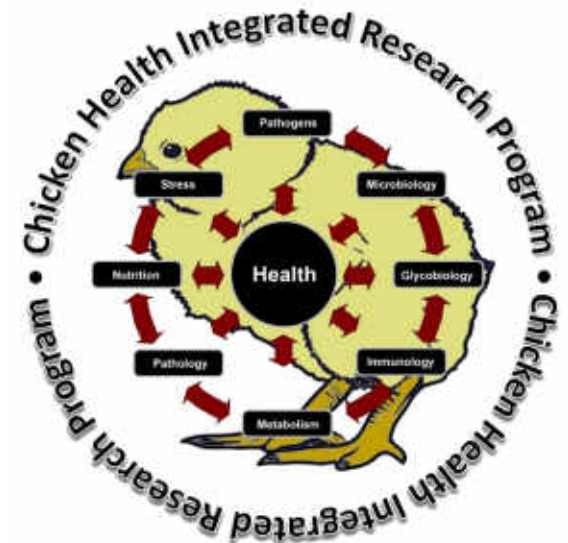


Research to Enhance Chicken Production Health

Douglas Inglis

Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada

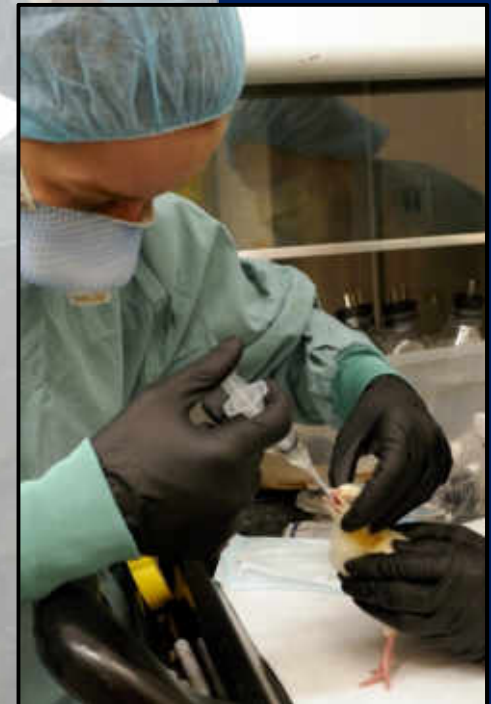


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Presentation outline

- Brief introduction to poultry research at the Lethbridge Research and Development Centre and University of Lethbridge
- Bird stress and disease
- Current research projects and recent outcomes
- Stress predisposition and biomarkers of health
- Mitigation of inflammation to manage disease
- Innovation strategy



Lethbridge Research and Development Centre

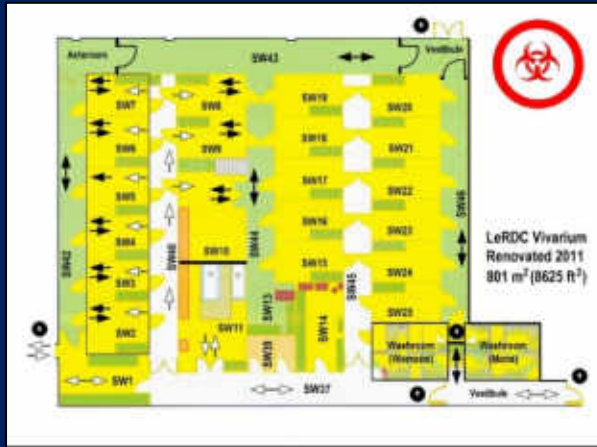


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Wade Abbott



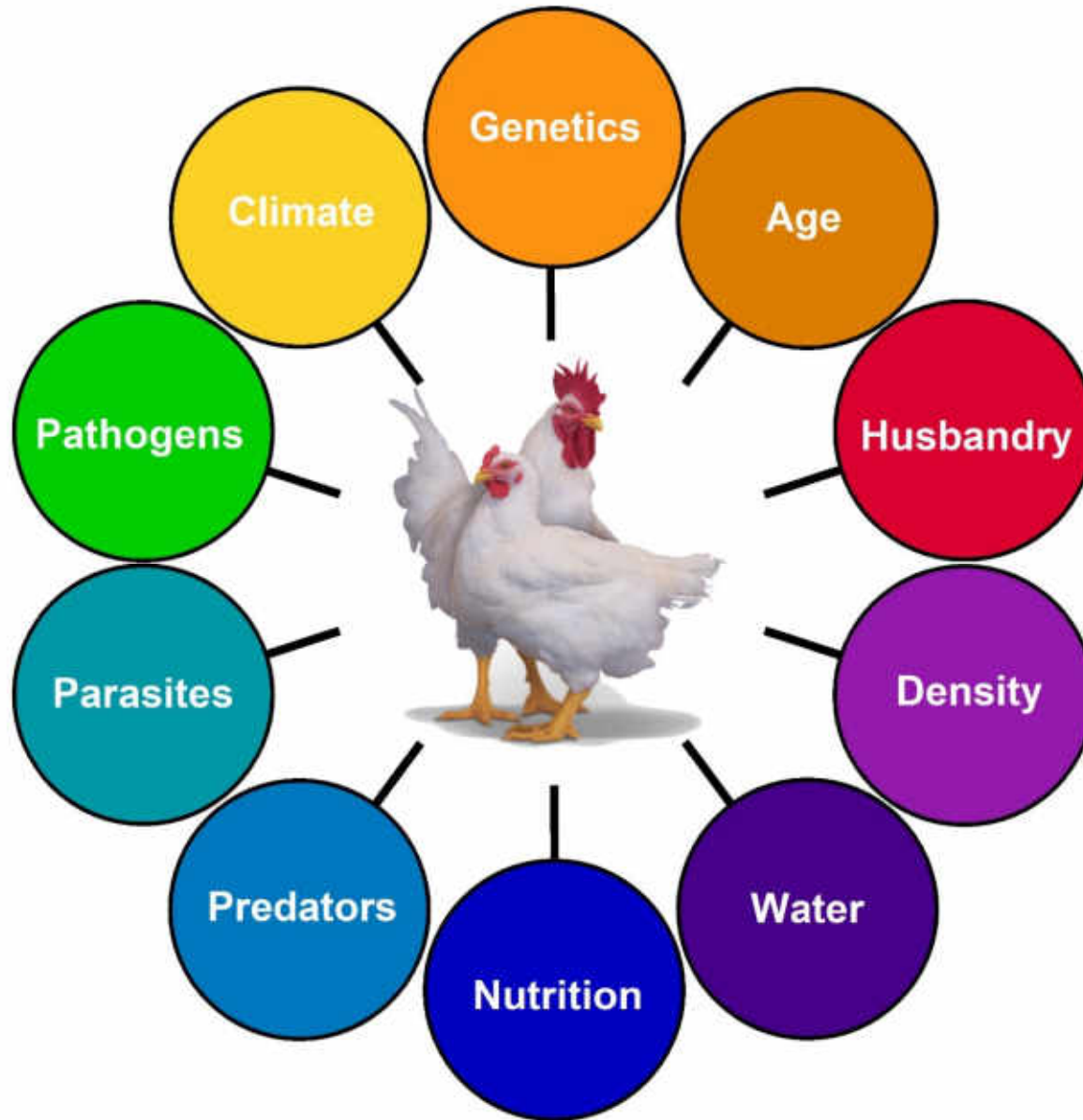
Sarah Zaytsoff



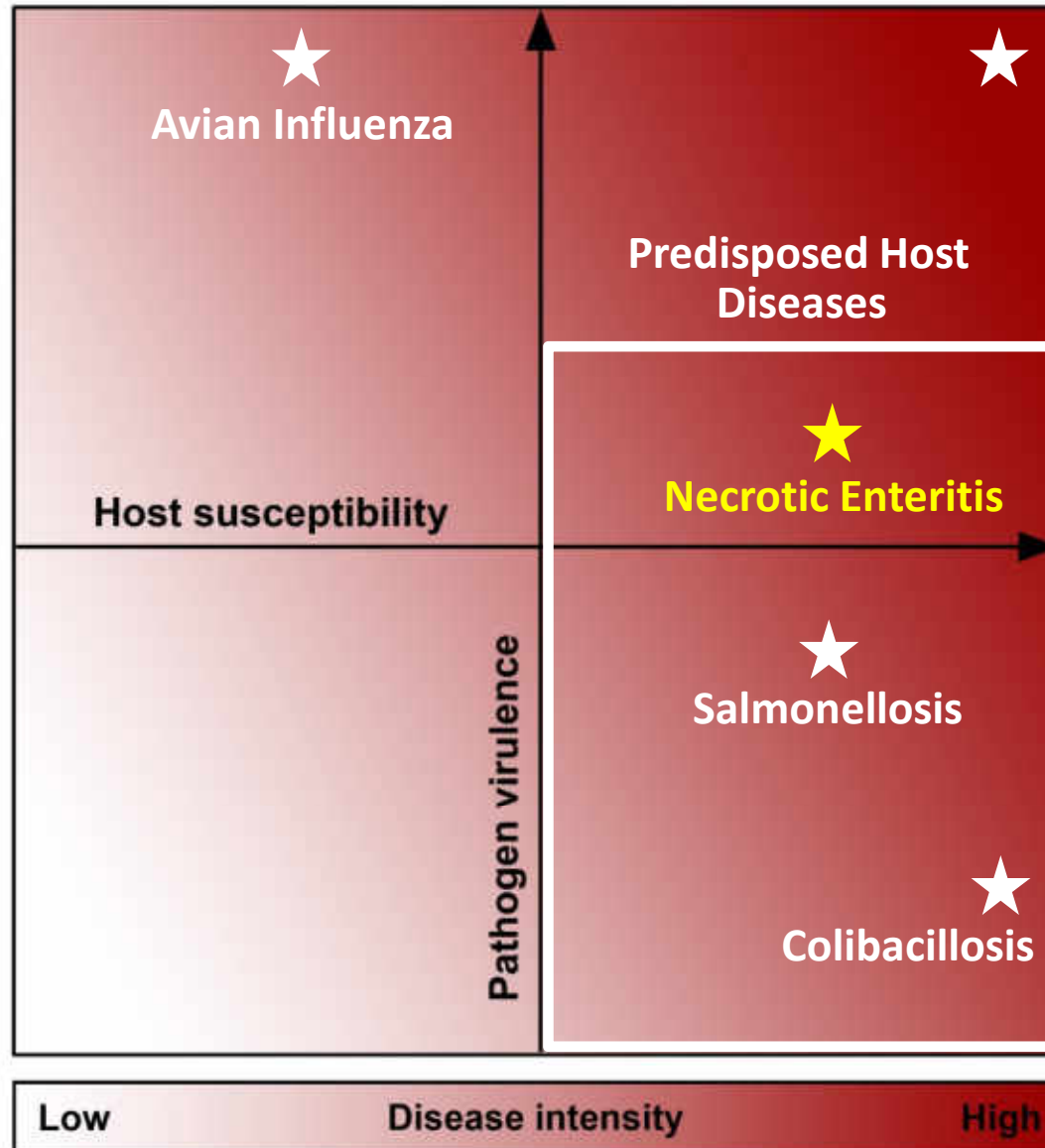
SW Alberta Producers



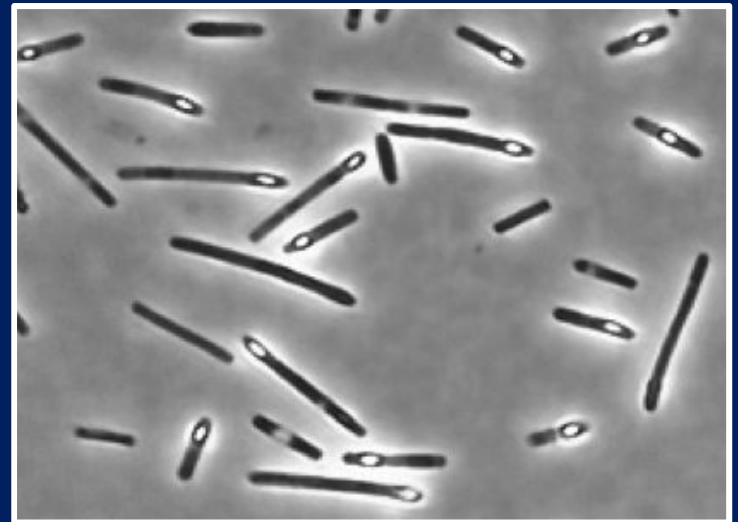
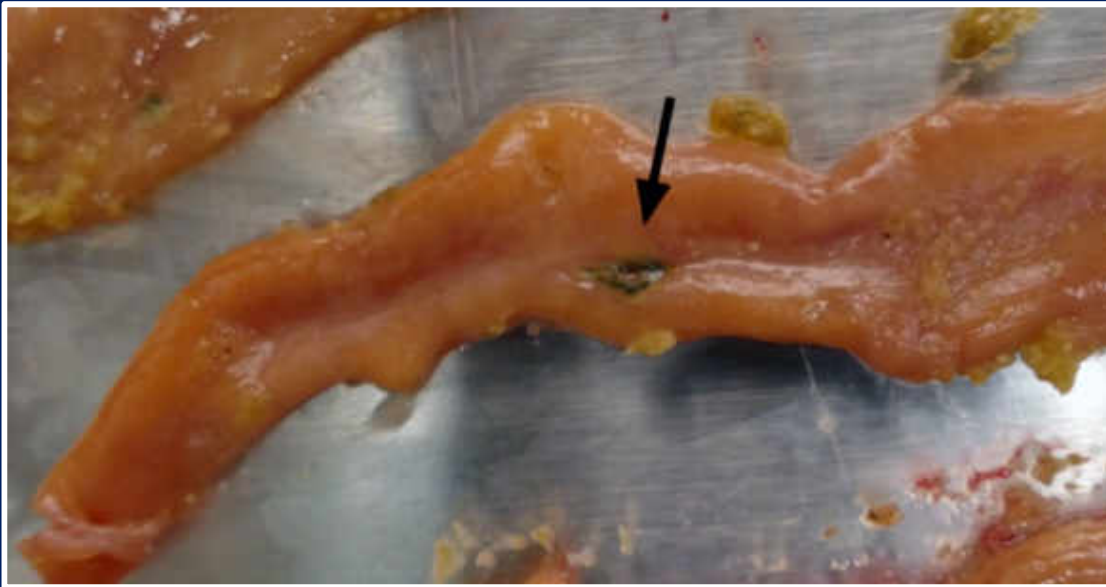
Many factors determine optimal bird health



