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FEFANA

Mechanisms involved in heat stress repose & Characterization of intervention strategies

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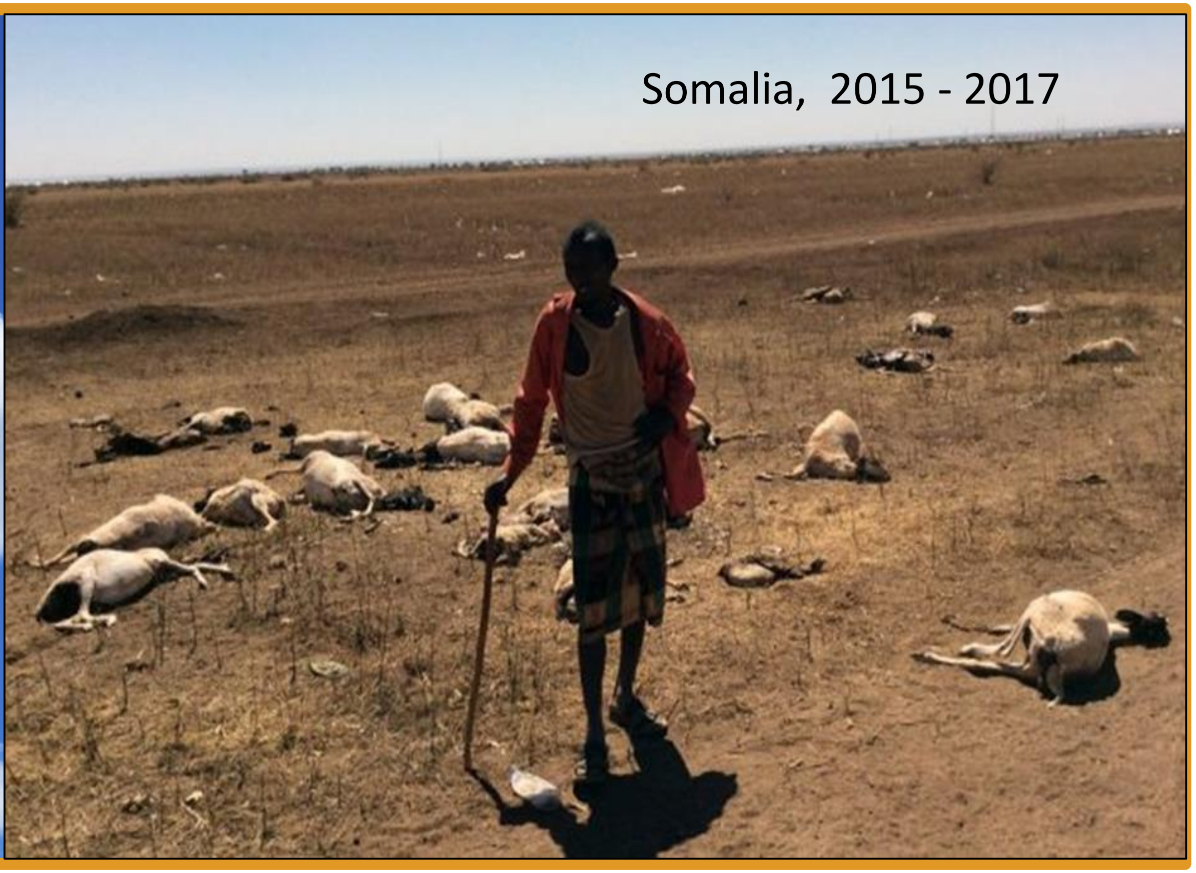
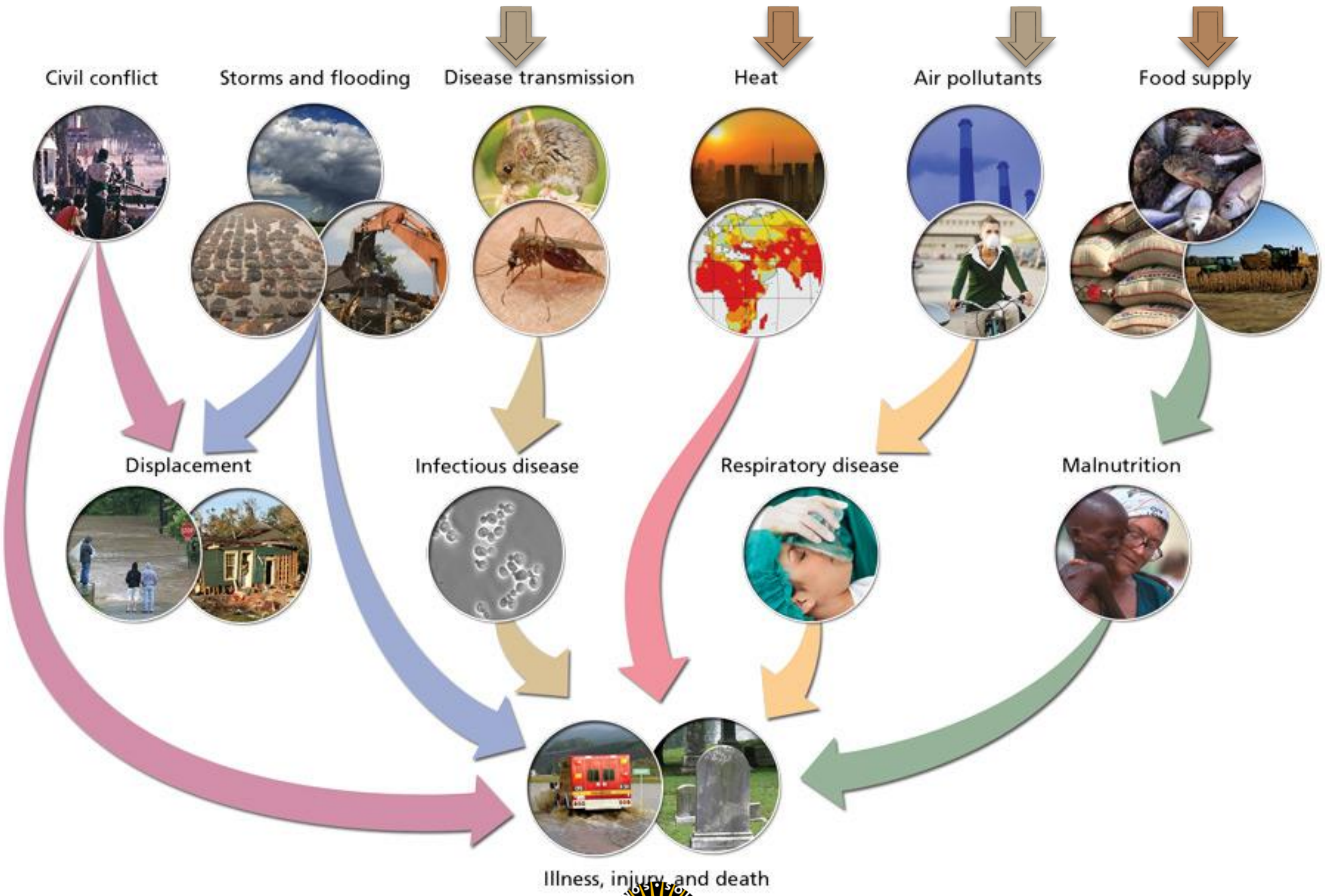
Heat stress -
A major component of worldwide climate change



