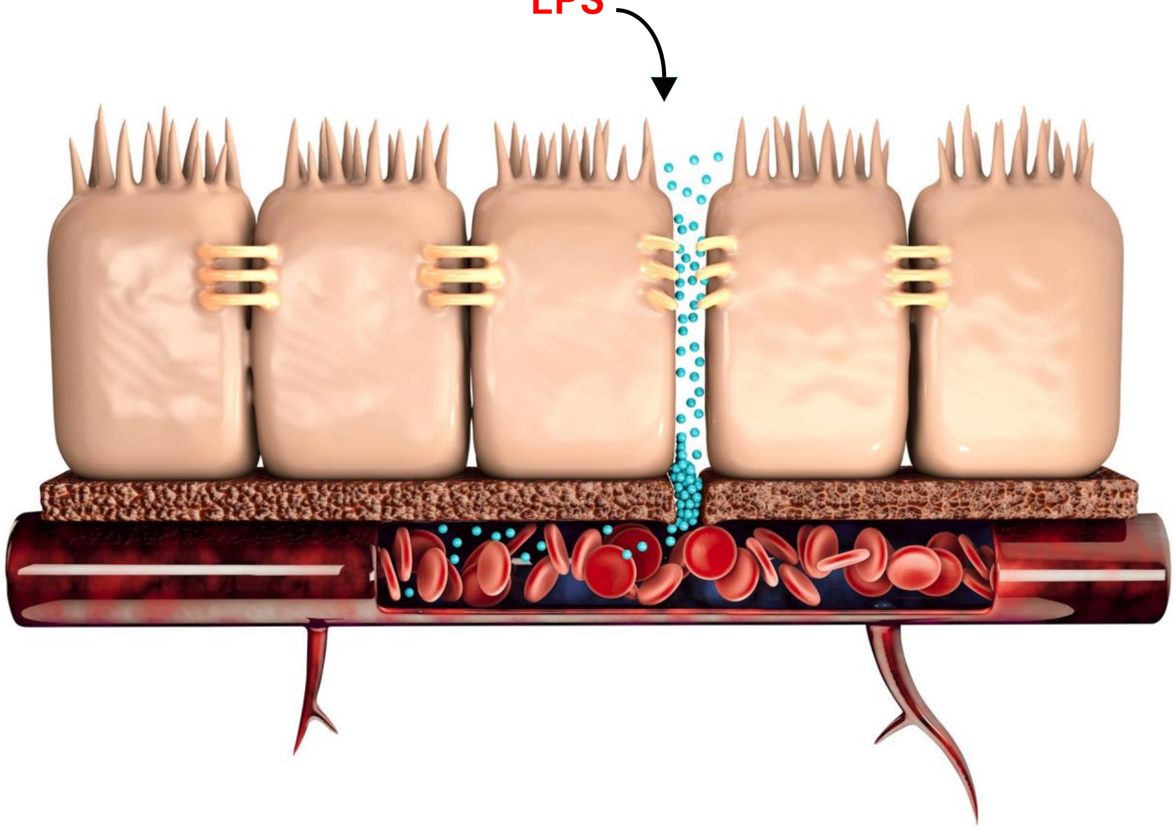




Bacterial chondronecrosis with osteomyelitis (BCO)

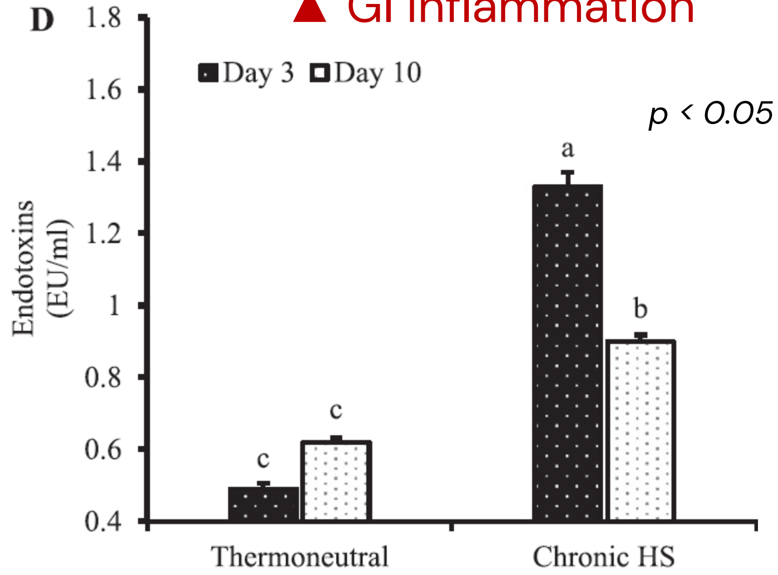
Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

Lipopolysaccharide Leakage



Source: *Nanto-Hara et al., 2020*

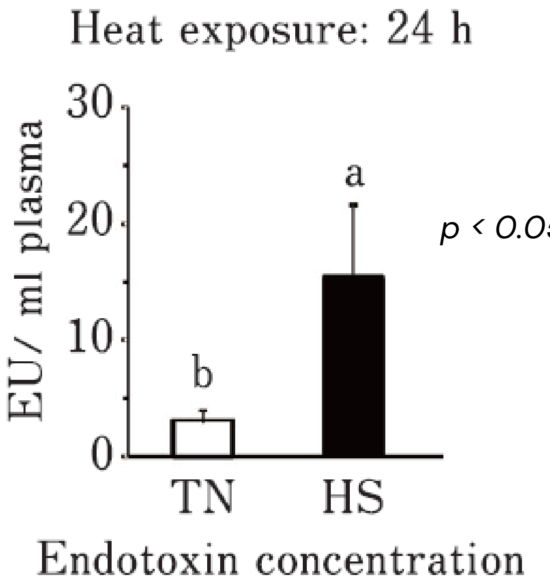
▲ GI inflammation



p < 0.05

* T° 30±2°C, 33-53% RH from D-26 to D-35, breed – Hubbard

Source: Alhenaky et al., 2017



p < 0.05

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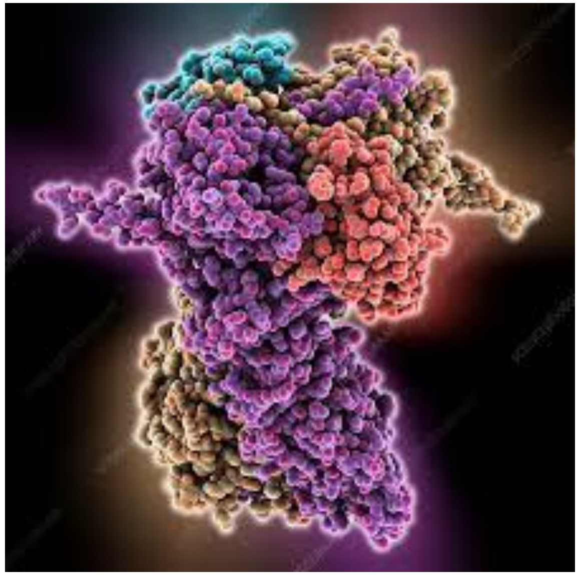
Effect of Heat Stress (HS) on Intestinal Integrity – Epithelium