Pathogen virulence vs host susceptibility

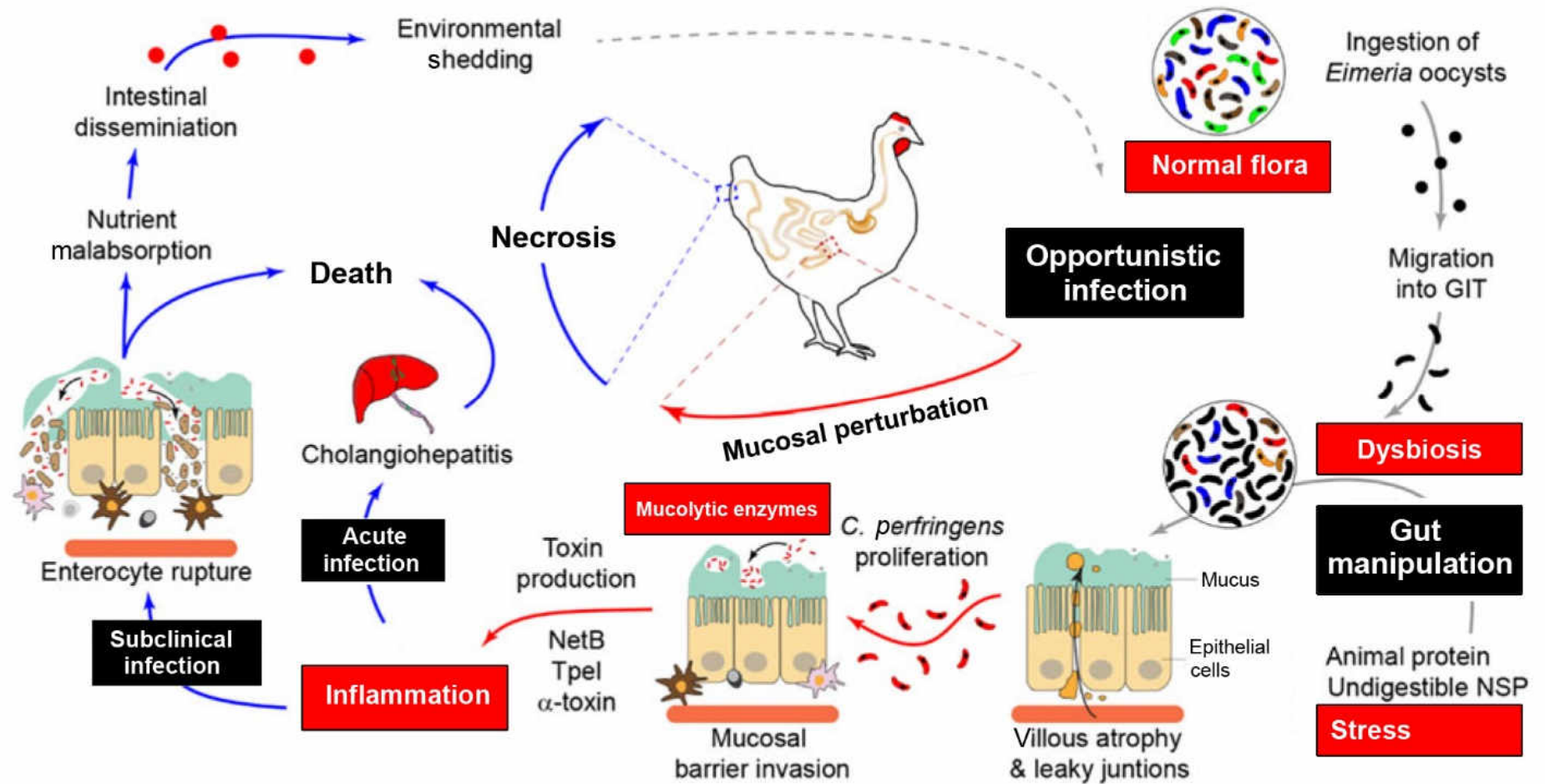


Necrotic enteritis incited by *Clostridium perfringens*



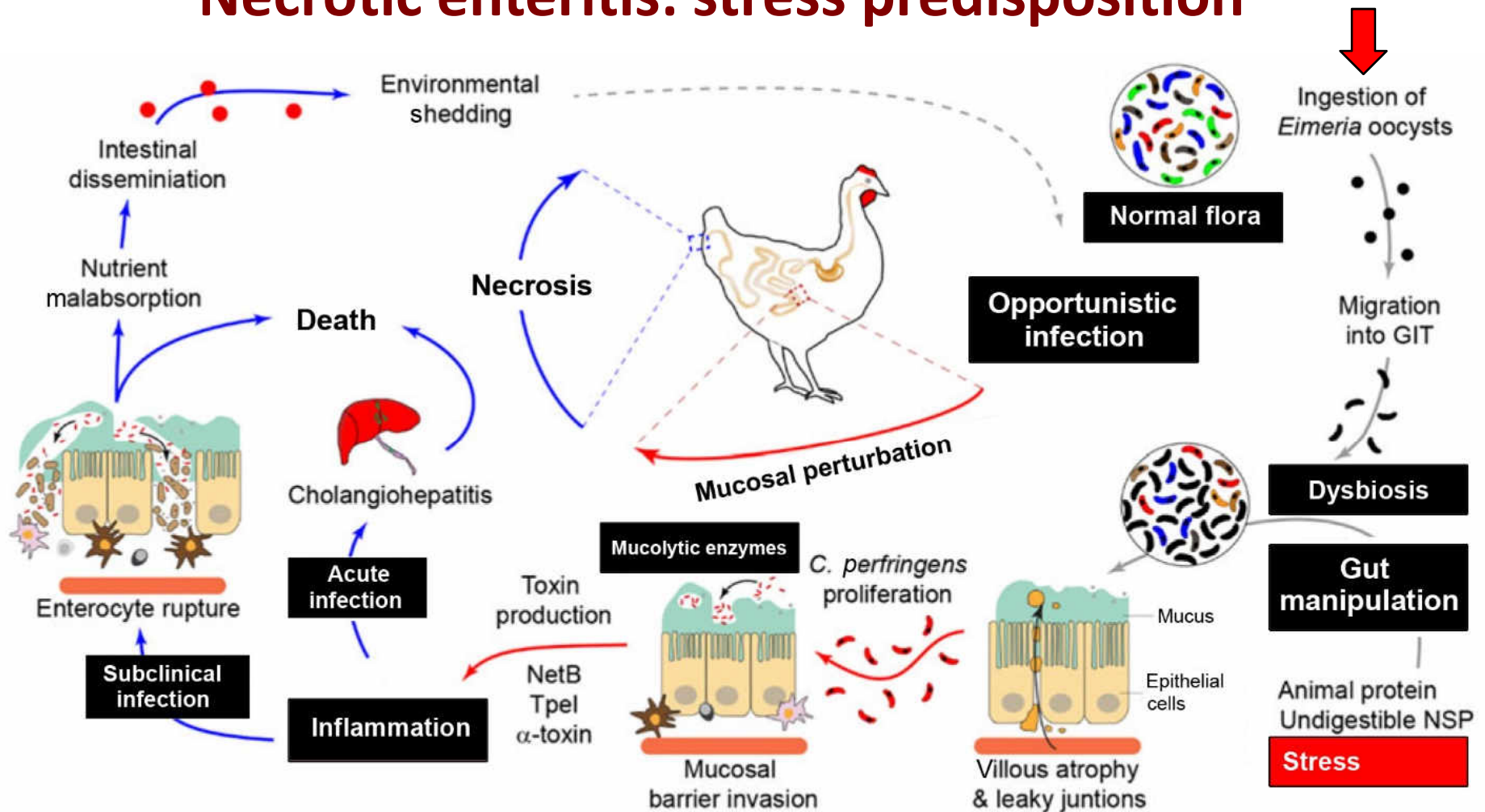
<https://commons.wikimedia.org/wiki/>

Necrotic enteritis pathogenesis



Alizadeh et al. 2022. Necrotic enteritis in chickens: a review of pathogenesis, immune responses and prevention, focusing on probiotics and vaccination. *Animal Health Research Reviews* 22:147-162.

Necrotic enteritis: stress predisposition



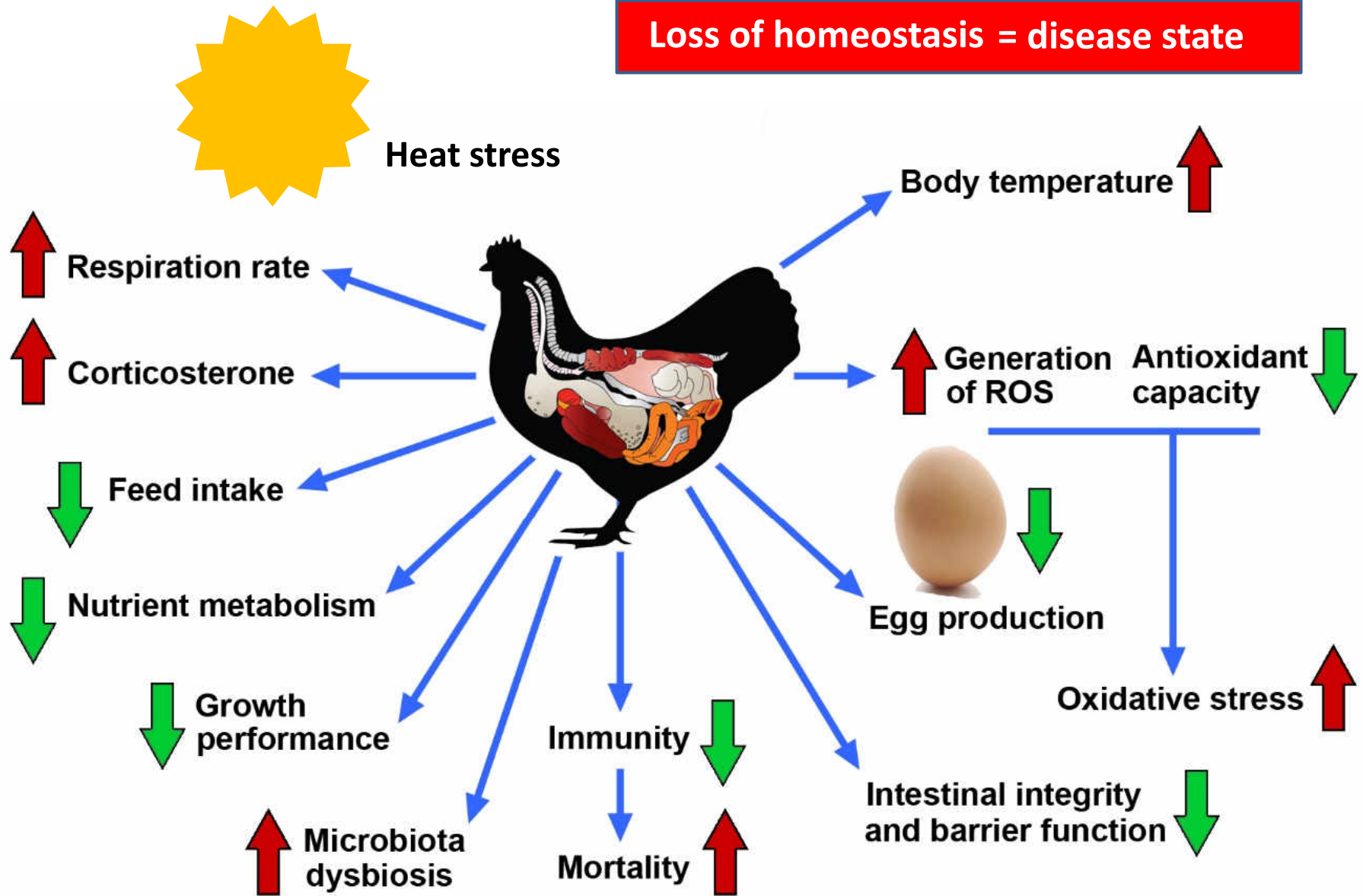
Stress as a crucial factor predisposing chickens to disease

Identification of biomarkers of stress and mechanisms involved

Tools for producers and development of rationale-based mitigations

Host predisposition due to stress

Loss of homeostasis = disease state



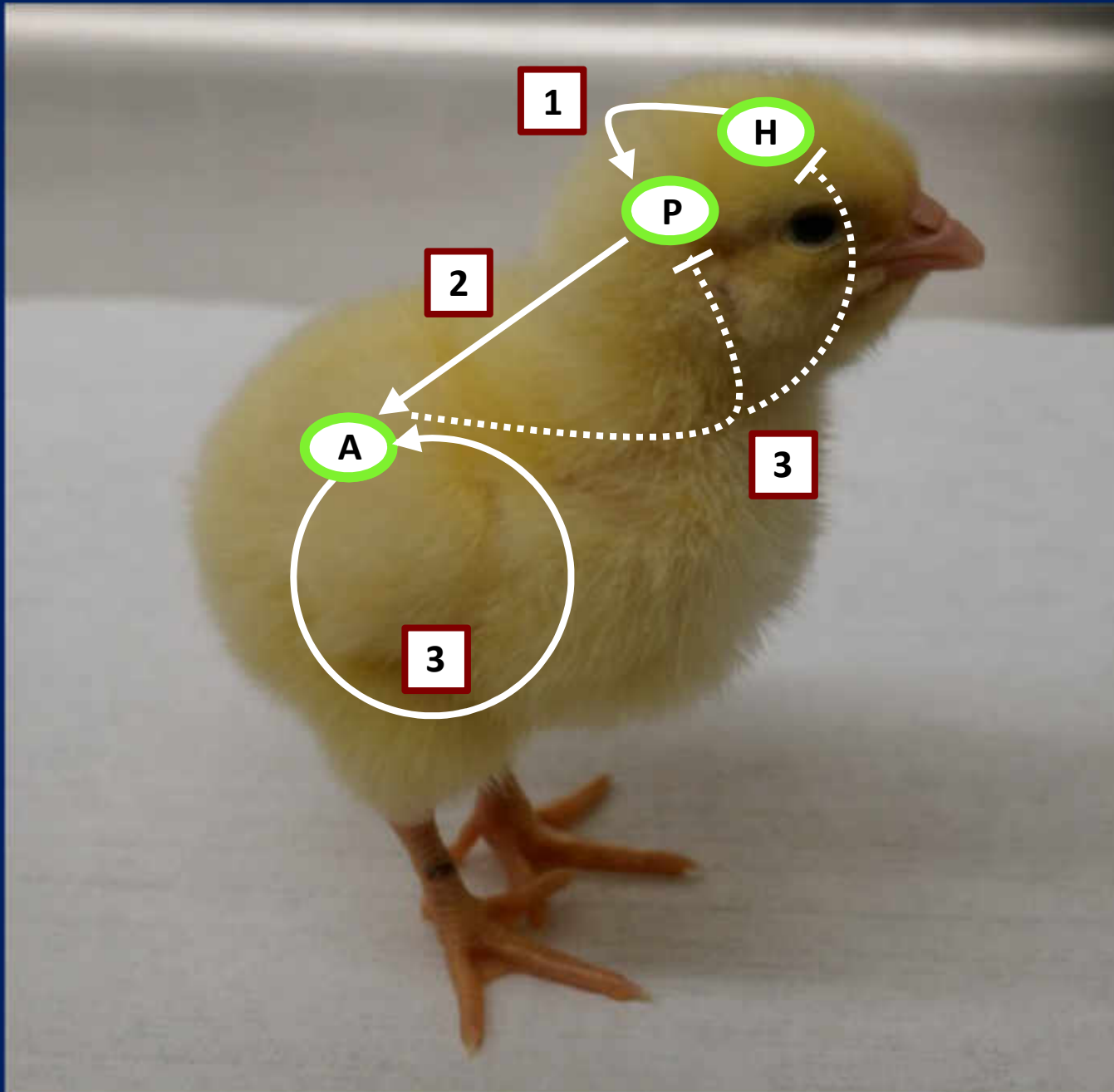
HPA stress axis

Endocrine organs:

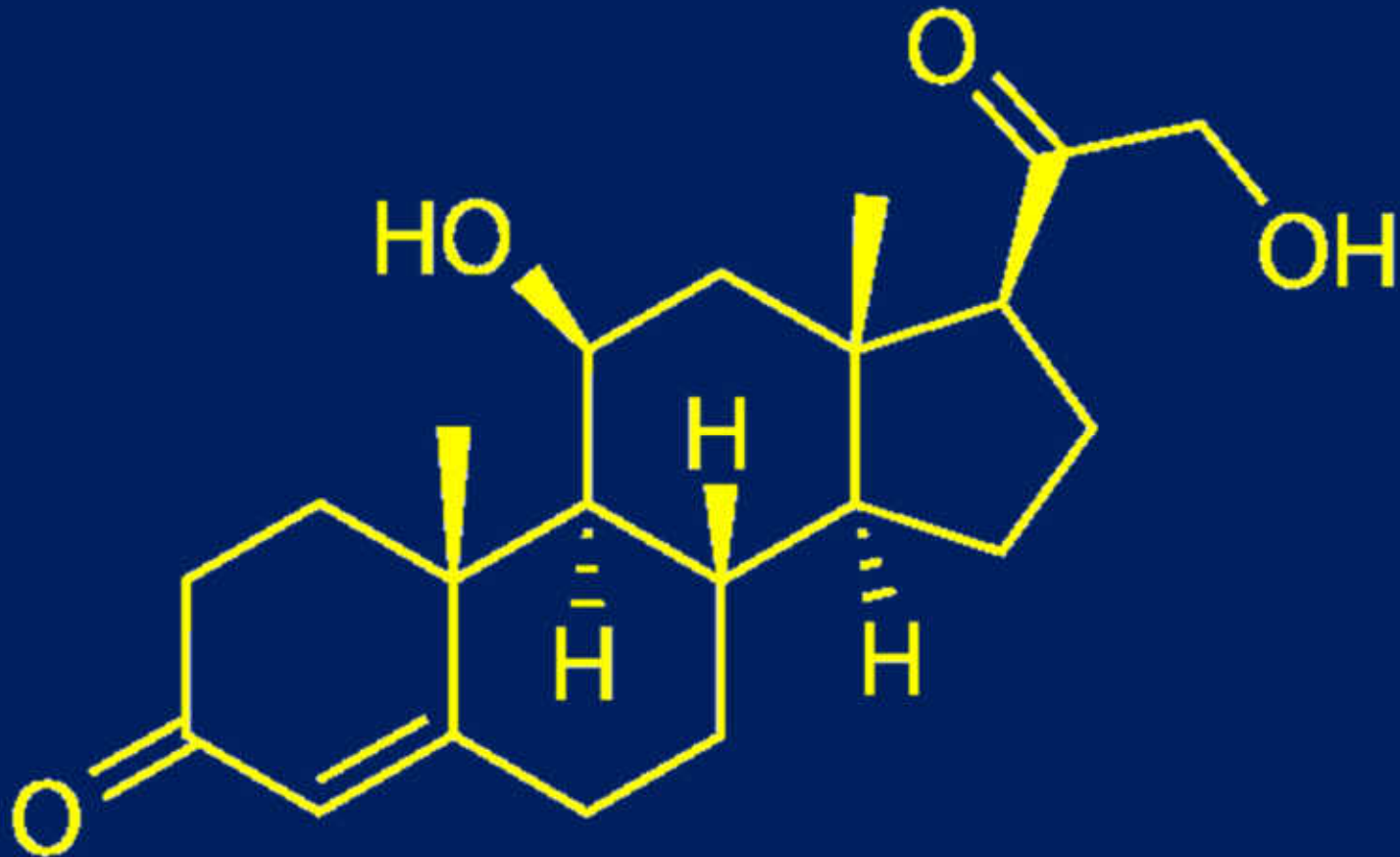
- H** Hypothalamus
- P** Anterior Pituitary
- A** Adrenal Gland

Hormones:

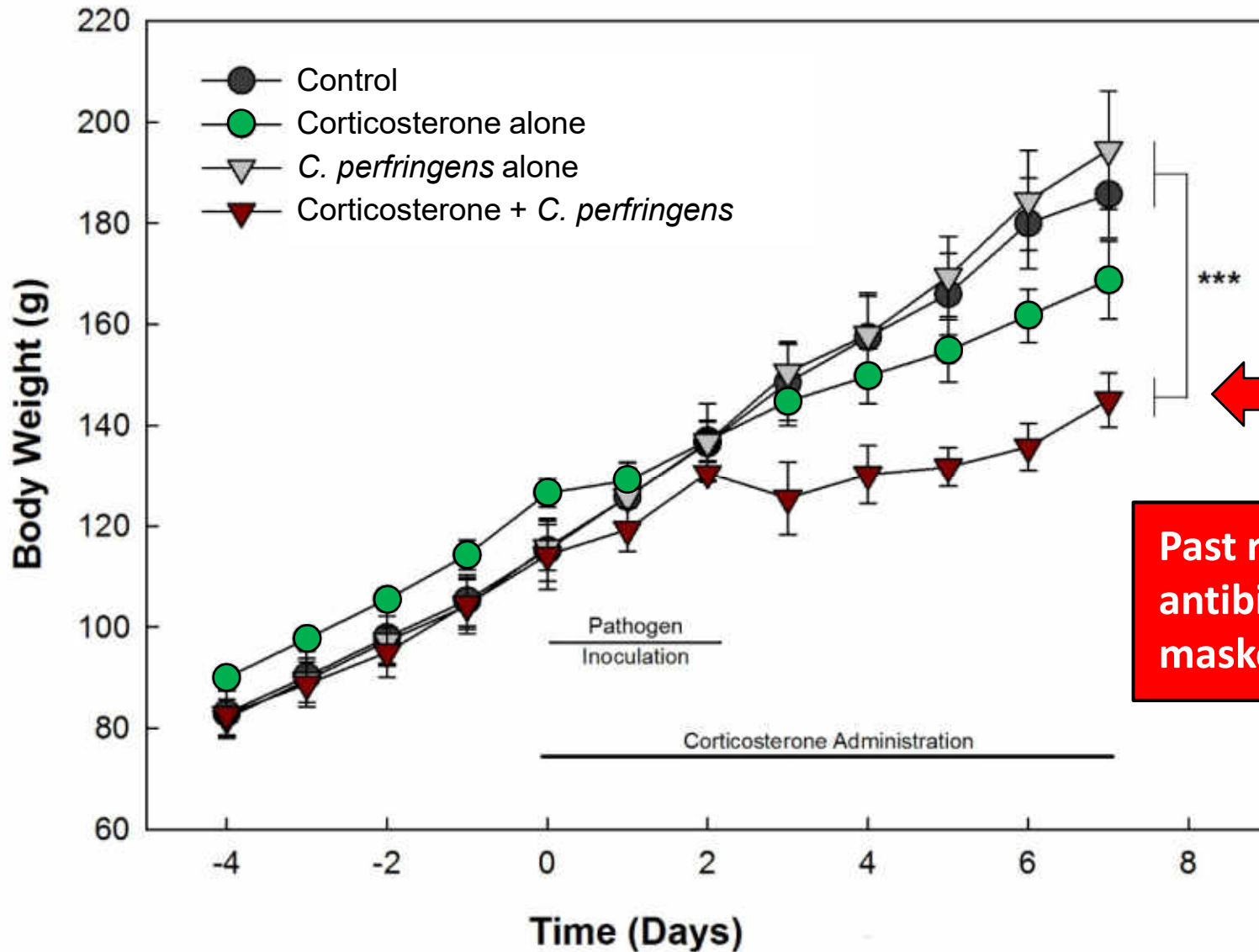
- 1** AVT & CRH
- 2** ACTH
- 3** Corticosterone



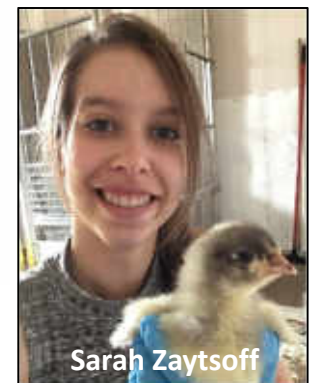
Corticosterone model of stress (CORT)



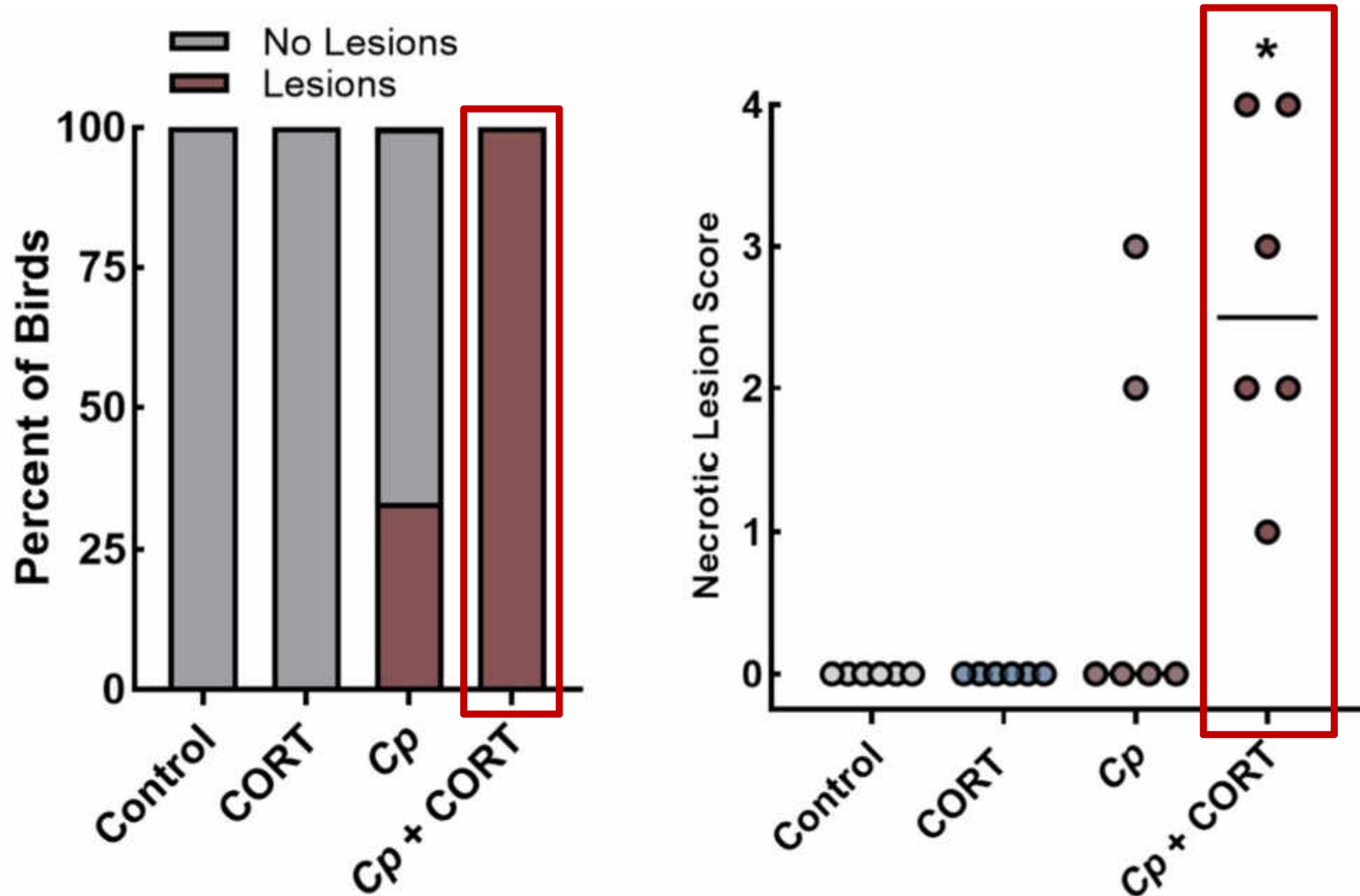
Chronic necrotic enteritis: stress predisposition



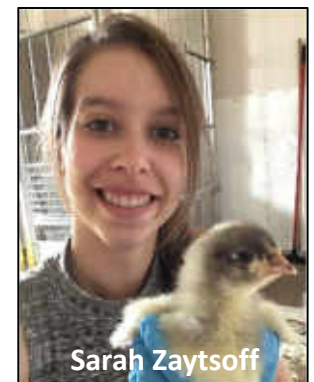
Past reliance on antibiotics has masked this effect

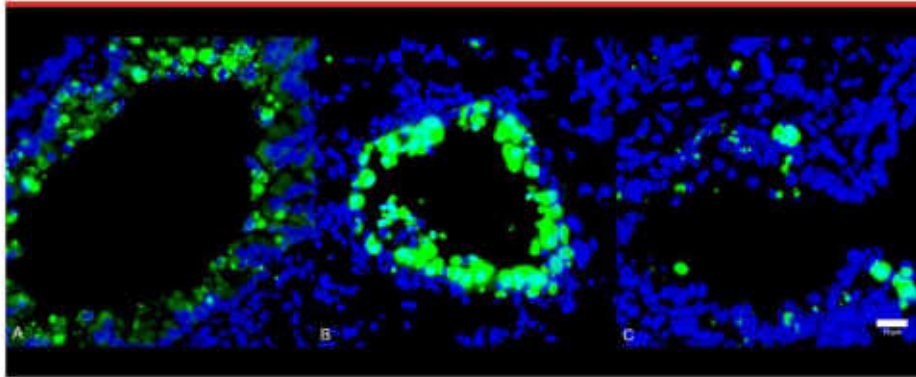


Acute necrotic enteritis: stress predisposition



All birds administered corticosterone developed acute necrotic enteritis (without co-infection or diet treatment)





Diabetes Marker Linked to COVID-19 Severity in Mice

A sugar that's less abundant in the blood of people with diabetes binds to SARS-CoV-2's spike protein and disrupts the virus's ability to fuse with cells.



Alejandra Manjarrez

May 16, 2022

Early on during the COVID-19 pandemic, experts noticed that people with diabetes were more likely to become hospitalized with the disease—the big question was why. Now, in vitro and in vivo work suggests that low concentrations of the metabolite 1,5-anhydro-D-glucitol (1,5-AG) may help explain the increased vulnerability of this population.