Climate change: Loss of arable land – loss of biodiversity

Increase in transmissible (vector-borne) diseases - <u>major animal stressor</u>

Projected overall impact of global climate changes



Physiological response to temperature stress

Exogenous stressors:
- High environmental temperature
- Strenuous exercise

HEAT STRESS - HEAT STROKE

Non controlled-may become lethal

Hyperthermia

Changes in temperature set point
- Fever (infection – IL-6))
- Drug induced (anaesthetics, psychotropic drugs…)

Rise in body temperature

Adaptive Response

Decrease in food/feed intake → decrease in water intake

DEHYDRATIION

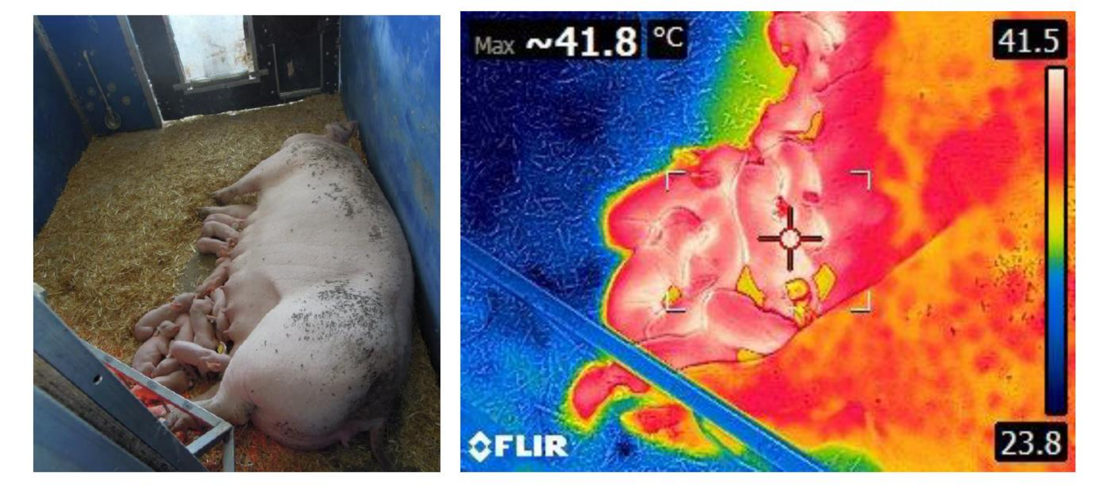
Increase respiration rate (panting) and heart rate

Repartitioning of blood flow favouring heat dissemination →
- **Peripheral vasodilation / central vasoconstriction**
- **Decease of splanchnic blood flow**
- **Decrease in pelvic blood flow (reproductive organs) →**

HYPOXIA → CELLULAR OXIDATIVE STRESS → loss of cell functions

Activation of a heat stress response

Activation of a heat stress response – visible clinical signs



Panting:
CO_2 loss → metabolic (blood) alkalosis

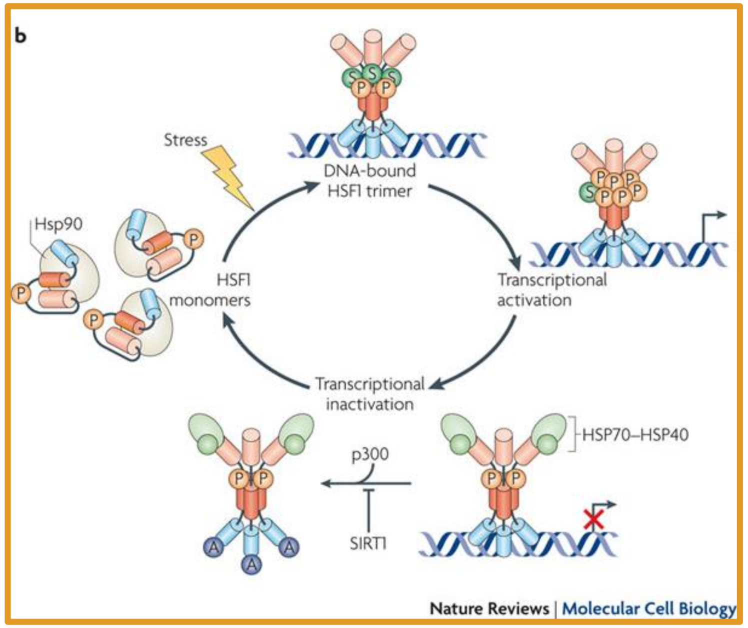
Lethargy → loss of appetite

Impairment of animal wellbeing, productivity and health

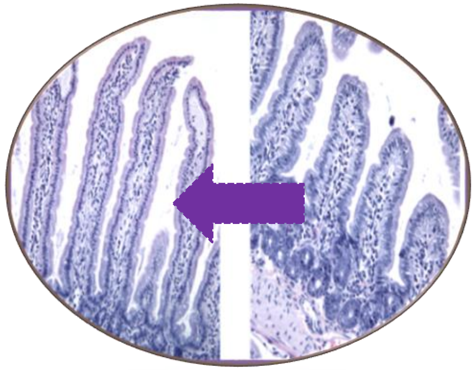
Activation of a heat stress response (key-elements) and repair mechanisms