What does it mean to the producers

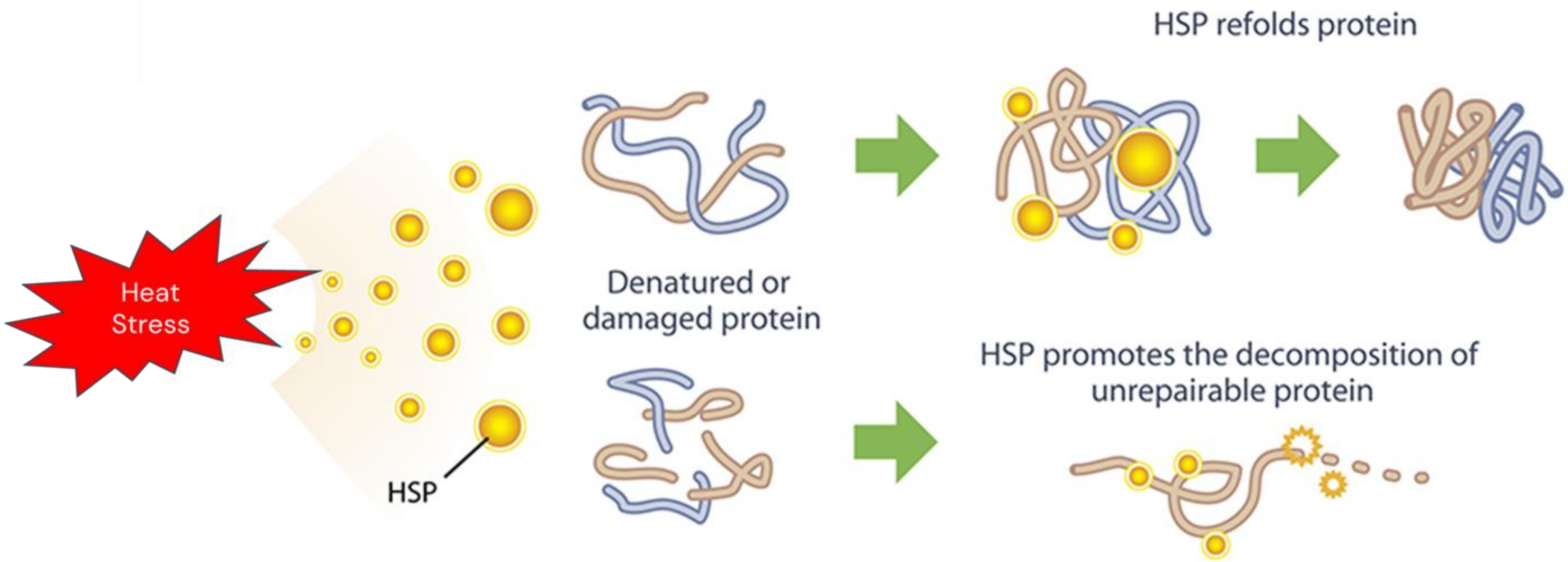
	Heat stress effect	Effects to Producers
GI epithelium	Altered GI epithelium morphology and enterocyte life cycle	Digestive and absorptive dysfunction ⇨ ↓ **performance**
		↑ paracellular permeability (↓ transepithelial electrical resistance and ↑ mucosa-to-serosa flux of markers) ⇨ **“leaky gut”**
	↓ GI epithelium integrity	LPS/endotoxin leakage → ↑ pro-inflammatory cytokines →GI inflammation and ↓ GI barrier ⇨ ↓ **performance**, ↑ **mortality**
		↓ **liver health and functionality**
		Endotoxemia → systemic inflammation, multi-organ failure, and septic shock ⇨ ↑ **mortality**, ↑ **culling**
		“Bacterial translocation” → bacterial chondronecrosis with osteomyelitis (BCO) lameness ⇨ ↓ **performance**, ↑ **culling**