Researchers reported last week (May 9) in *Nature Metabolism* that 1,5-AG, a monosaccharide used as a blood biomarker for human diabetes mellitus because levels of the sugar are significantly lower in people with diabetes, binds to the spike protein of SARS-CoV-2 and prevents the virus from fusing with human cells—the crucial first step in cell entry.

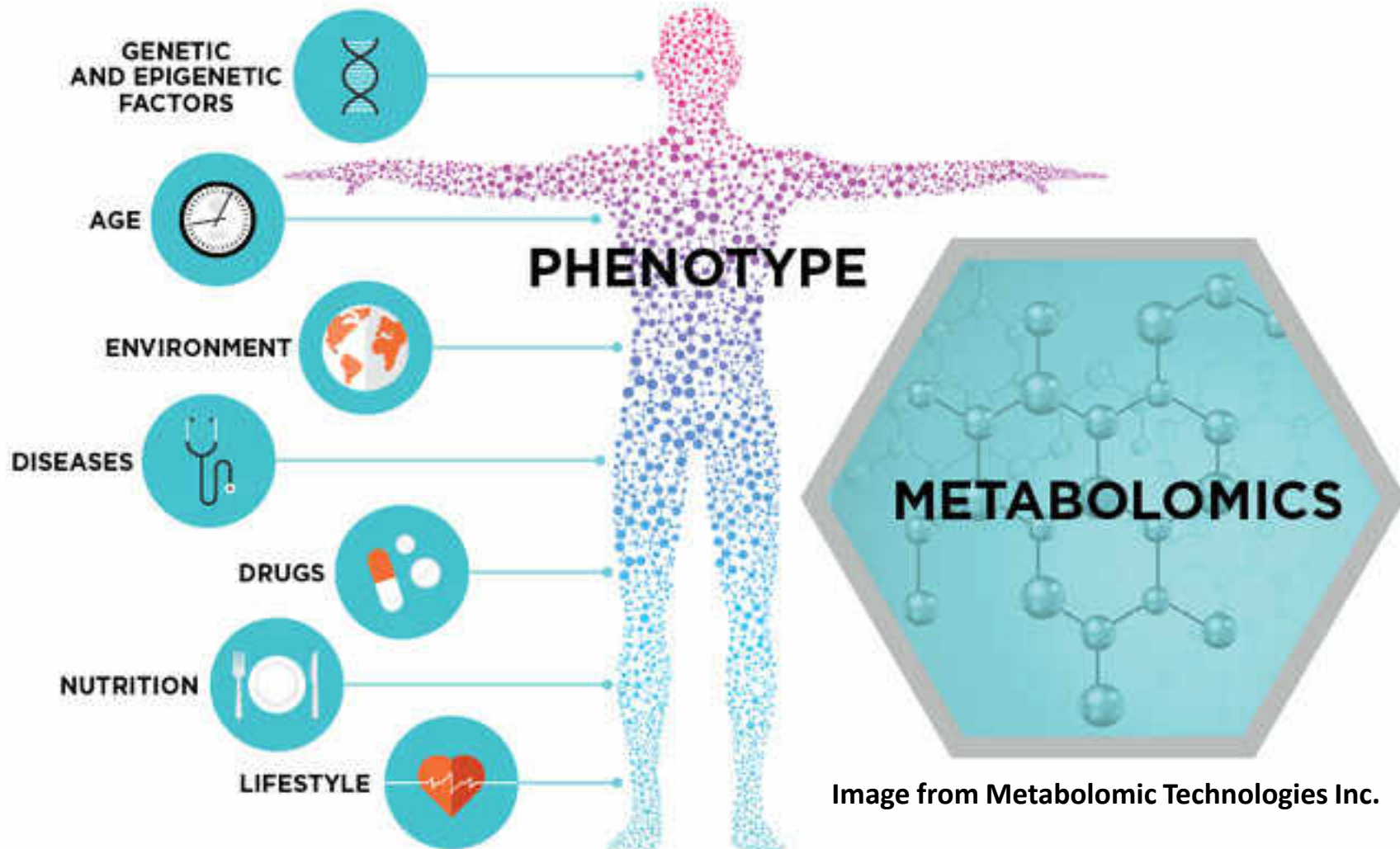
ABOVE:

Comparison of immune cell infiltration (green) into lung tissue during SARS-CoV-2 infection in healthy (A), diabetic (B), and 1,5-AG-treated diabetic (C) mice.

MODIFIED FROM FIG. 4G IN *NAT METAB*, DOI: 10.1038/s42255-022-00567-z, 2022.

Biomarkers and Health

- Identification of biomarkers predictive of bird health = before disease occurs
- Development of a diagnostic tool for producers
- Informed implementation of mitigation strategies proactively
- Objective evaluation of management practices on farm



Metabolomics is the systematic study of all chemical processes concerning metabolites, providing characteristic chemical fingerprints that specific cellular processes yield, by means of the study of their small-molecule metabolite profiles



① Stress exposure



Tony Montana



U of Lethbridge



③ Metabolite extraction



② Sample collection

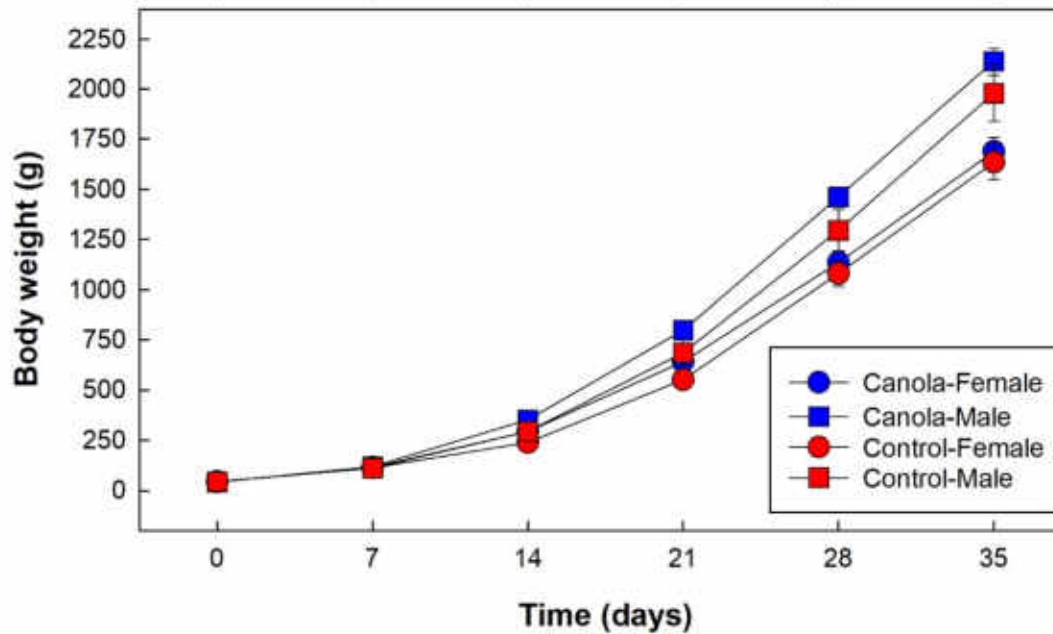
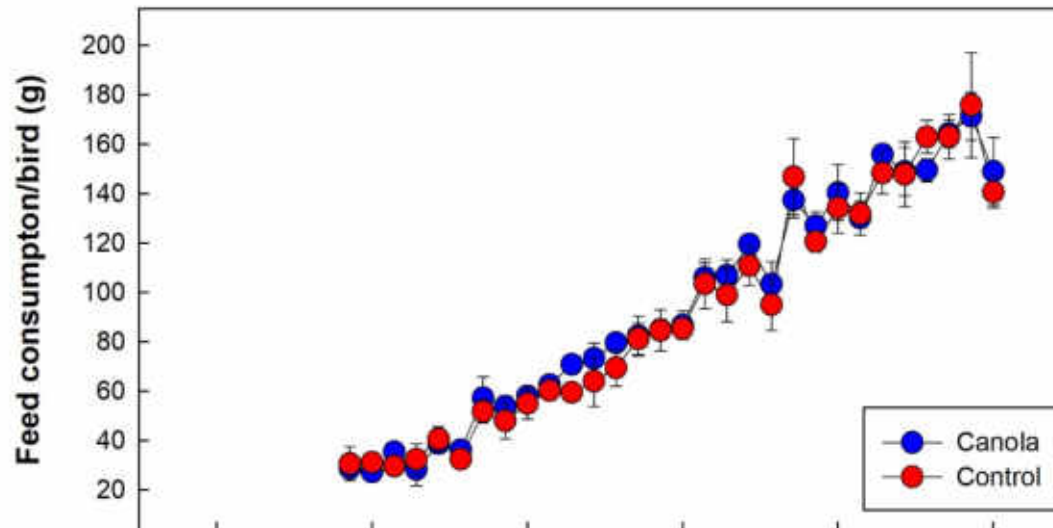


④ Metabolite detection using a 700 MHz Bruker Advance III HD Nuclear Magnetic Resonance Spectrometer (NMR)

⑤ Metabolite and pathway identification



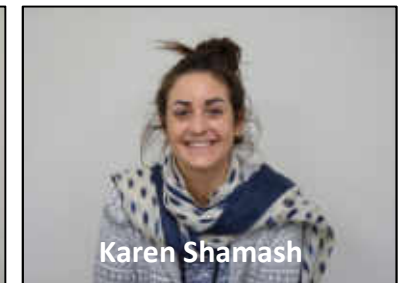
Impact of canola meal on broilers: an example



- Canola meal in the diet (5%) did not affect feed consumption nor body weight gain
- Birds were outwardly healthy