Repartitioning of blood flow
Tissue HYPOXIA
Cellular oxidative stress

Activation of HSF and expression of heat shock proteins (chaperons to protect cellular proteins)

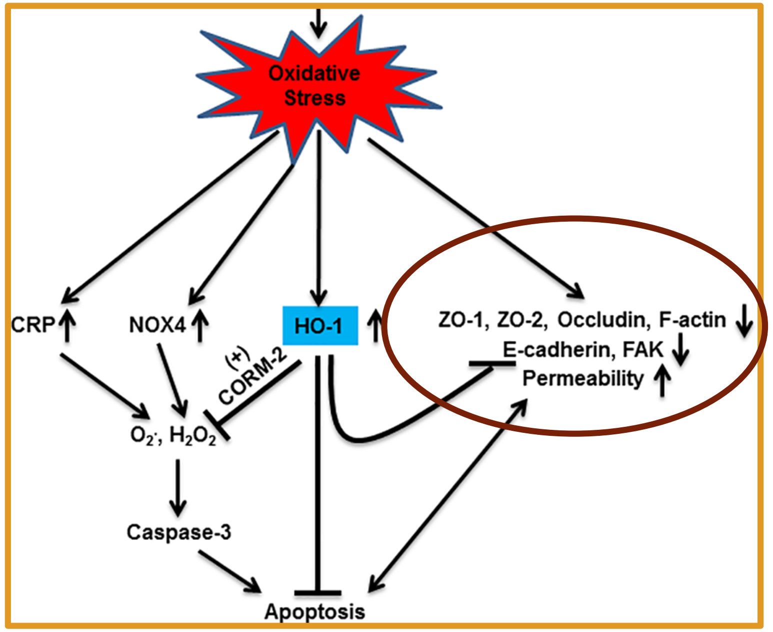


The HSF1 activation and attenuation cycle, involving trimerization, multiple post-transcriptional modifications and feedback from heat shock proteins (HSPs).



Intestinal effects
(& repair)

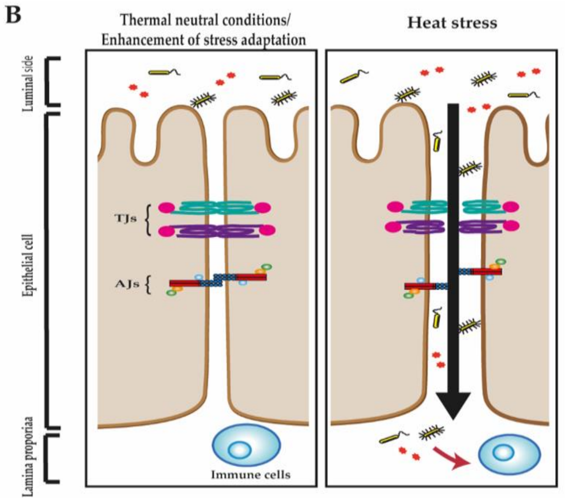
Activation of HO-1 (heme oxygenase) (Stimulation of anti-oxidant pathways)



HO-1 inhibits the cleavage of TJ- proteins and blocks apoptosis

(1) The poultry model:
in vivo proof of principle

Hypothesis:
Heat stress impairs the intestinal barrier integrity (TJ) resulting in gut leakage



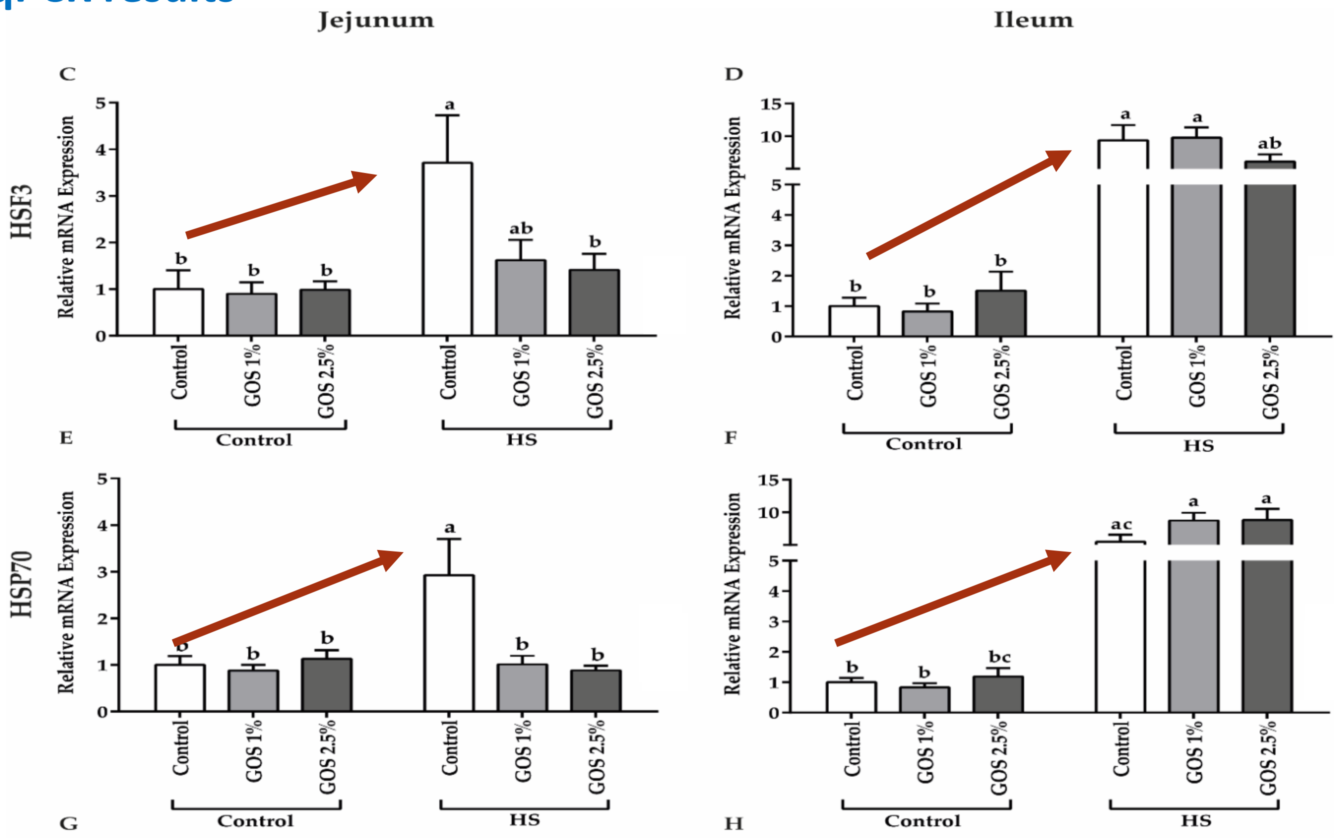
Experimental groups:
- Control (normal temperature 23-25°C)
- Heat stress (39°C, 5 days, 8h per day)