Effect of Heat Stress (HS) on Molecular Biomarkers

The role of Heat Shock Protein

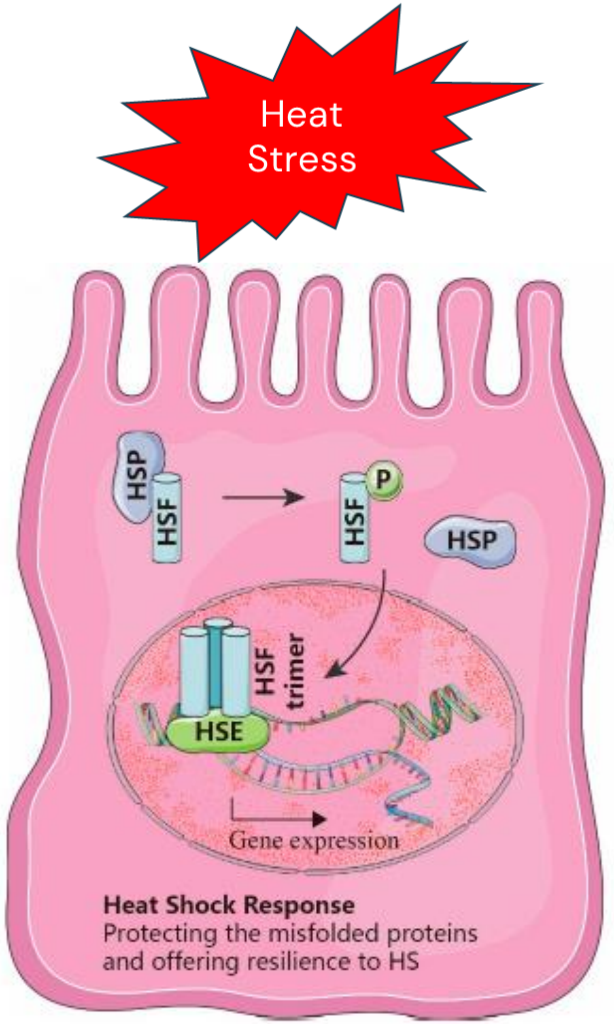


Heat Shock Protein



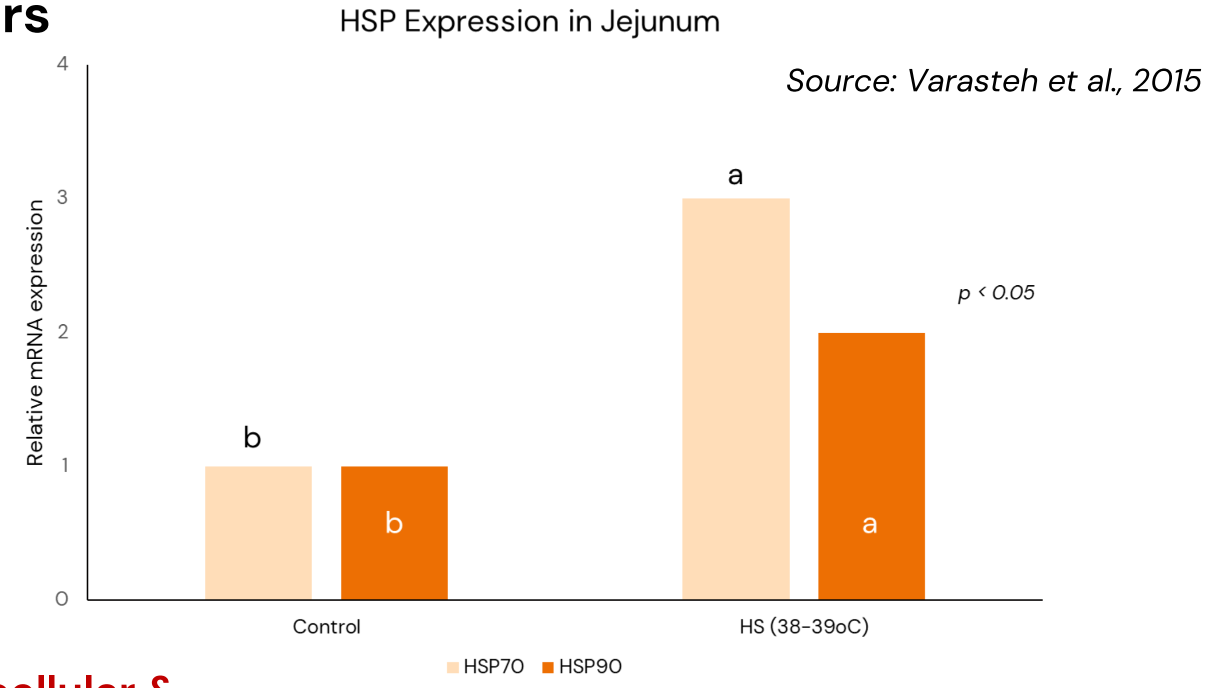
Adapted from http://www.animoup.info

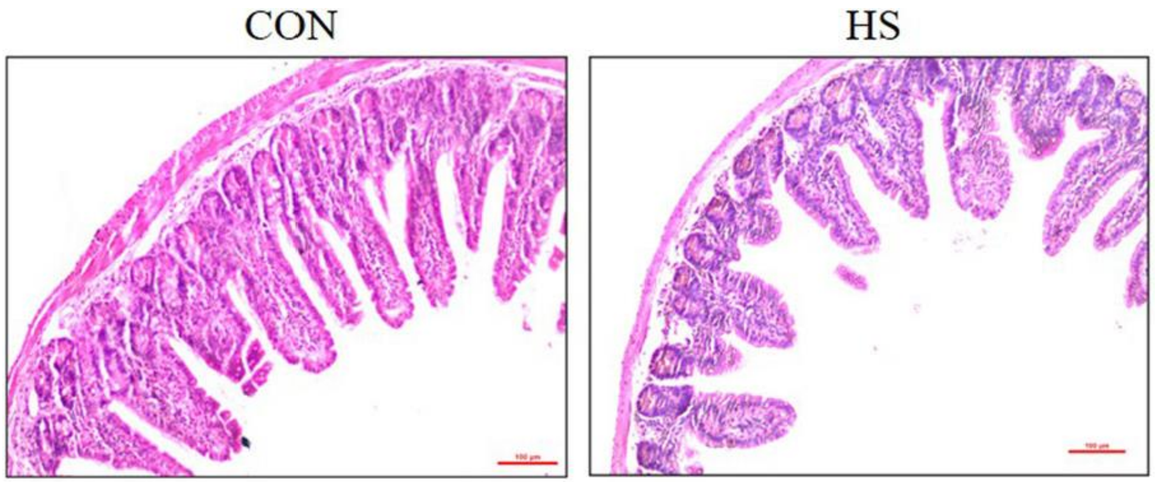
Effect of Heat Stress (HS) on Molecular Biomarkers



Cell under HS conditions activate heat shock response pathway and produce HSPs to protect the misfolded proteins and offer resilience to HS

▲ HSPs: indicating ▲ cellular & tissue injury





Source: Lu et al., 2022

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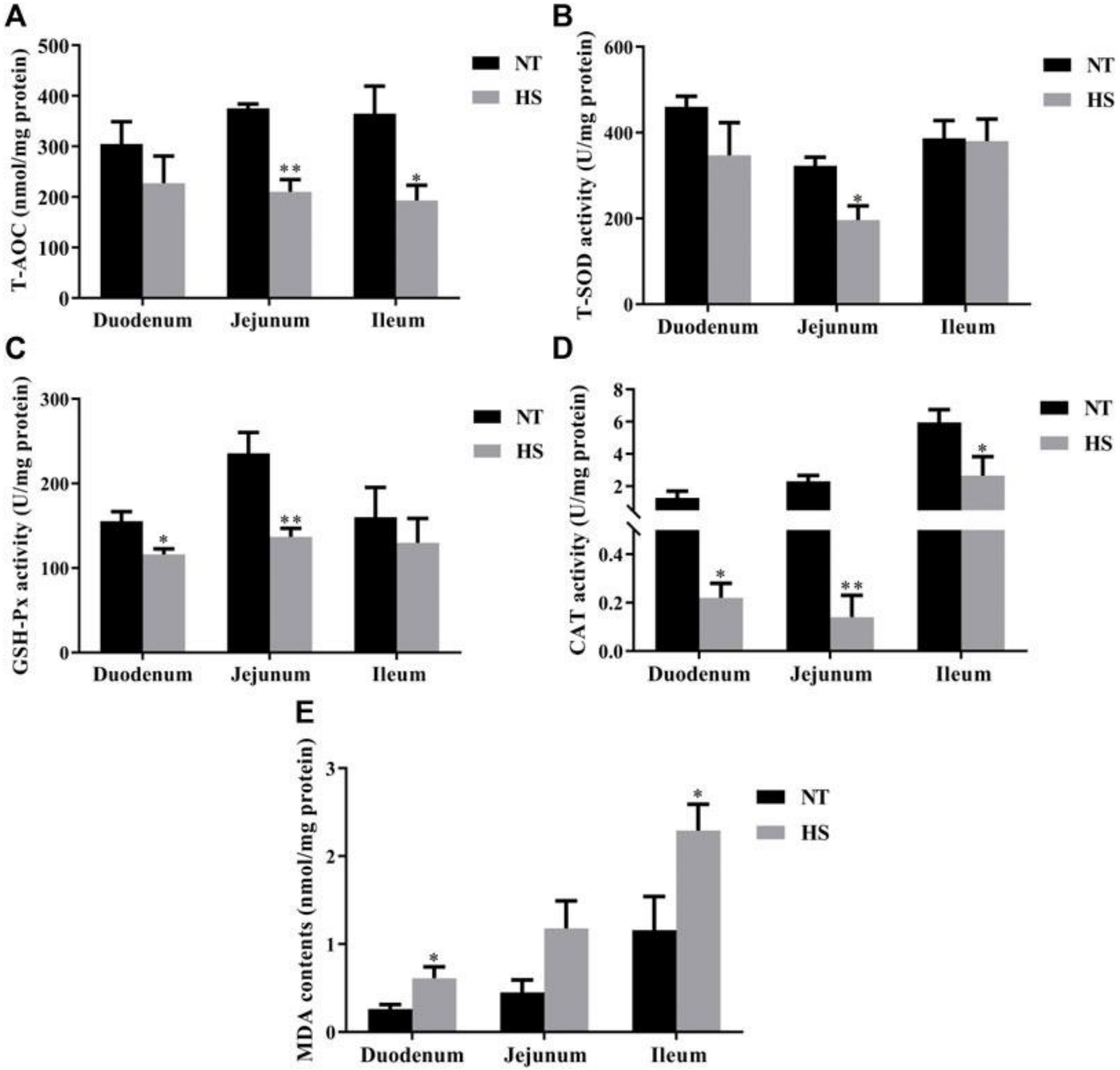
Effect of Heat Stress (HS) on Molecular Biomarkers