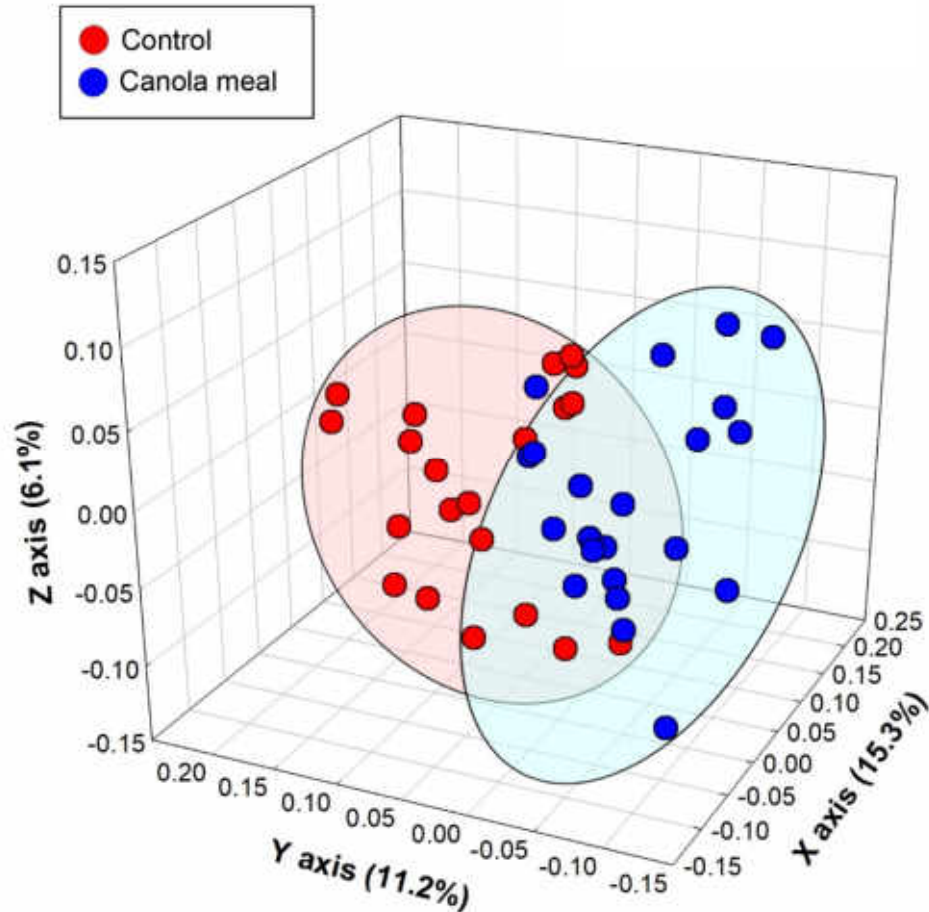


Tara Shelton



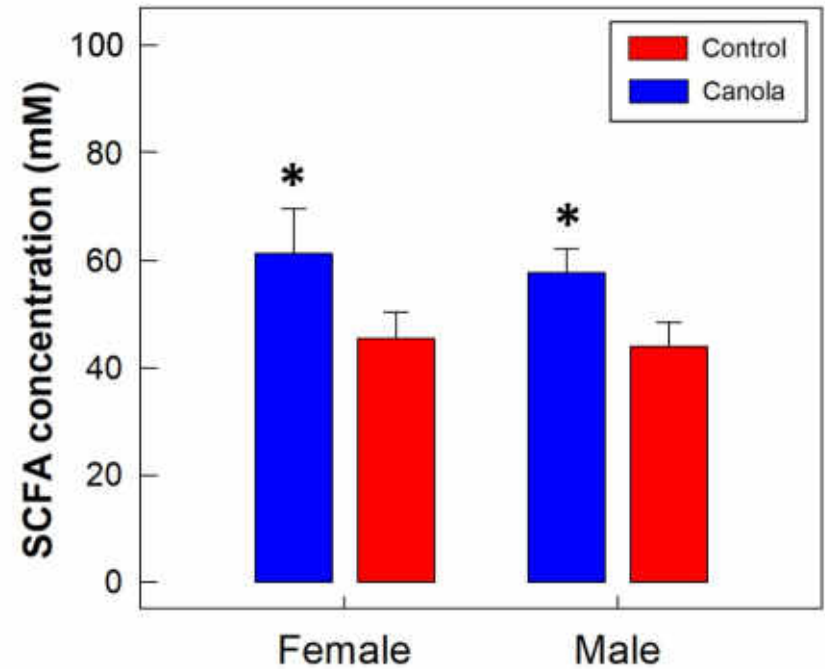
Karen Shamash

Canola meal affected cecal bacterial function



Bacterial community structure

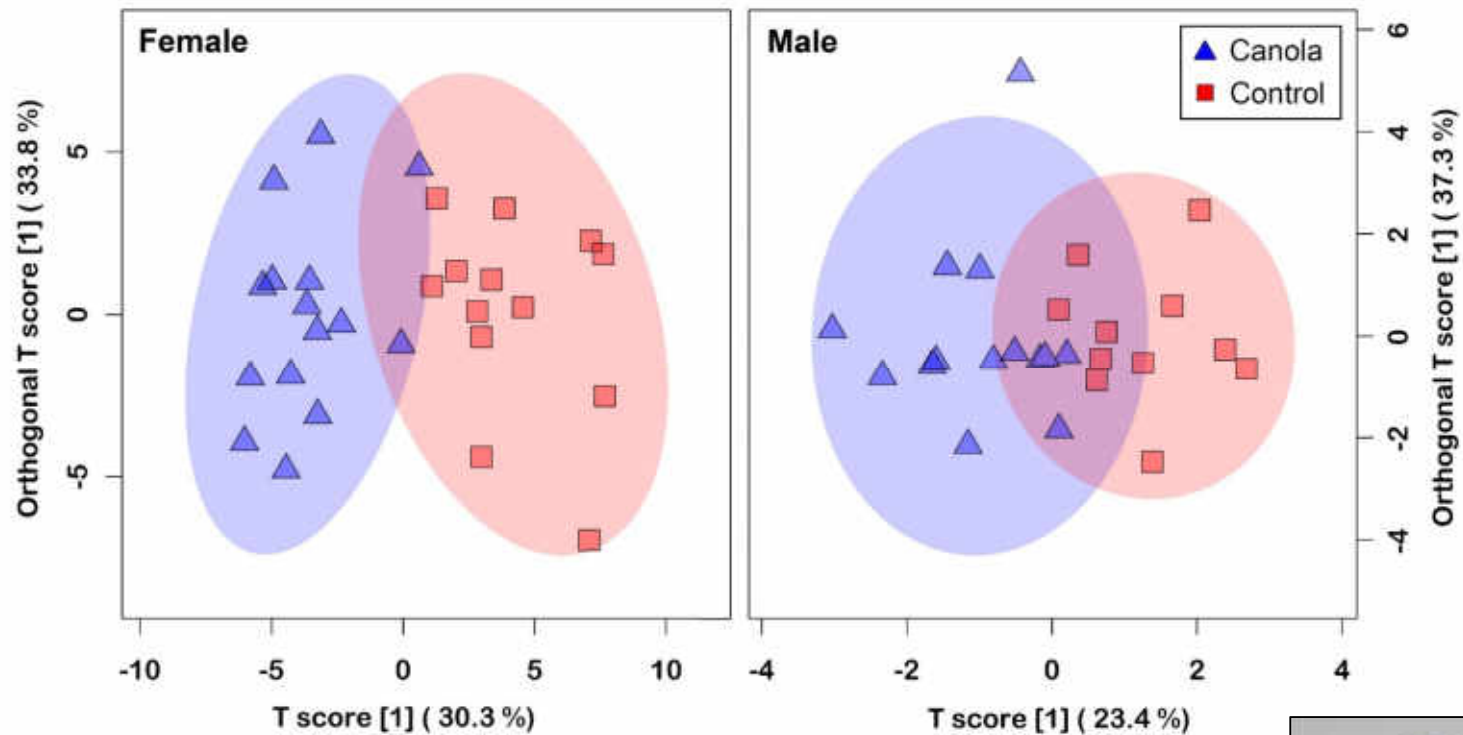
Total short-chain fatty acids



Jenny Gusse

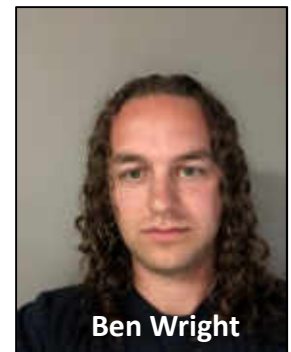
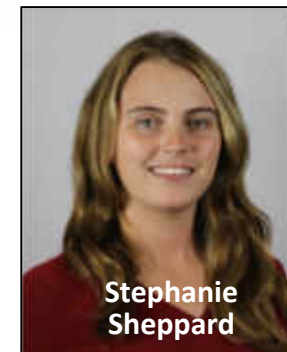
Canola meal affected the metabolome of broilers

- Canola meal affected the metabolome of all samples examined
- Cecal digesta, liver, kidney, breast muscle, and **pancreas**



Remember: each marker represents one bird, and the shaded ellipses represent the 95% confidence intervals for each group

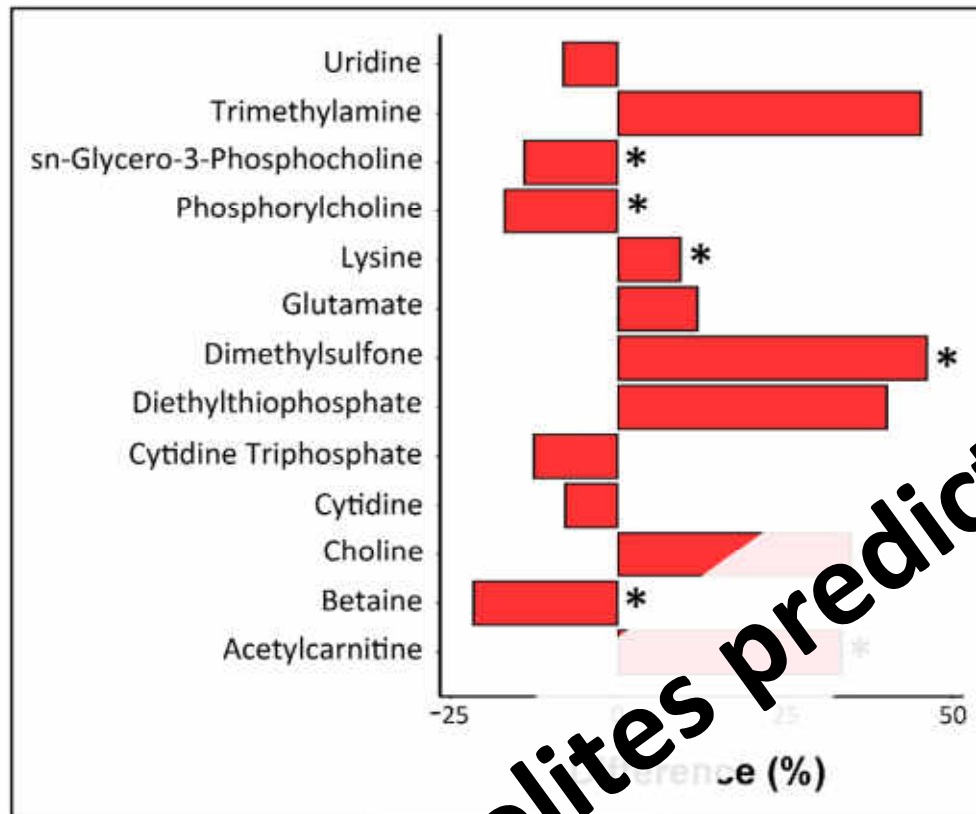
Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA)



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Canola meal affected the metabolome of broilers



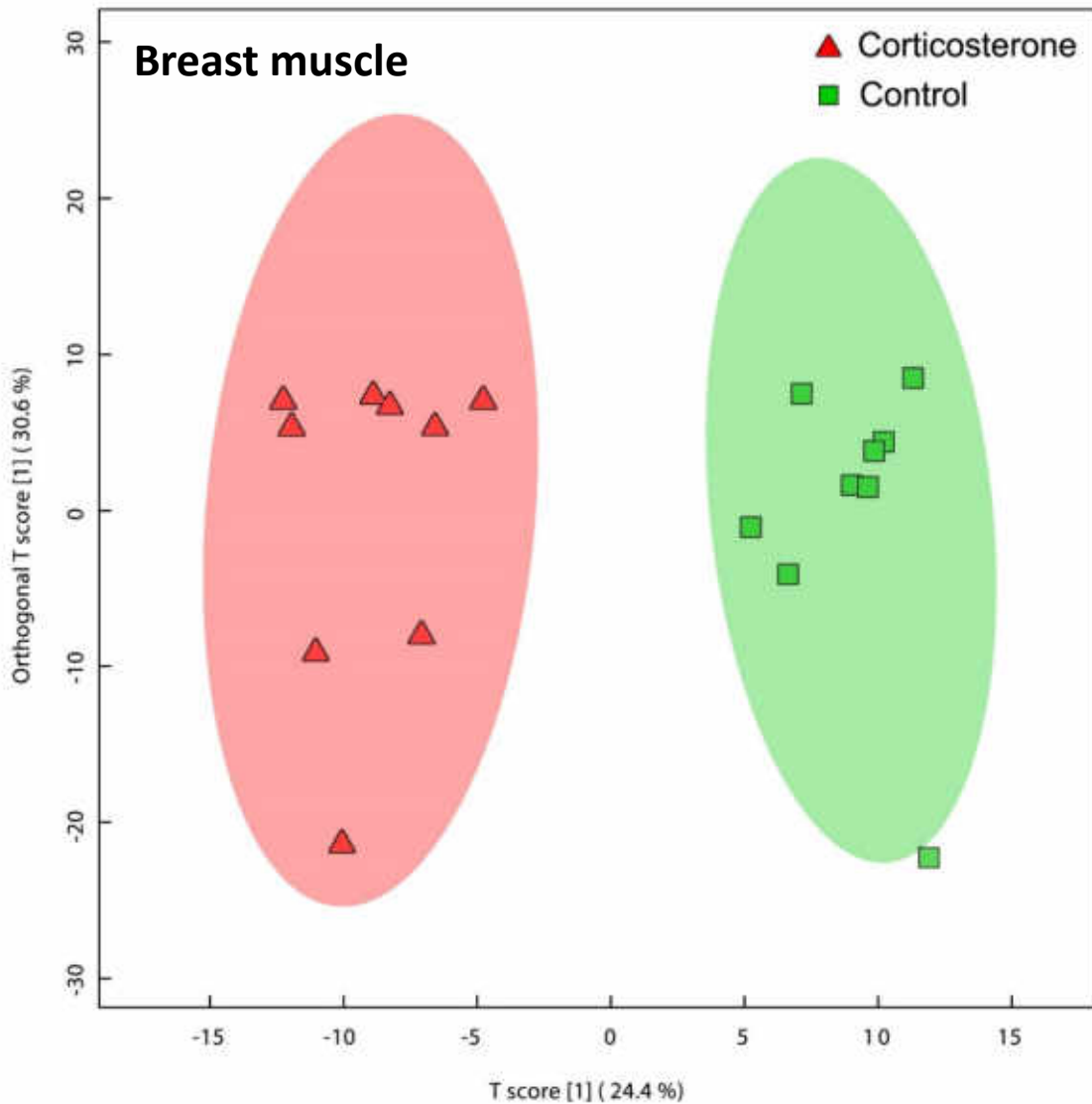
Metabolites predictive of disease

- Metabolites linked to patterns of pancreatitis
- Examples: sn-glycerol-3-phosphocholine, phosphorylcholine, lysine, dimethylsulfone, choline, betaine, and acetylcarnitine
- **Note:** Betaine, which can be synthesized from choline, along with acetylcarnitine and dimethylsulfone, have antioxidant effects that counter pancreatitis-like effects



Biomarkers of stress and bird health





Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA)

Corticosterone incited stress significantly altered the metabolome of all tissues examined, including breast muscle (metabolites associated with reduced quality)

Example illustrates the power of metabolomics and the profound impacts that stress has on birds (meat quality)

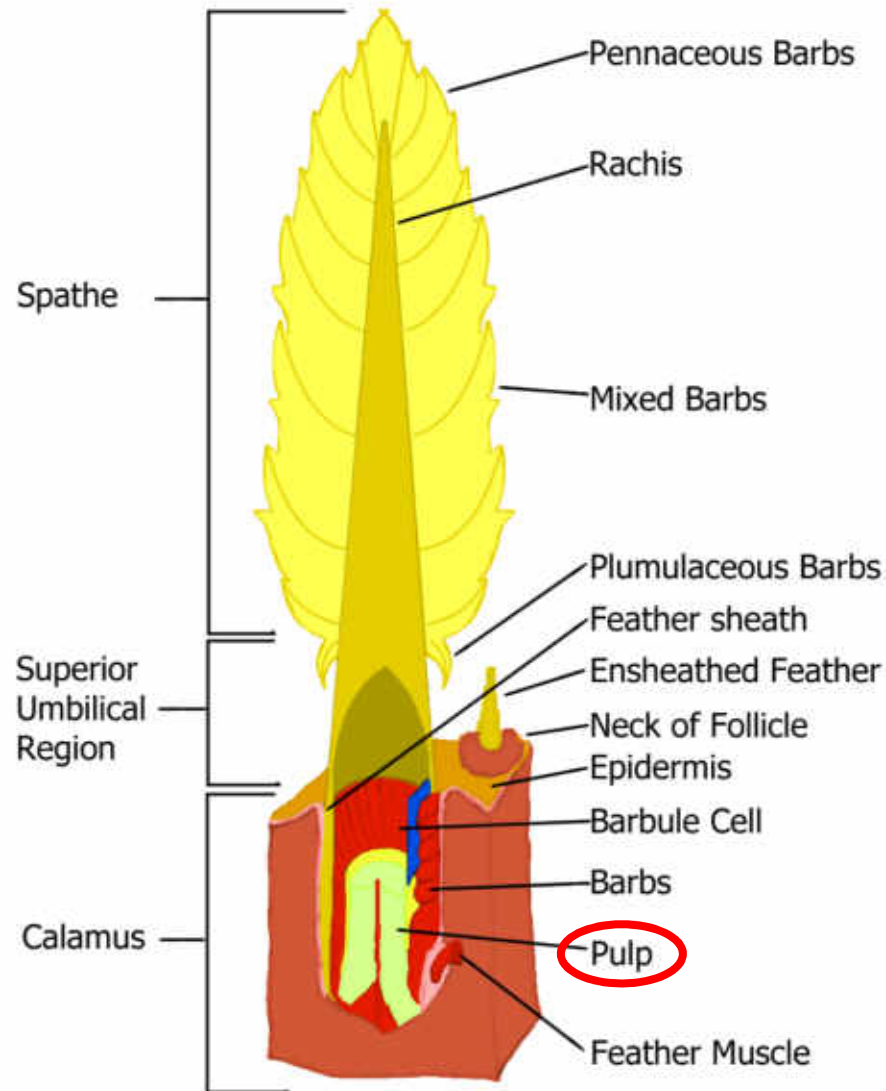


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Metabolomics for on farm application



- Feathers are readily available
- Feather pulp is easy to collect
- **Questions:**
- Can metabolite spectra be produced from feather pulp?
- Is the feather pulp metabolome comparable to that of other samples such as blood?
- Is the feather pulp metabolome sensitive to biological factors?