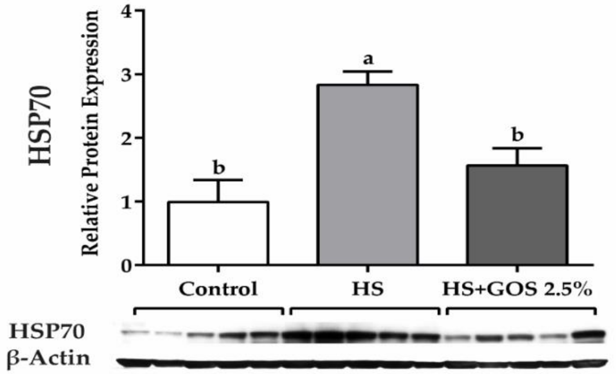
Test parameters in jejunum and ileum:
Gene (and protein) expression:
- HSFs, HSPs
- Tight junction and adherens junction proteins
- Inflammatory markers

Intervention model:
GOS (galacto- oligosaccharides)
stimulating TJ expression &assemblage

Heat shock response in chicken intestines

HSF3 & HSP 70 expression: qPCR results



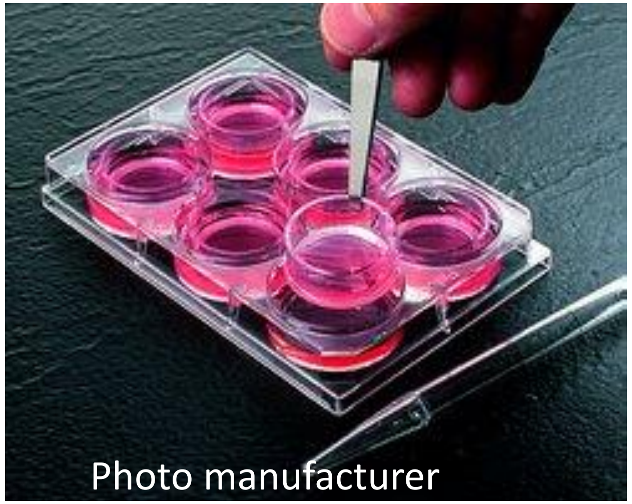


HSP70- protein levels (jejunum)

Heat stress upregulates the **HSF3** and **HSP70** expression
GOS increases resilience to heat shock in the jejunum

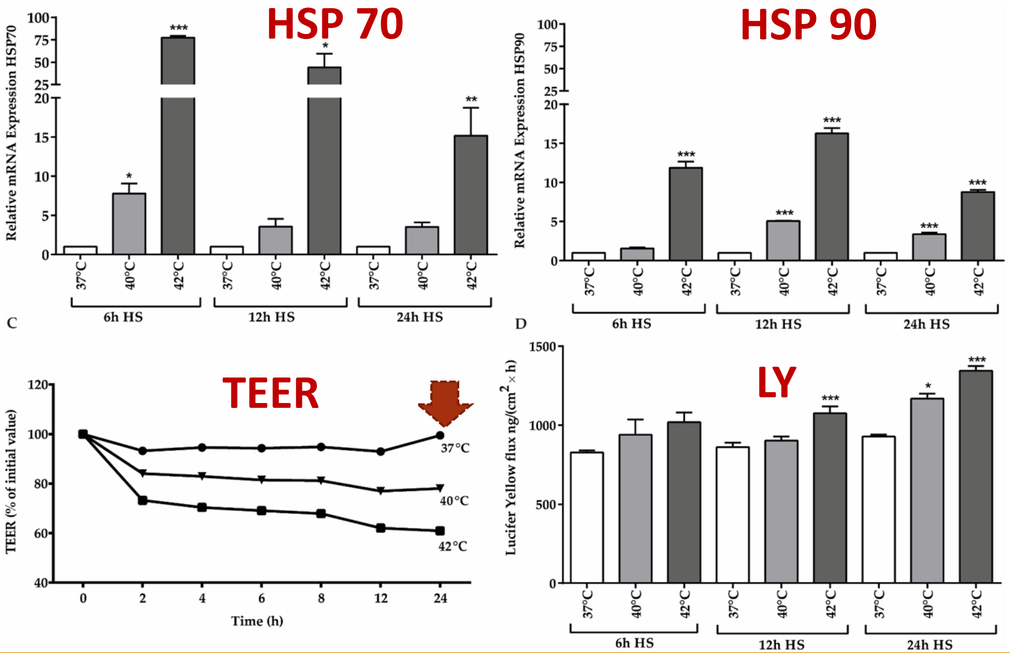
For more details see Varasteh et al., 2017, & Dos Santos et al. 2019

I(2) n vitro model: rapid testing of potential intervention strategies



Parallel incubations at **37°, 40° and 42° Celsius**

Temperature-dependent HSP expression



Temperature-dependent decrease in TEER
followed by an increase in Lucifer yellow transport

Caco-2 cells (intestinal epithet cell line) grown on transwell inserts:

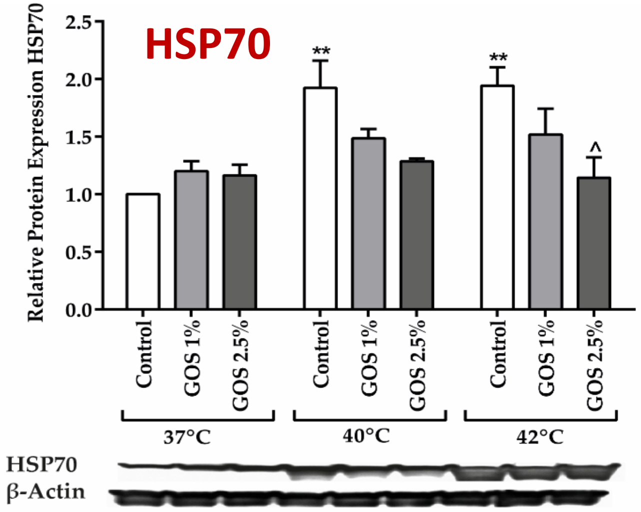
- **Well characterized cell line (drug research)**
- **Well established model for the measurement of functional parameters of transmembrane transport & gut leakage**

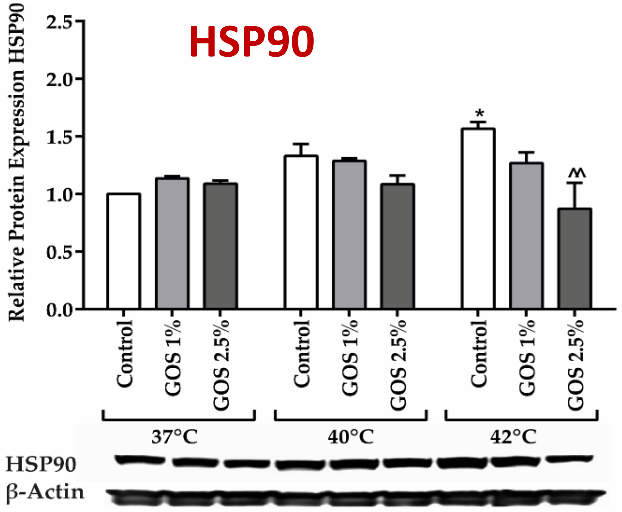
Heat stress: protective effects of GOS in the Caco-2 cell model

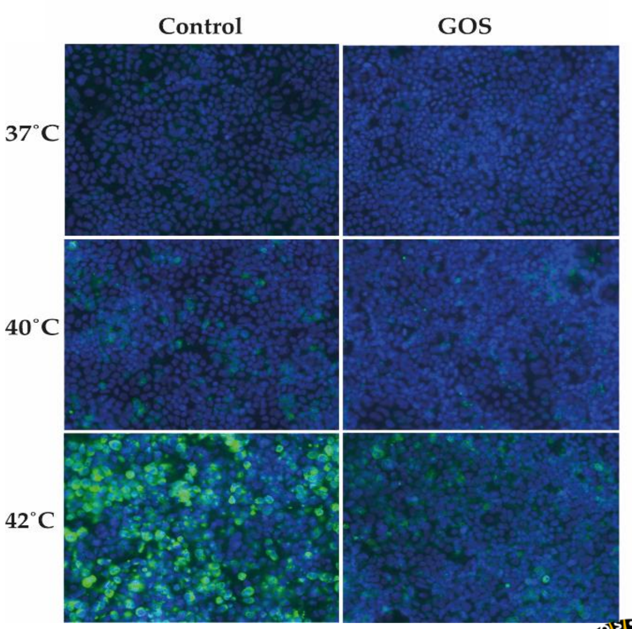
mRNA (qPCR)

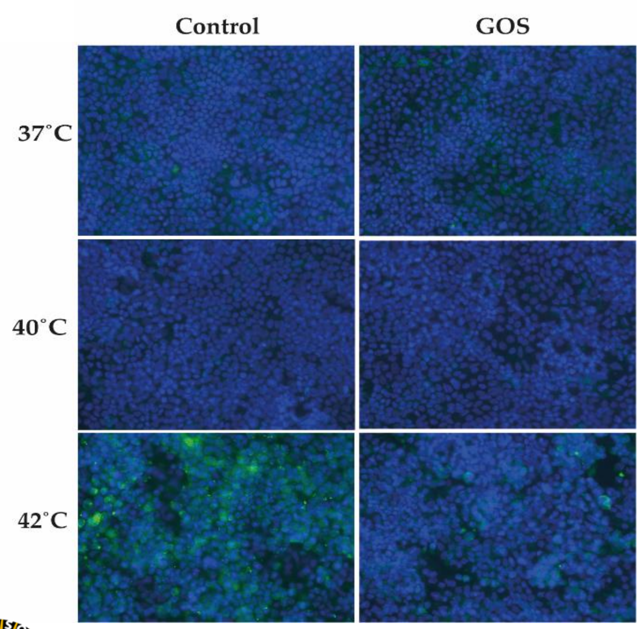
Protein expression
(Western blots)