**p<0.05*
***p<0.01*

▲ HSPs:
▼ T-AOC
▼ T-SOD
▼ GSH-Px
▼ CAT
▲ MDA (oxidative stress marker)

Antioxidant enzymes

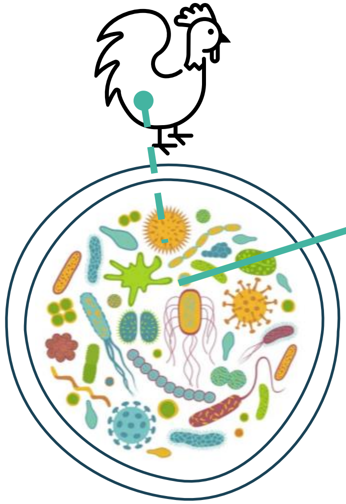
Source: Liu et al., 2022

Effect of Heat Stress (HS) on Molecular Biomarkers

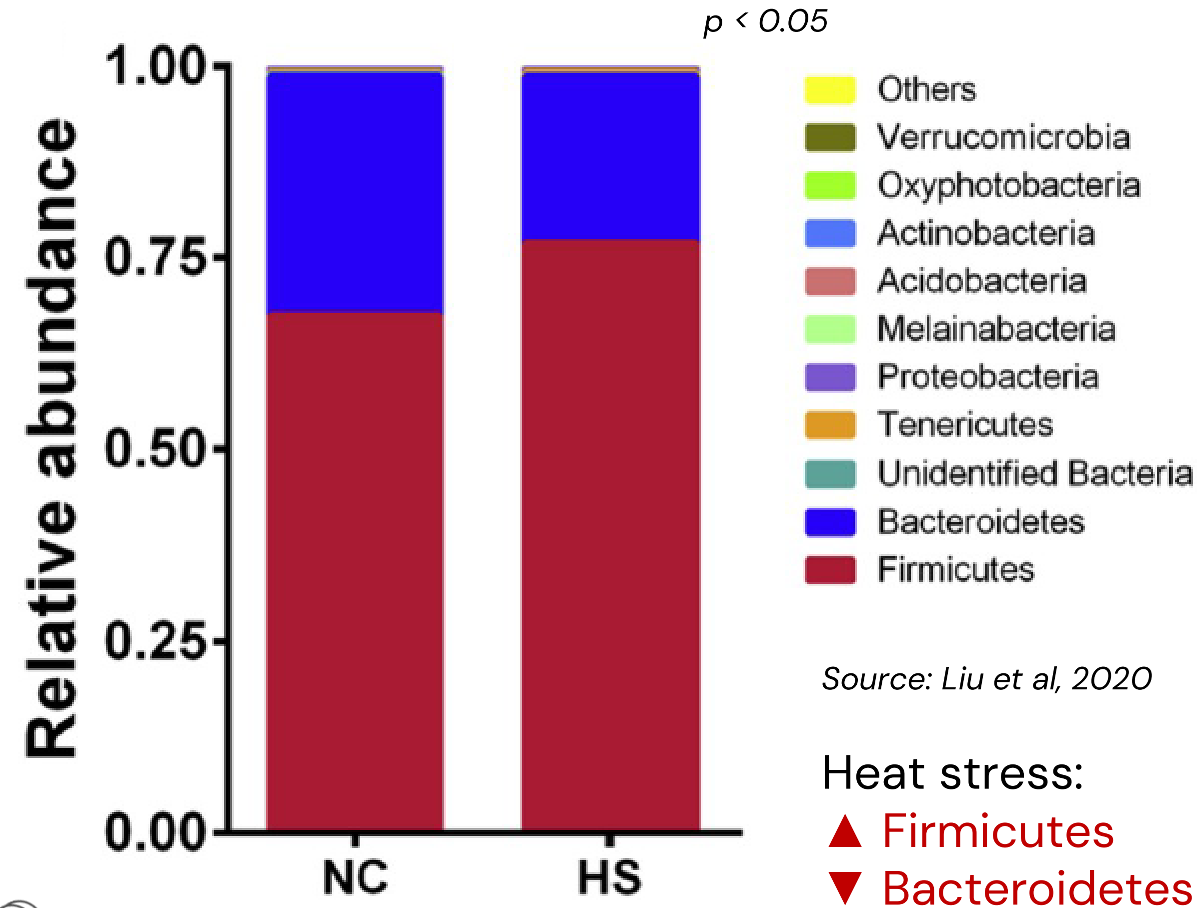
What does it mean to the producers

	Heat stress effect	Effects to Producers
Molecular Biochemistry	↑ heat shock proteins production – HSP70, HSP90	↓ **performance**
		↑ cellular stress and tissue injury → compromise immune system → susceptibility to diseases ⇨ ↓ **health and performance,** ↑ **medication cost,** ↑ **mortality**
	↓ antioxidant enzymes – SOD, GSH-Px, CAT ↑ oxidative stress marker - MDA	↑ production of ROS → ↑ oxidative stress and lipid peroxidation → cellular damage ⇨ ↓ **health and performance**

Effect of Heat Stress (HS) on Intestinal Microbiota



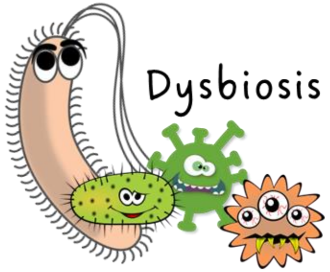
Major phyla in poultry's gut microbiota:
- *Firmicutes*
- *Bacteroidetes*
- *Proteobacteria*
- *Actinobacteria*
- *Cyanobacteria*



Source: Liu et al, 2020

Heat stress:
▲ Firmicutes
▼ Bacteroidetes

Changes in gut microbiota induced by heat stress:
- Structure:
  - Species richness, diversity, network, etc.
- Composition:
  - *Firmicutes : Bacteroidetes* ratio, abundance of taxa, etc.
- Metabolic functions:
  - Metabolism pathway, signaling pathways, etc.
- Microbial metabolites:
  - Short-chain fatty acids, amino acids, etc.