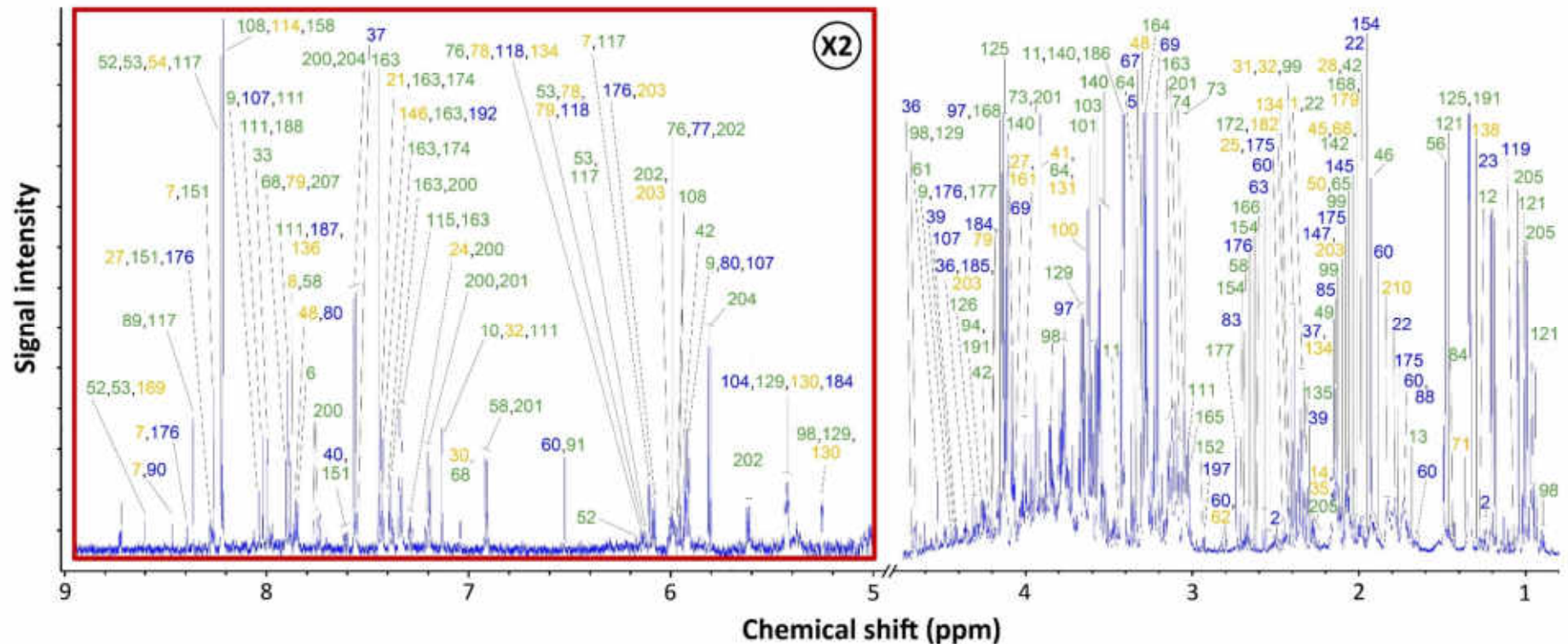
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Kate Brown

Can metabolite spectra be produced from feather pulp?



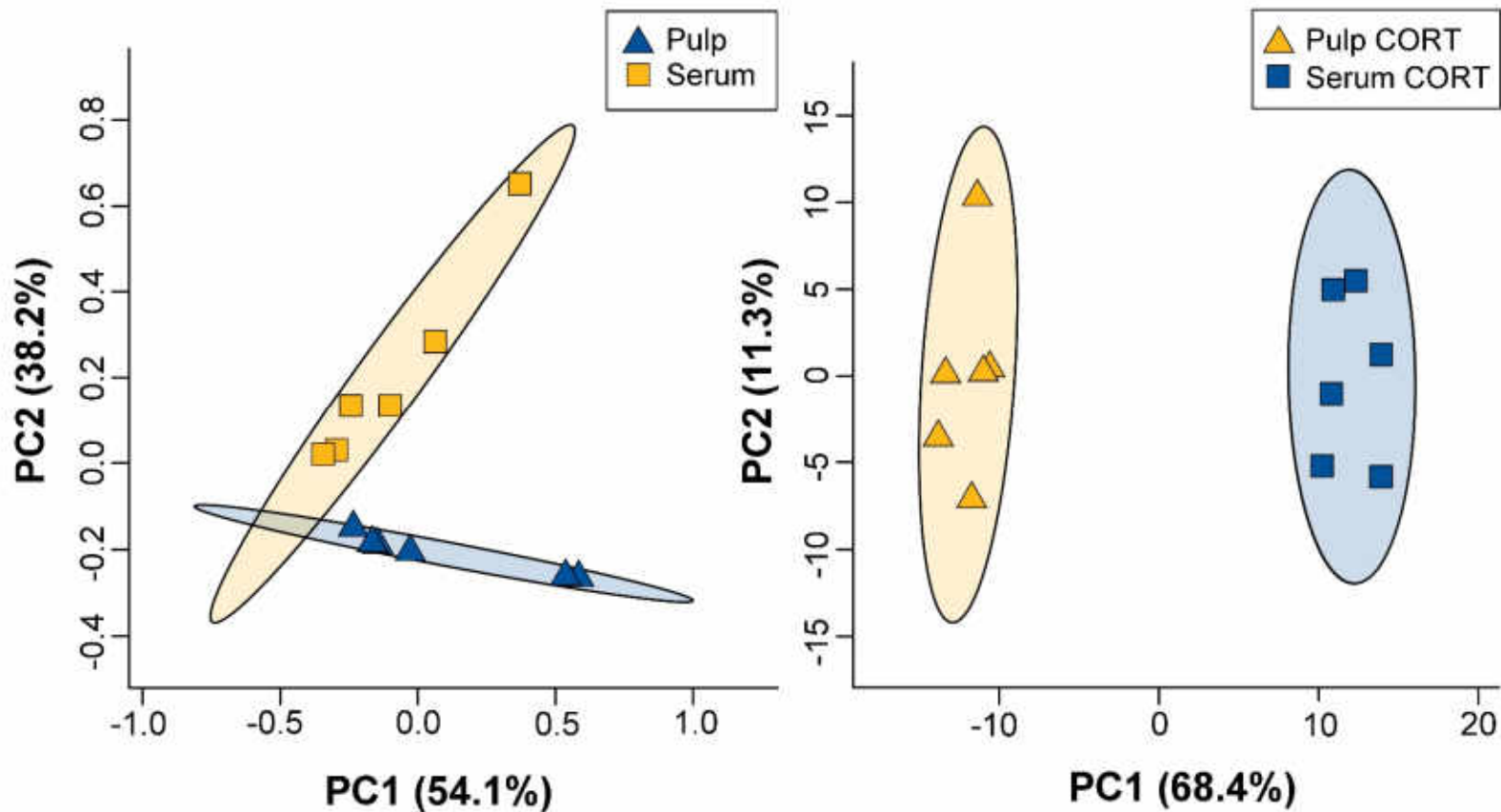
Yes



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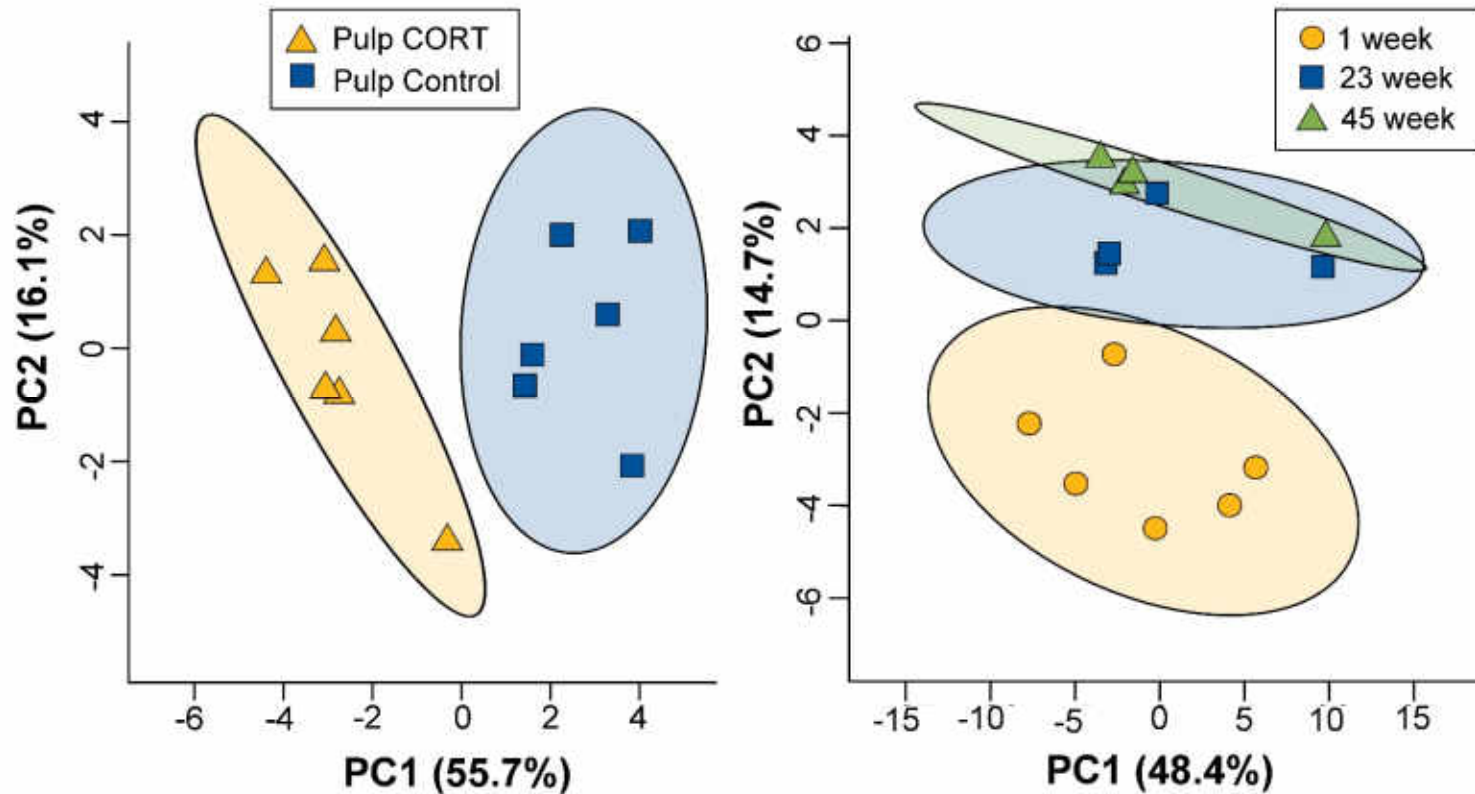
Is the feather pulp metabolome comparable to that of other samples such as blood?



No ... it's potentially superior



Is the feather pulp metabolome sensitive to biological factors?



Yes



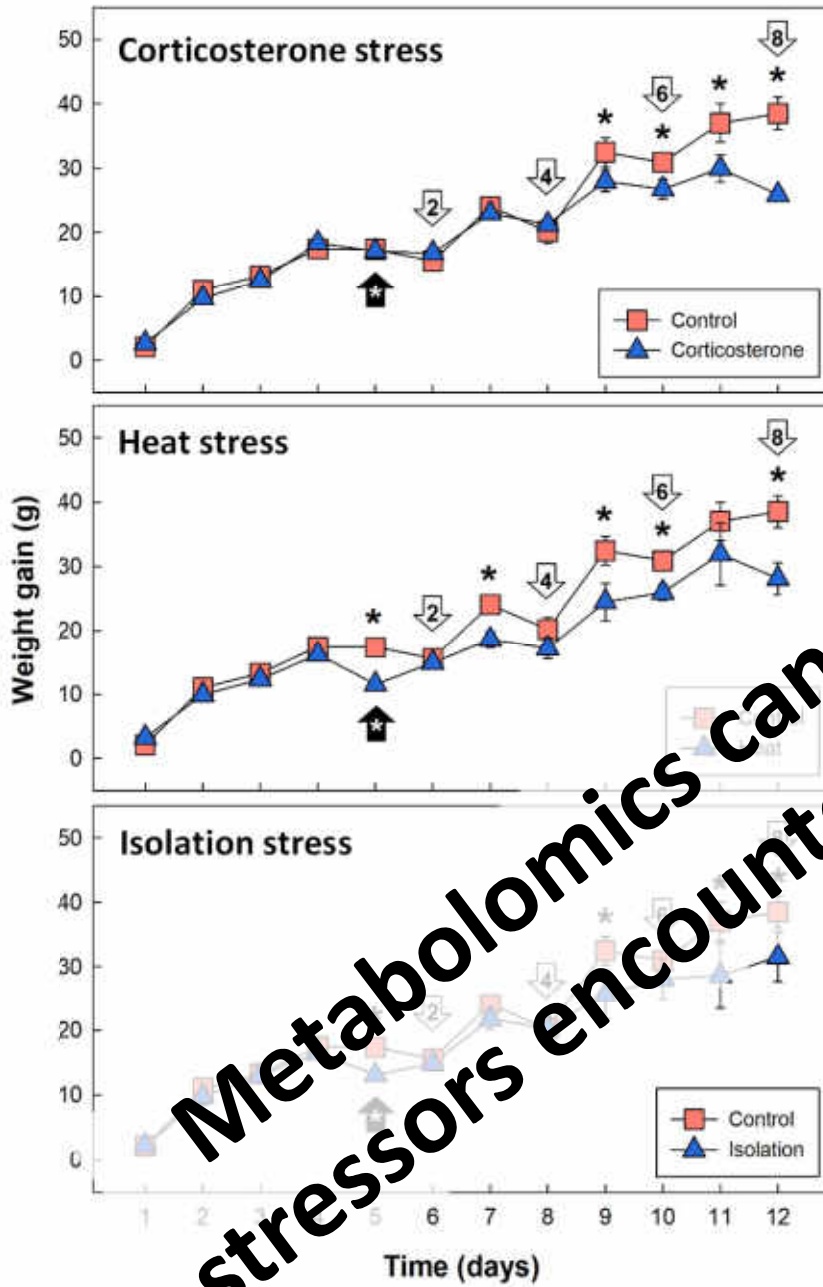
Production stressors vs corticosterone stress

- Is the corticosterone stress model relevant?
- Can metabolomics be applied to identify biomarkers of stress in production settings?