Immuno-histochemical
staining

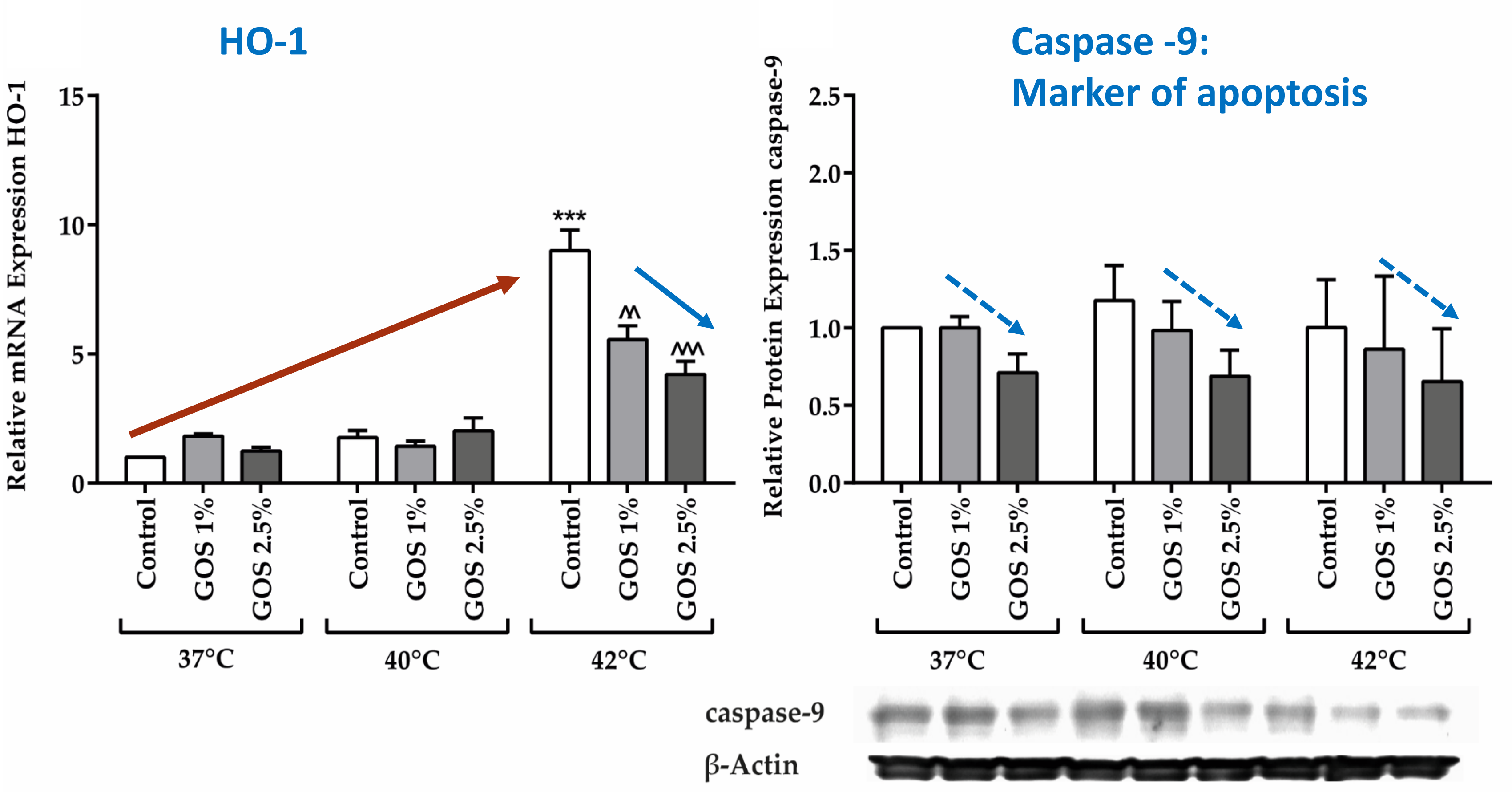








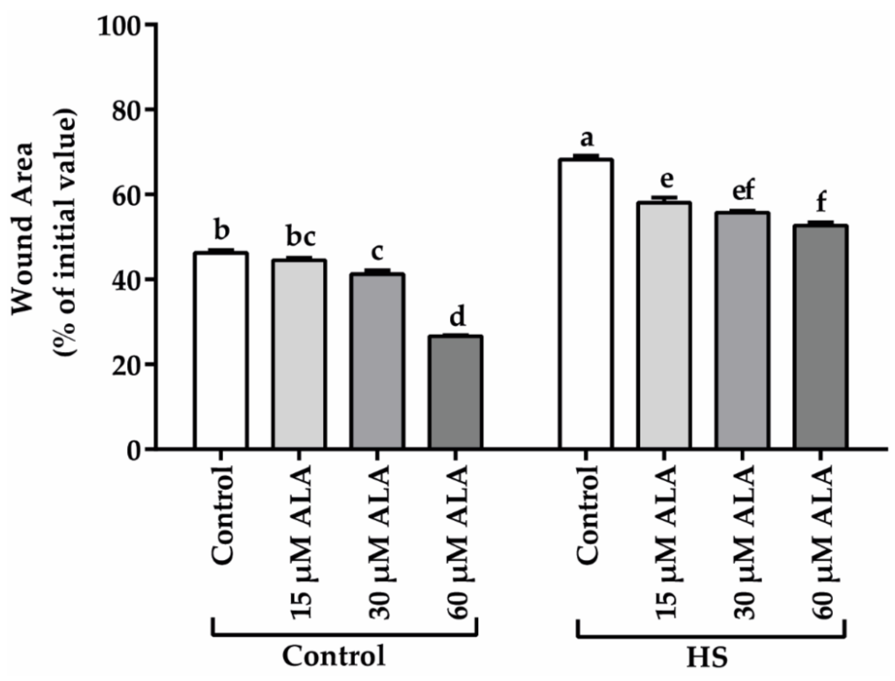
Oxidative stress cascade:
HO-1 and Caspase-9



Reduction of oxidative stress response by GOS (42°) - induction of apoptosis marginal in this model