▲ F/B ratio = microbial imbalance

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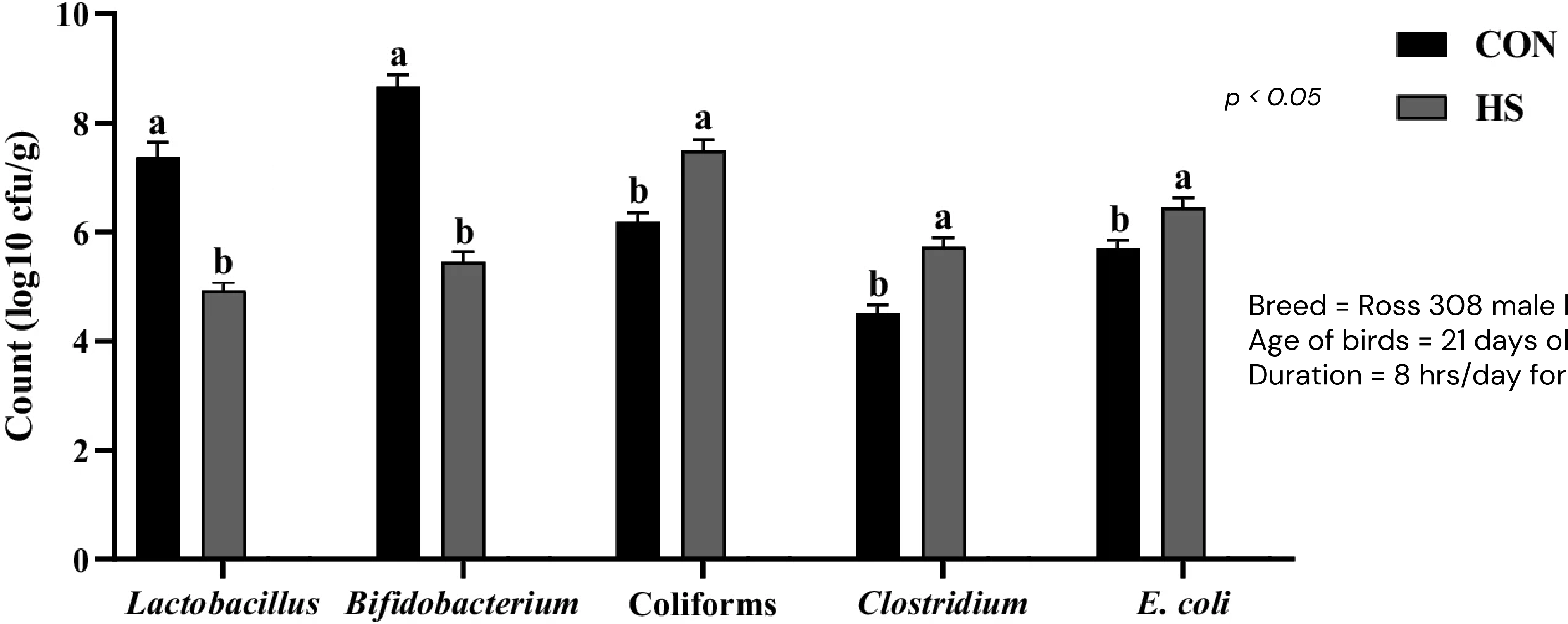
Effect of Heat Stress (HS) on Intestinal Microbiota



$p < 0.05$

Breed = Ross 308 male broiler
Age of birds = 21 days old, HS = 33±1°C
Duration = 8 hrs/day for 22 days