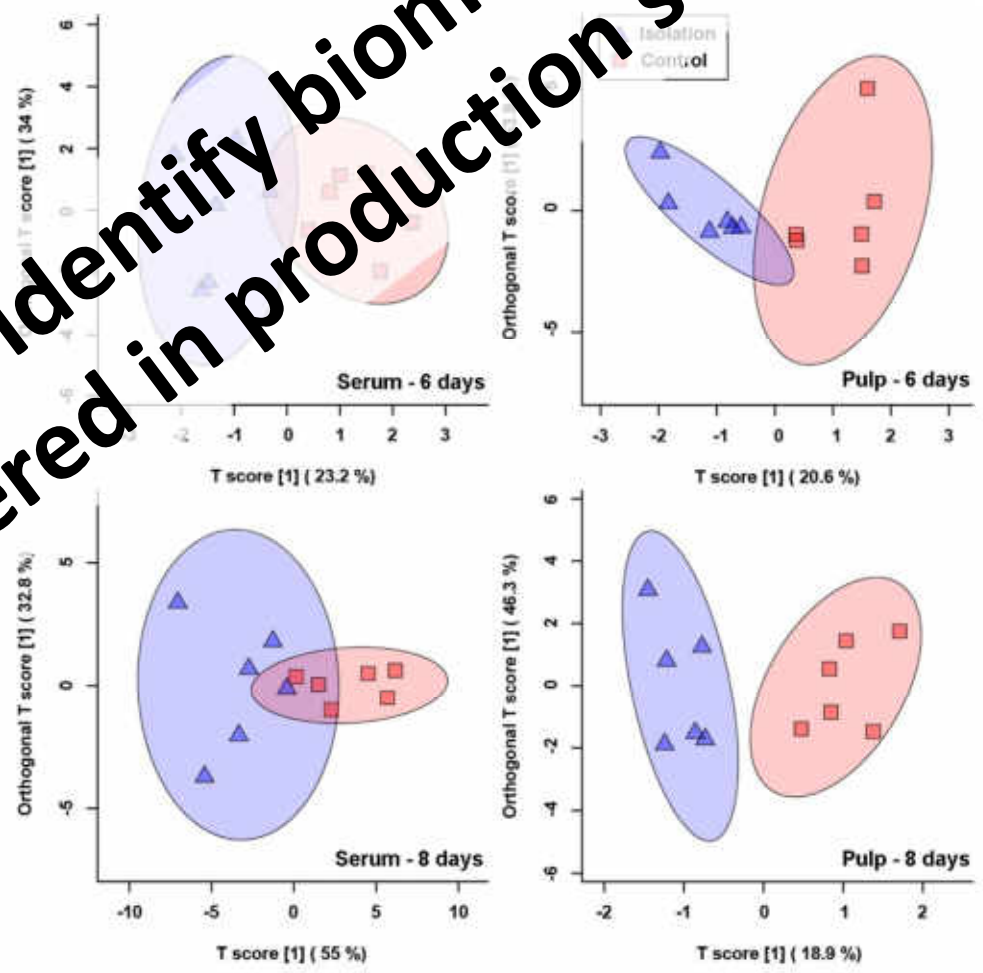
Kate Brown

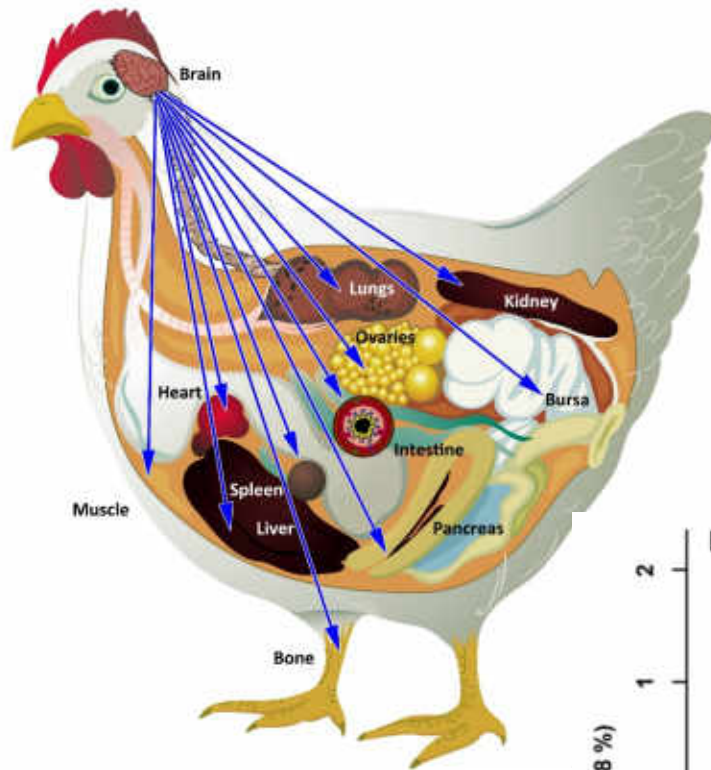




All stressors affected the metabolome of tissues as a function of time: liver, kidney, breast muscle, serum, and spleen pulp (different metabolites and pathways)

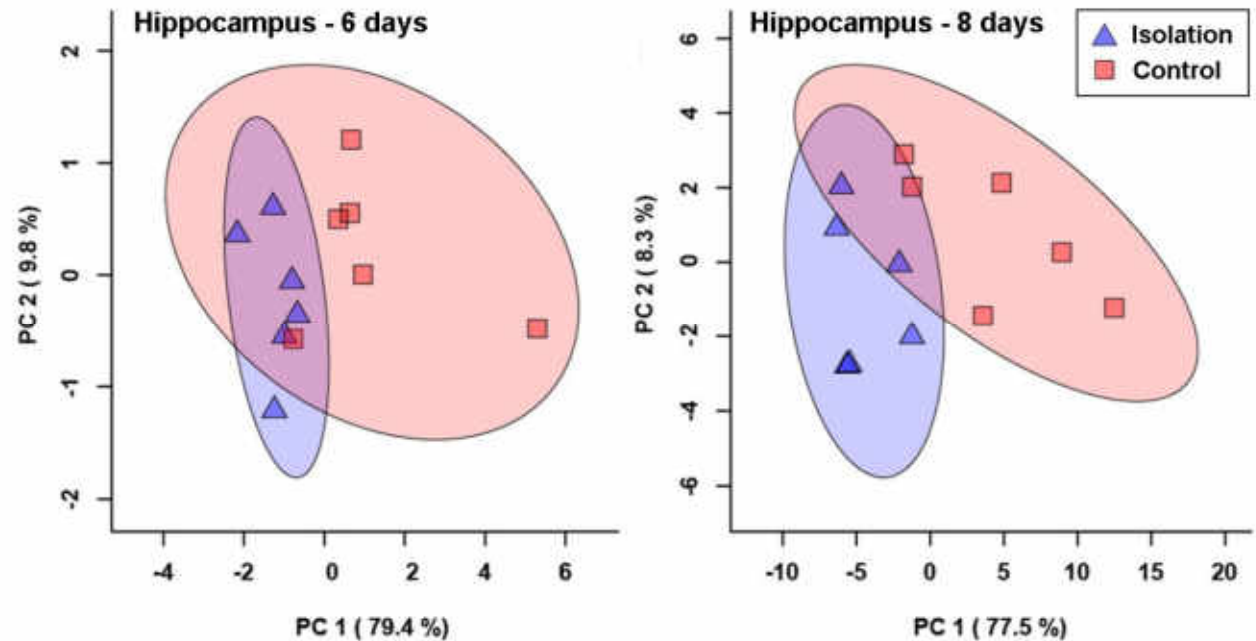
Metabolomics can identify biomarkers of stressors encountered in production settings





Homeostasis

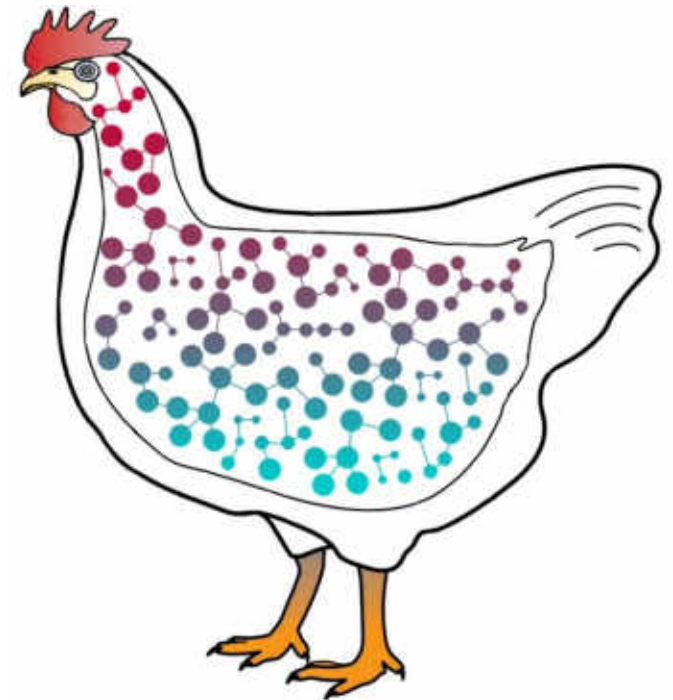
- Brain regulates homeostasis via direct and indirect communication with organs and tissues = overall bird health
- Body-wide cascade
- Stress effects on metabolomics of the brain?



All stressors impacted the brain metabolome, including the hippocampus = learning and memory, and an indicator of health disorders

Metabolomics and diagnostic tool development

- A variety of stressors impacted the metabolome of layers and broilers
- The metabolome was altered before adverse health impacts were manifested
- Findings illustrate the potential for using metabolomics to predict health outcomes in production
- Collation of specific metabolites predictive of disease manifestation is in progress (with validation in production settings)
- **Goal** = develop a diagnostic tool for use on farm
- Allow producers to monitor the health status of a flock and implement control measures in advance of disease
- Objective evaluation of husbandry strategies



Intestinal Health

31

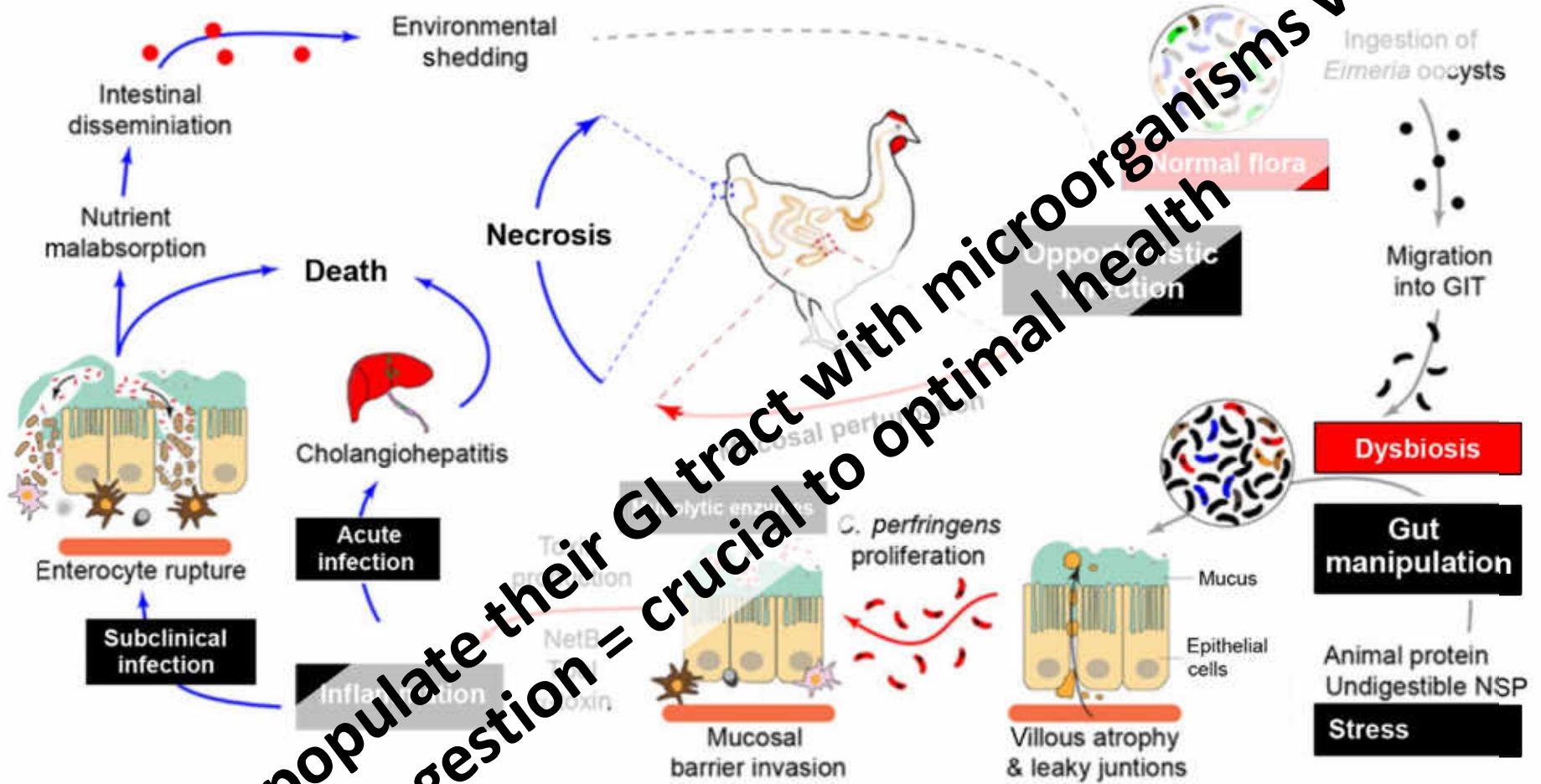
Intestinal bacteria are crucial to optimal host health

Intestinal bacteria are critical for optimal immune competency

Colonization resistance = is the mechanisms whereby the intestinal microbiota protects the host against incursion by harmful microorganisms

Dysbiosis = disruption to the microbiota decreasing colonization resistance

Necrotic enteritis: microbiota manipulation

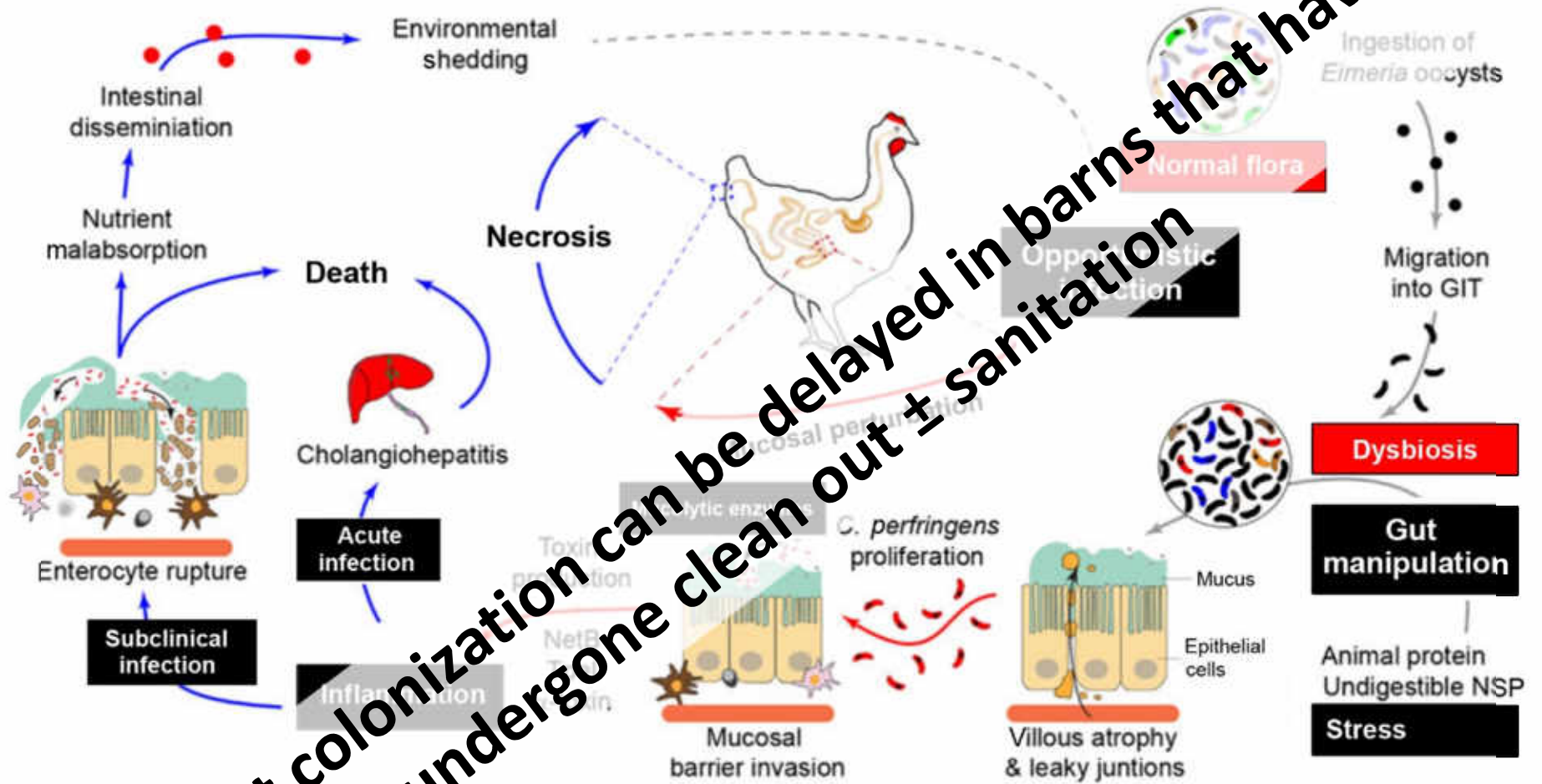


Chicks populate their GI tract with microorganisms via ingestion = crucial to optimal health

Can a one-time administration of the microbiota from a healthy adult donor to day-old chicks achieve colonization resistance?