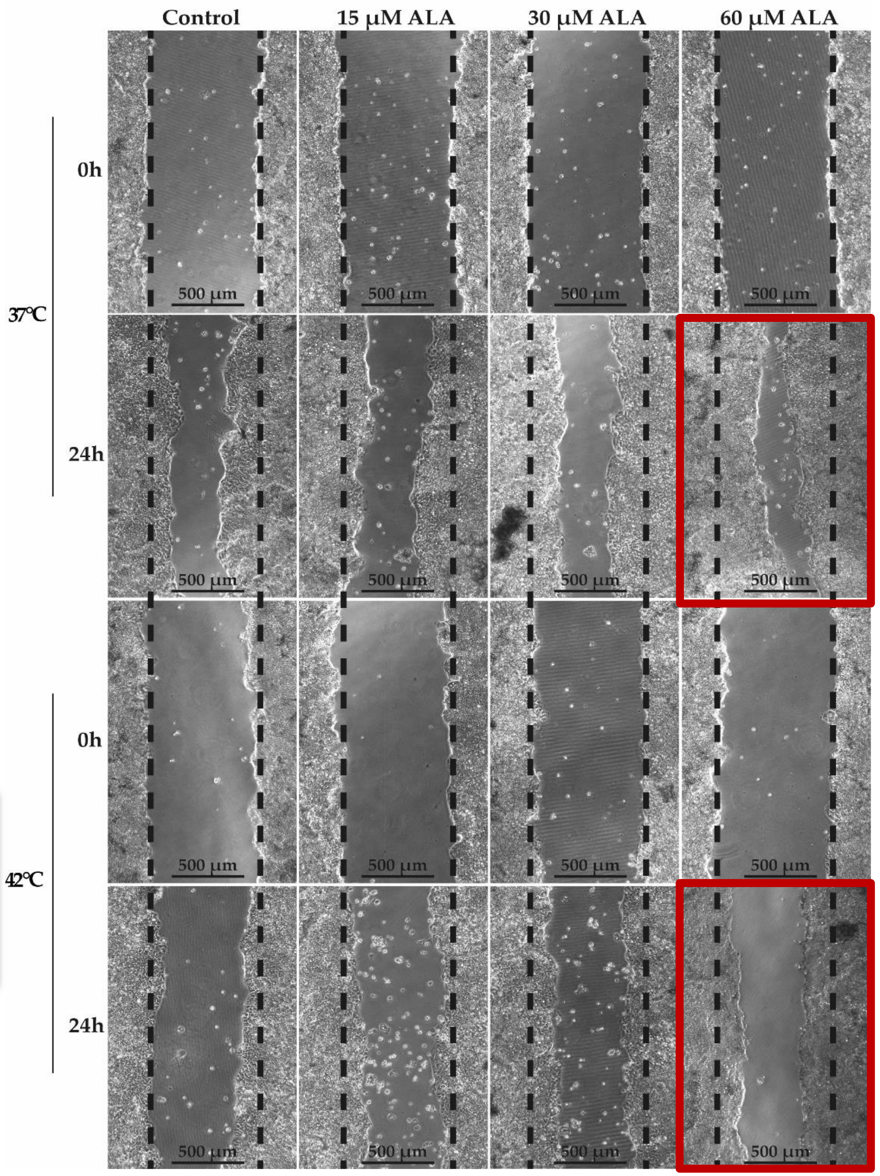
(2b) Modification of the Caco-2 cell model:

The "wound healing" assay: focus on cell proliferation & regeneration



Repair after 24 hrs (37°)

Marginal repair under heat stress (42°)



Model experiments with α-Lipoic acid

Conclusions and recommendations

Climate change increases the risk for heat stress in animals
Heat stress affects animal health, welfare, productivity and resilience to infectious diseases.

The intestinal tract is specifically affected due to a rapid reduction on splanchnic blood flow, resulting in hypoxia and the induction of a stress response (HF, HSP, HO apoptosis …)

The resulting "Leaky gut syndrome" is characterized by

- **loss of barrier integrity → increased risk for entry of pathogens and antigens**
- **inflammatory response → decreased nutrient utilization & fluid and mineral imbalances)**

Next to an optimization of housing conditions (air –conditioning) whenever possible, dietary intervention strategies seem to offer the opportunity to mitigate adverse effects of heat stress in animals - thereby also increasing animal health and welfare.

A broad tool box (*in vitro* and *in vivo* assays) is available to support the development of effective strategies

With special thanks to

Journal of Functional Foods
Volume 16, June 2015, Pages 265–277

Galacto-oligosaccharides exert a protective effect against heat stress in a Caco-2 cell model

Soheil Varasteh[a, b], Saskia Braber[a], Johan Garssen[b, c], Johanna Fink-Gremmels[a]
Show more

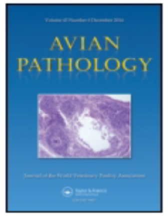
http://dx.doi.org/10.1016/j.jff.2015.04.045

Eur J Nutr (2018) 57:1577–1589
DOI 10.1007/s00394-017-1442-y

ORIGINAL CONTRIBUTION

α-Lipoic acid prevents the intestinal epithelial monolayer damage under heat stress conditions: model experiments in Caco-2 cells

Soheil Varasteh[1,2] · Johanna Fink-Gremmels[1] · Johan Garssen[2,3] · Saskia Braber[2]



Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: https://www.tandfonline.com/loi/cavp20

Quantitative histo-morphometric analysis of heat-stress-related damage in the small intestines of broiler chickens

PLOS | ONE

RESEARCH ARTICLE

Differences in Susceptibility to Heat Stress along the Chicken Intestine and the Protective Effects of Galacto-Oligosaccharides

Soheil Varasteh[1,2], Saskia Braber[1]*, Peyman Akbari[1,2], Johan Garssen[2,3], Johanna Fink-Gremmels[1]

1 Division of Veterinary Pharmacy, Pharmacology and Toxicology, Utrecht University, Utrecht, The Netherlands, 2 Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands, 3 Nutricia Research, Utrecht, The Netherlands

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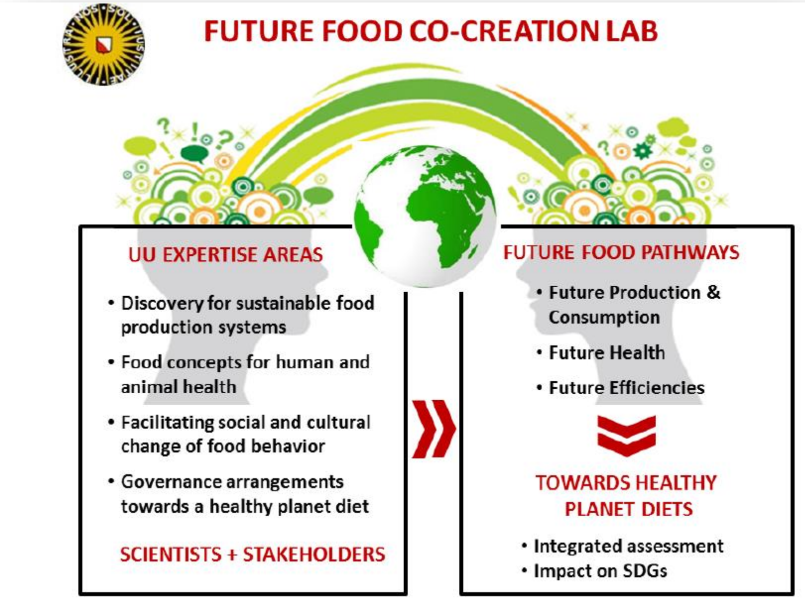
Regiane, Soheil, Saskia and many others