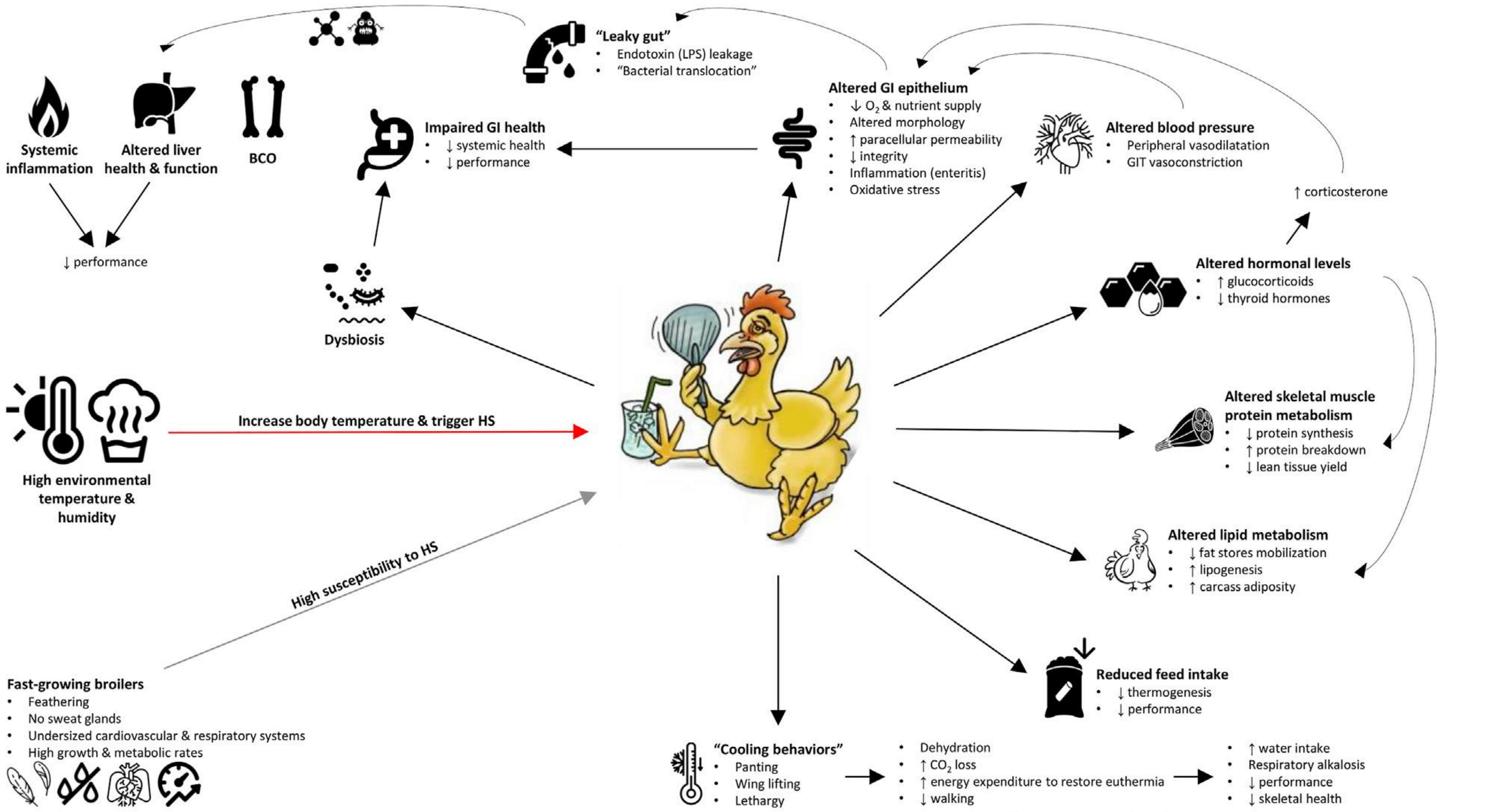
Source: Sulaiman et al, 2023

Effect of Heat Stress (HS) on Intestinal Microbiota

What does it mean to the producers

	Heat stress effect	Effects to Producers
GI microbiota	Perturbation of GI ecosystem and microbial community stability	Dysbiosis → GI barrier dysfunction and GI inflammation ⇨ ↓ **health and performance, ↑ medication cost, ↑ mortality**
		↑ susceptibility to GI pathogen colonization → GI disorder (e.g. necrotic enteritis) ⇨ ↓ **health and performance, ↑ medication cost, ↑ mortality**

Summary



Icon source: ●✖■ Noun Project

Picture modified from: https://www.durvet.com/blog/chill-out-summertime-safety-for-backyard-poultry/

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Mechanism-based Intervention Strategies on Heat Stress

GOAL: reduce ROS production, improve antioxidant defense system, stabilizing gut microbiota

<u>Microbial modulation</u>

i. Probiotics
   - "living microorganisms which exert health promoting benefits when administered in adequate amounts" (Reid, 2016)
   - *Lactobacilli, Bifidobacteria, Bacilli, Streptococci, Aspergillus, Candida, Saccharomyces* etc.

ii. Prebiotics
   - "selectively fermented ingredients that results in specific changes in the composition and/or activity of the GI microbiota, thus conferring benefit(s) upon host health (Valcheva et al., 2016)

iii. Synbiotics
   - "mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and activity of beneficial microorganism in the gut" (Gyawali et al., 2019)

Mechanism-based Intervention Strategies on Heat Stress

The effects of pro-/prebiotics on intestinal integrity and immunomodulation

	Name	Integrity	Immunomodulation	Other Effect(s)
Pro-biotics	*Lactobacillus* species	TEER↑ Intestinal permeability↓ ZO-1↑ occludin↑ E-cadherin↑ claudin-2↑ claudin-3↑ Morphological damage↓ β-catenin↑	IL-10↑ IL-27↑ IL-1↓ IL-6↓ TNF-α↓ NF-κB activation↓ Corticosterone↓ IgA secreting cells↑ Intraepithelial lymphocytes↓	Integrin-p38 MAPK activation↑ HSP expression↑ Antioxidative capacity↑ Nutrient transporters↑ Mucin genes transcription and protein production↑
	Bifidobacterium species			
	Bacillus species			
	E. coli Nissle	ZO-2 dissociation↓		-
	Streptococcus thermophiles	occludin delocalization↓		-

	Name	Integrity	Immunomodulation	Other Effect(s)
Pre-biotics	HMO	ZO-1↑ occludin↑ JAM-A↑ Crypt proliferation↑ Intestinal permeability↓	IL-10↑ TLR-4↓ NF-κB translocation↓ p38 MAPK activation↓	Mucus production↑ HIF-1α↓ Cleaved caspase-3↓ EGFR activation↑
	GOS	TEER↑ Intestinal permeability↓ occludin↑ claudin-3↑ E-cadherin↑	IL-6 mRNA↓ IL-8 mRNA↓ TLR-4↓ IL-33↓ CXCL-8↓ CXCL-1↓ CXCL-2↓	HSP expression↓ Populations of probiotics↑ HO-1 expression↓
	MOS	Intestinal permeability↓ permeability↓ Villus height↑	-	Goblet cells↑ Populations of probiotics↑ *E. coli* load↑
	COS	Intestinal permeability↓ Morphological damage↓	-	-
	FOS	TEER↑ Intestinal permeability↓ occludin↑ ZO-1↑	-	Colonic SCFA concentration↑ Mucosal damage↓

Source: Lian et al, 2020

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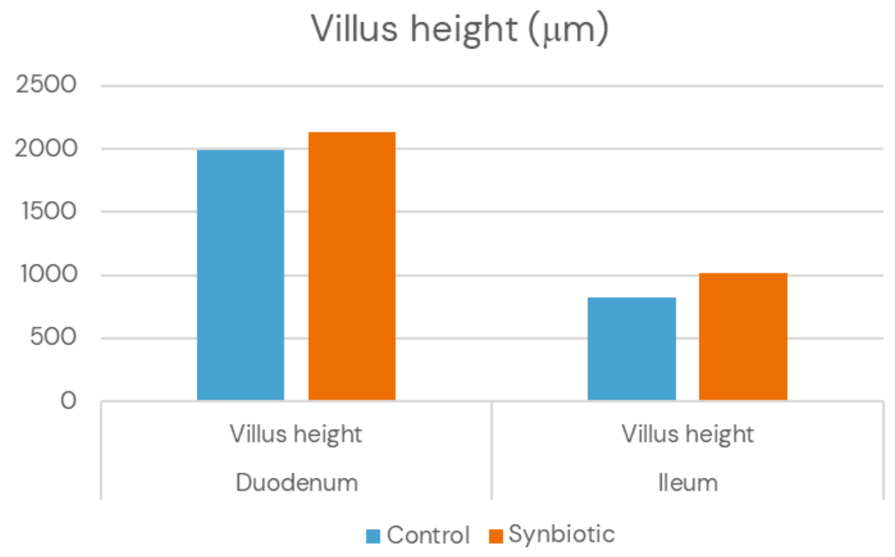
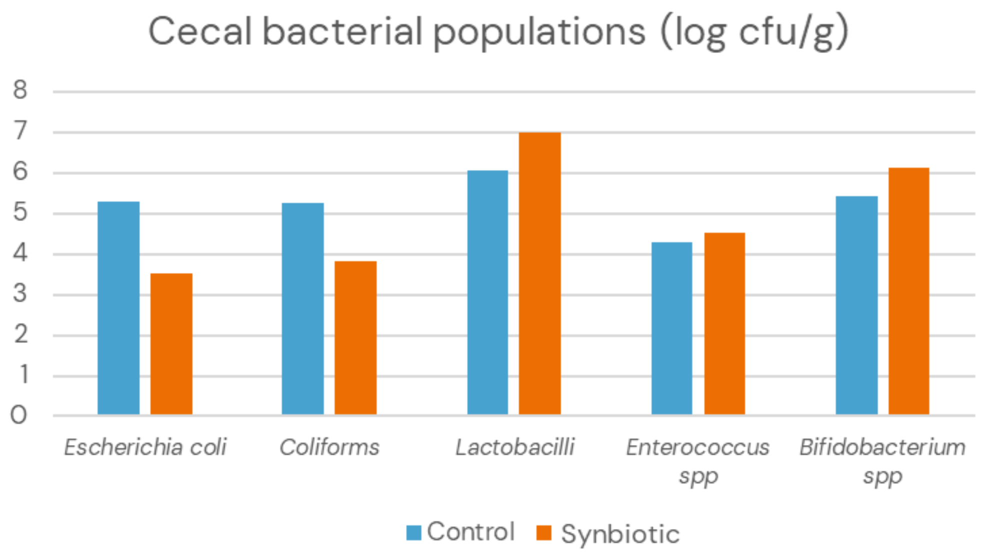
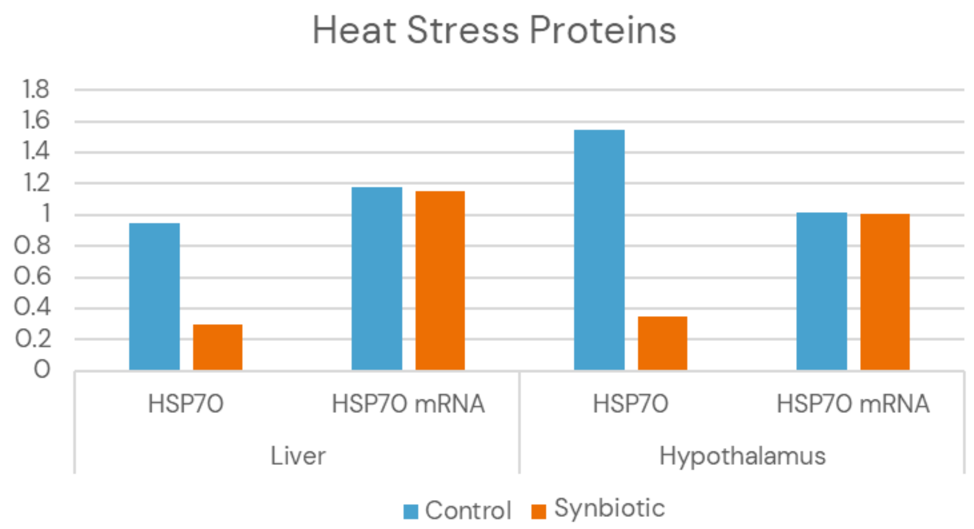
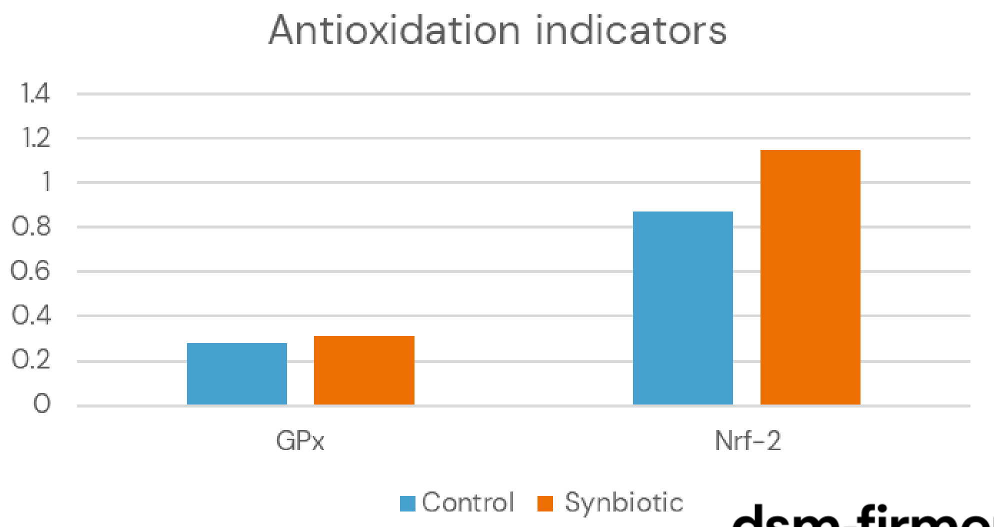
Effects of Synbiotics on Intestinal Health of Heat-Stressed Birds







Source: Jiang et al, 2020



Source: Mohammed et al, 2019

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Mechanism-based Intervention Strategies on Heat Stress

GOAL: reduce ROS production, improve antioxidant defense system, stabilizing gut microbiota