Thank you



***Future Food Utrecht**: www.uu.nl/en/research/*
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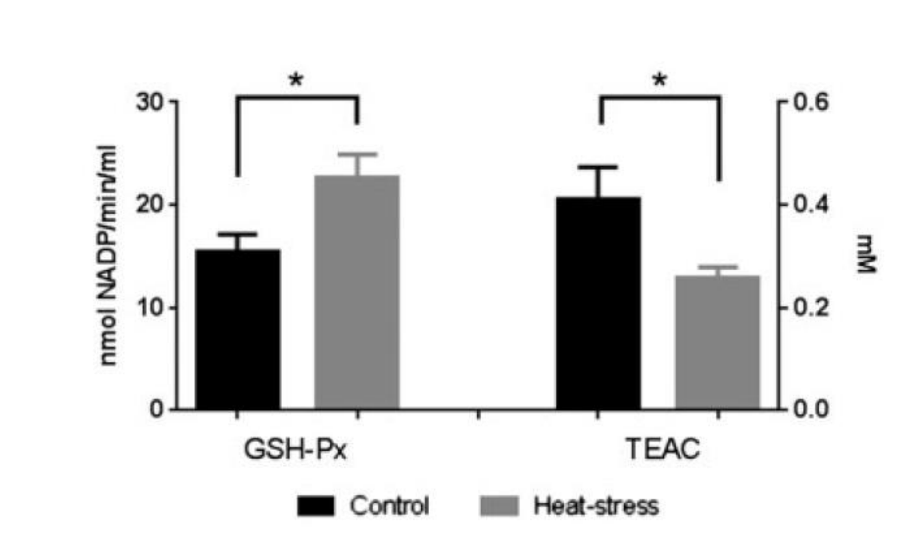
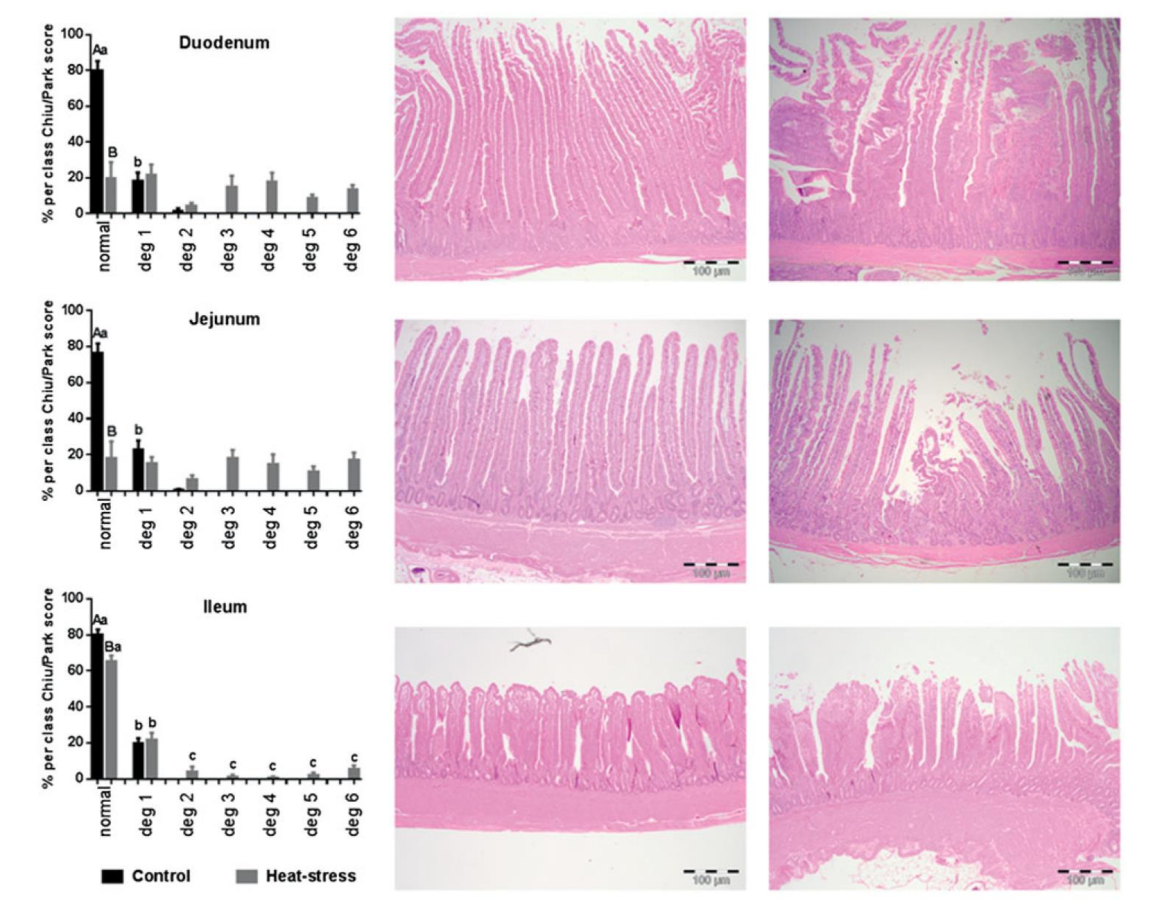
The Institute for Risk Assessment Sciences (IRAS) is an interfaculty institute of the Faculties of Medicine and Veterinary Medicine.
World Health Organization Collaborating Centre for Research on Environmental Health Risk Assessment.

The 1st attempt:

Conventional serum parameters &

Histo-morphometry





Santos et al., 2015

Challenges for livestock farming in a changing world

Declining resources
Feed security
Feed safety
World market prices

Precision feeding
genetic potential
productivity
disease resilience

Consumer expectations and demands

Increasing Awareness:
- Food safety and AMR
- Animal Health and Welfare
- Environmental impact (CO_2, CH_4)
- Intensive farming & biodiversity
- Feed sourcing information (GMO)

One Health Objectives:
taking care of humans, animals and the environment