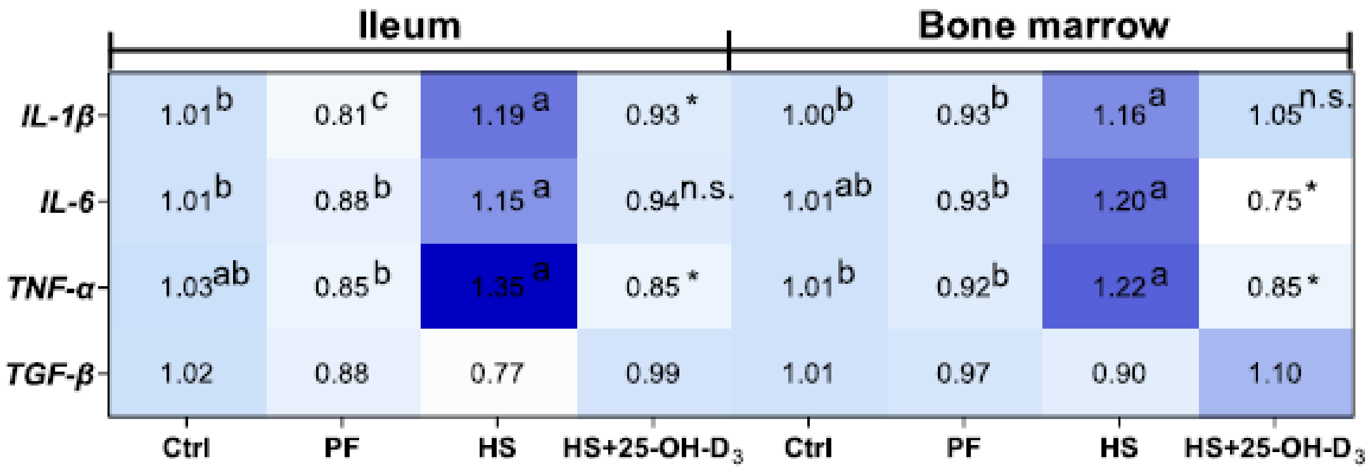
Vitamin Supplementation

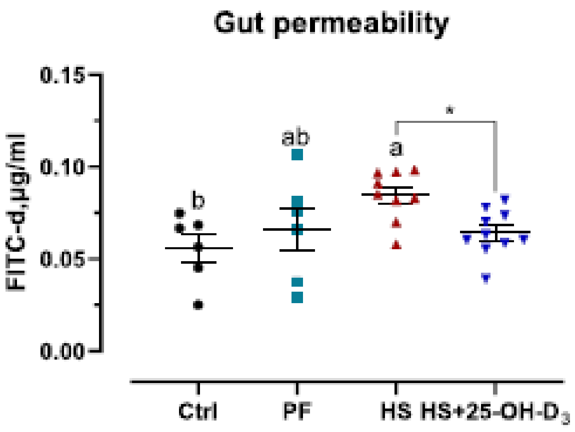
i. 25-hydroxy vitamin D_3 (25-(OH)D_3)
   - A highly effective metabolite of vitamin D_3
   - Decreased expression of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α)
   - Increased expression of anti-inflammatory cytokines (TGF-β)

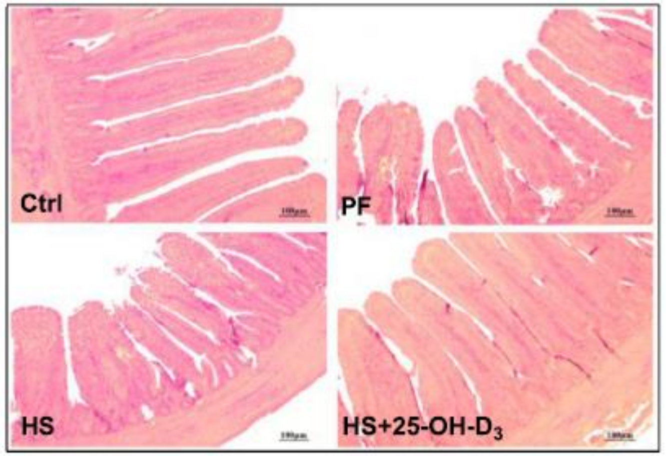
ii. Vitamin E (a-tocopherol acetate)
   - Lower physiological stress released by corticosterone and catecholamines
   - Protection against lipoperoxidative damage by free radicals
   - Improved immune responsiveness, proliferation and function of immune cells (e.g. macrophages, plasma cell, lymphocytes, etc.)

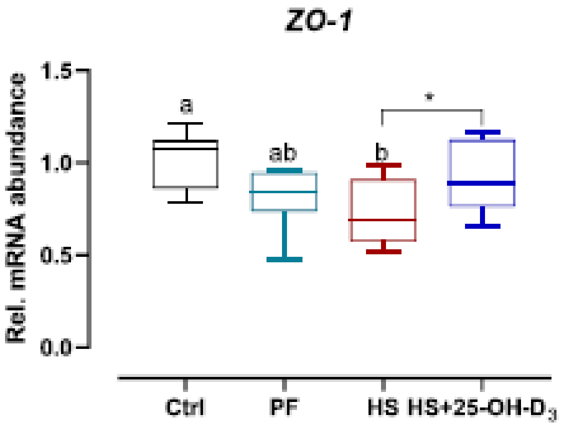
iii. Vitamin C (ascorbic acid)
   - water-soluble antioxidant
   - Improved antioxidant status and decreased lipid peroxidation
   - Important in regulating normal rectal and body temperature during heat stress period
   - Facilitate the utilization of nutrients and enhance nutrient digestibility

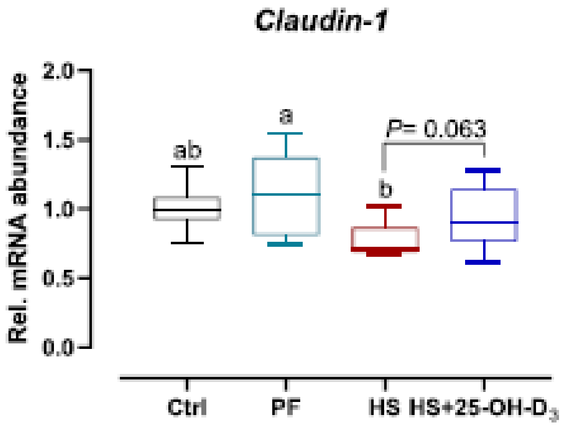
Effects of 25-(OH)D_3 on Intestinal Health of Heat-Stressed Birds











Source: Zhang et al, 2021

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Mechanism-based Intervention Strategies on Heat Stress

GOAL: reduce ROS production, improve antioxidant defense system, stabilizing gut microbiota

Phytobiotic/Phytochemicals/Herbs

Phytochemicals/Herbs	Effect on heat-stress birds
Ginger	• Increased biochemical blood parameters • Enhanced growth performance • High antibacterial potential • Increment of digestibility, palatability, metabolism, and health status of broilers
Garlic	• Increased intestinal villi • Reduce crypt depth
Lycopene	• Powerful antioxidant • Increase cell growth and immune response • Anti-inflammatory, immune-booster effect • Augment antioxidant enzymes e.g. SOD and GSH-Px • Dropping MDA level
Resveratrol (trans-3,5,4'-trihidroxiestilbeno)	• Improvement in FI and BWG under chronic heat stress • Lower HSP27, HSP70 and HSP90 mRNA • Increase antioxidant enzymes SOD, CAT, and GSH-Px • Diminished MDA • Amplify *Lactobacillus* and *Bifidobacterium*, and lessen *Escherichia coli* • Enhances intestinal epithelial barrier function and tight junction via modulating mRNA expression of related genes
Cinnamon powder	• Enhanced daily gain and antioxidative status (SOD, CAT, and total antioxidant capacity) • Reduce the concentration of MDA
Thyme essential oil	• Improve growth performance • Augmented humoral immune response, and relative weight of lymphoid organs (spleen, thymus, and bursa of Fabricius)

Nutritional Manipulation for Heat Stress Alleviation in Poultry

- Feeding management
- Water management
- Increasing feed density
- Amino acid supplementation
- Vitamin supplementation
- Mineral supplementation
- Phytochemicals and herbal additives
- Pre-, pro- and synbiotic

- Promotes growth performance
- Increases productivity
- Anti-stress effect
- Scavenges free radicals
- Promotes antioxidant defense
- Anti-inflammatory effect
- Stimulates immunocompetence
- Regulates heat shock response
- Antimicrobial effects
- Improves nutrient digestibility
- Protects intestinal health
- Restores blood metabolites
- Promotes behavior and welfare
- Improves thermoregulatory response
- Reduces mortality

Adapted from Onagbesan et al., 2023

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