Microbiota = all the bacteria within the GI tract (>1000 species)

Necrotic enteritis: microbiota manipulation

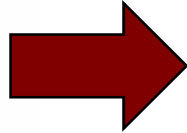


GI tract colonization can be delayed in barns that have undergone clean out ± sanitation

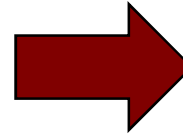
Can a one-time administration of the microbiota from a healthy adult donor to day-old chicks achieve colonization resistance?

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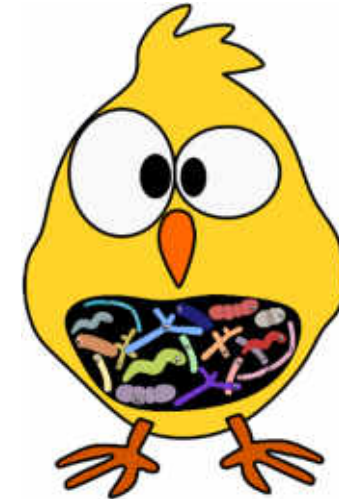
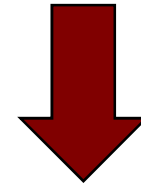
Donor ceca



Microbiota propagation
Free from pathogens



Complex
microbiota



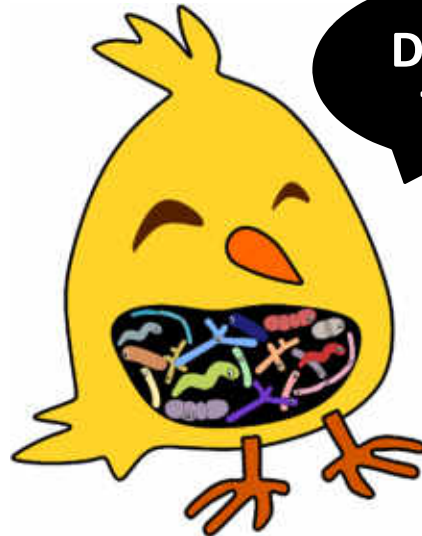
Microbiota administration \pm
(1-day old chick)



Pathogen inoculation \pm



Disease
free



Necrotic Enteritis



Sarah Zaytsoff

- Bacterial residents in the intestines of poultry are anaerobes
- Growing these bacteria requires specialized equipment



Paul Moote



Kathaleen House

**Poultry Anaerobic Bacteriology Laboratory
Lethbridge Research and Development Centre**

The “artificial intestine” used to propagate the poultry microbiota

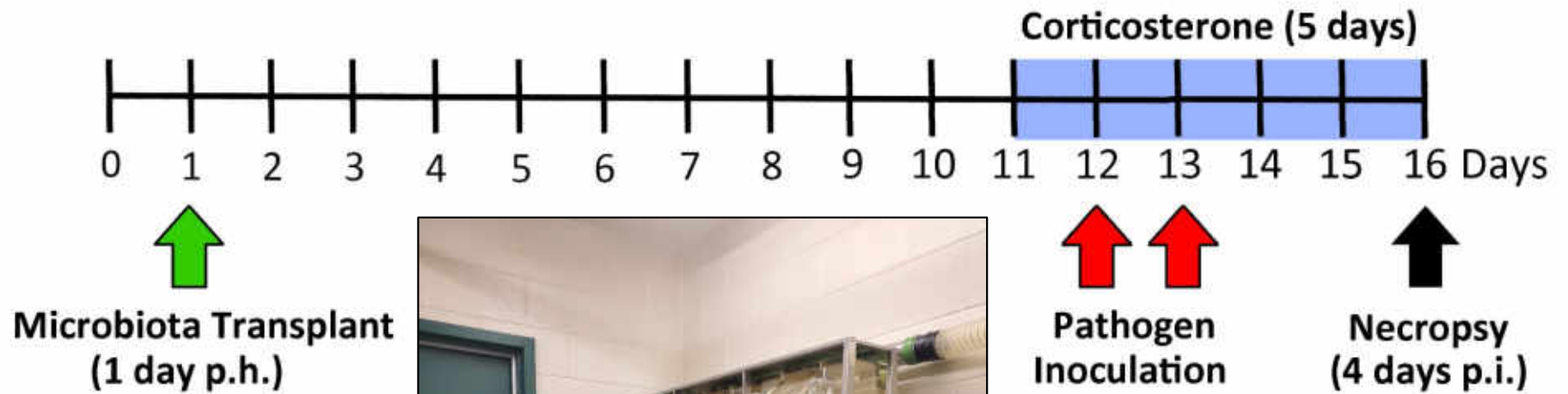


The “artificial intestine” used to propagate the poultry microbiota

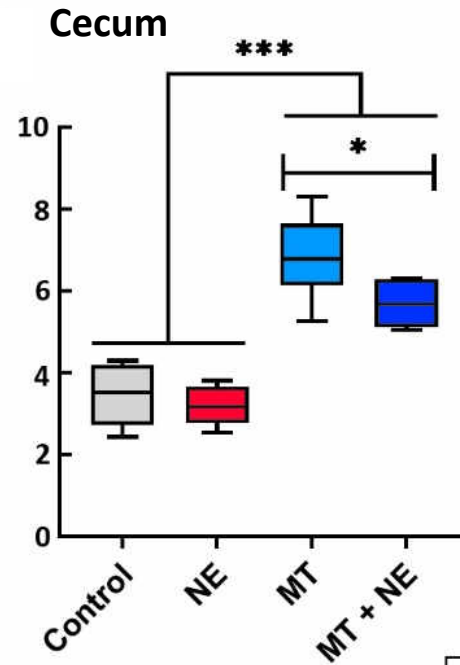
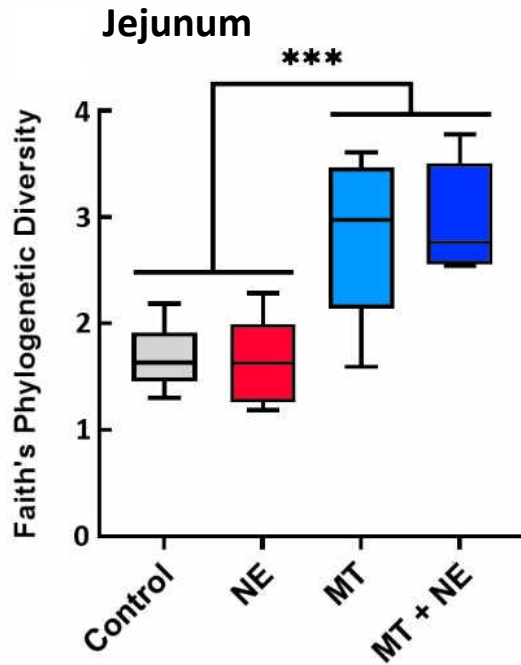


Administration of the poultry microbiota to a day-old broiler chick

Experiment time course

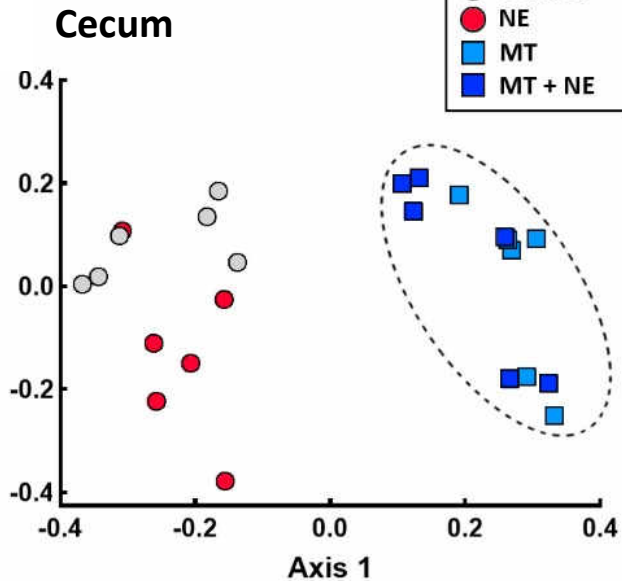
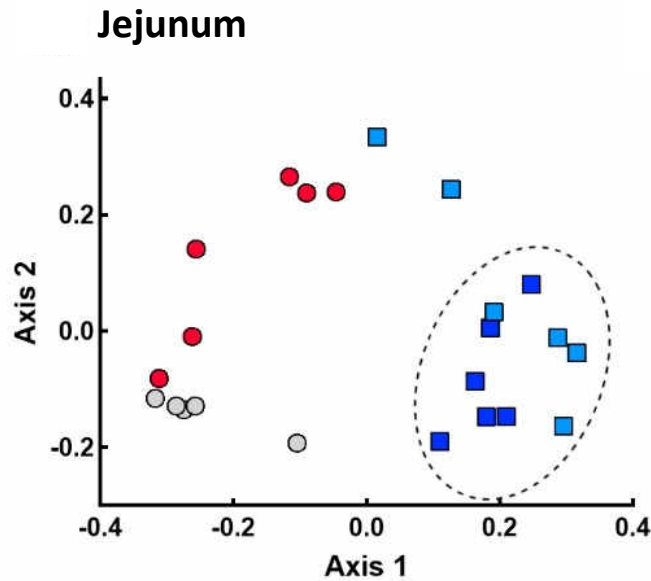


Bacterial community diversity

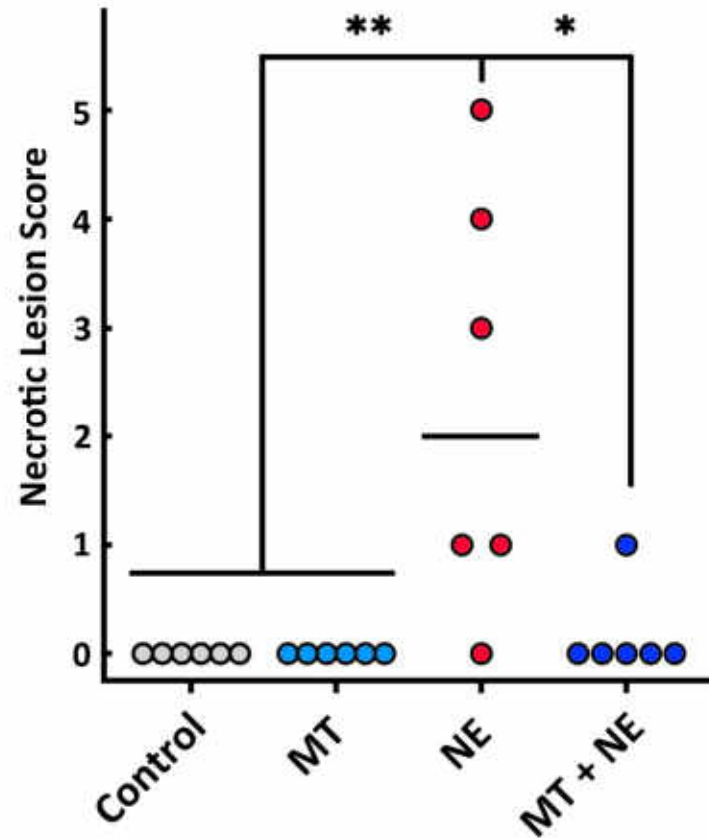
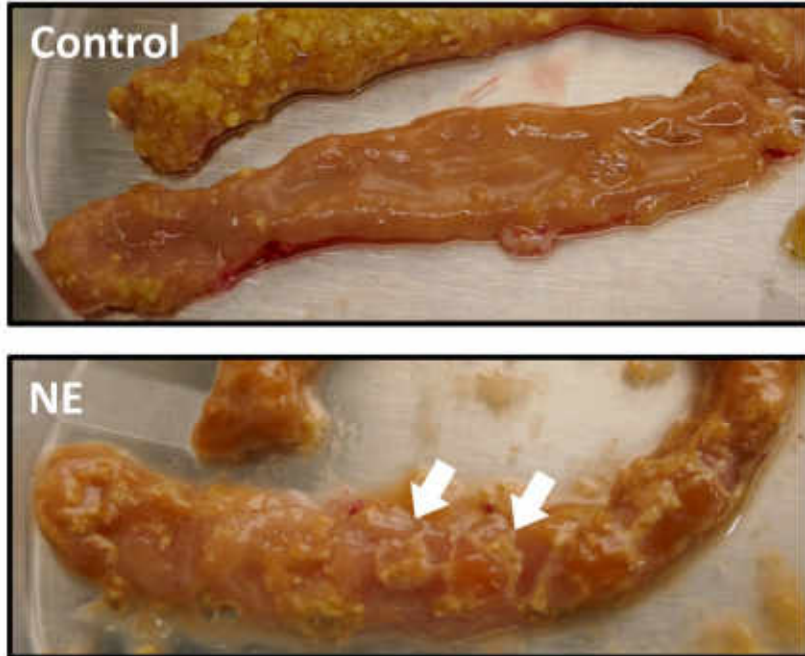


NE = Necrotic enteritis
MT = Microbiota Transplant

Bacterial community structure



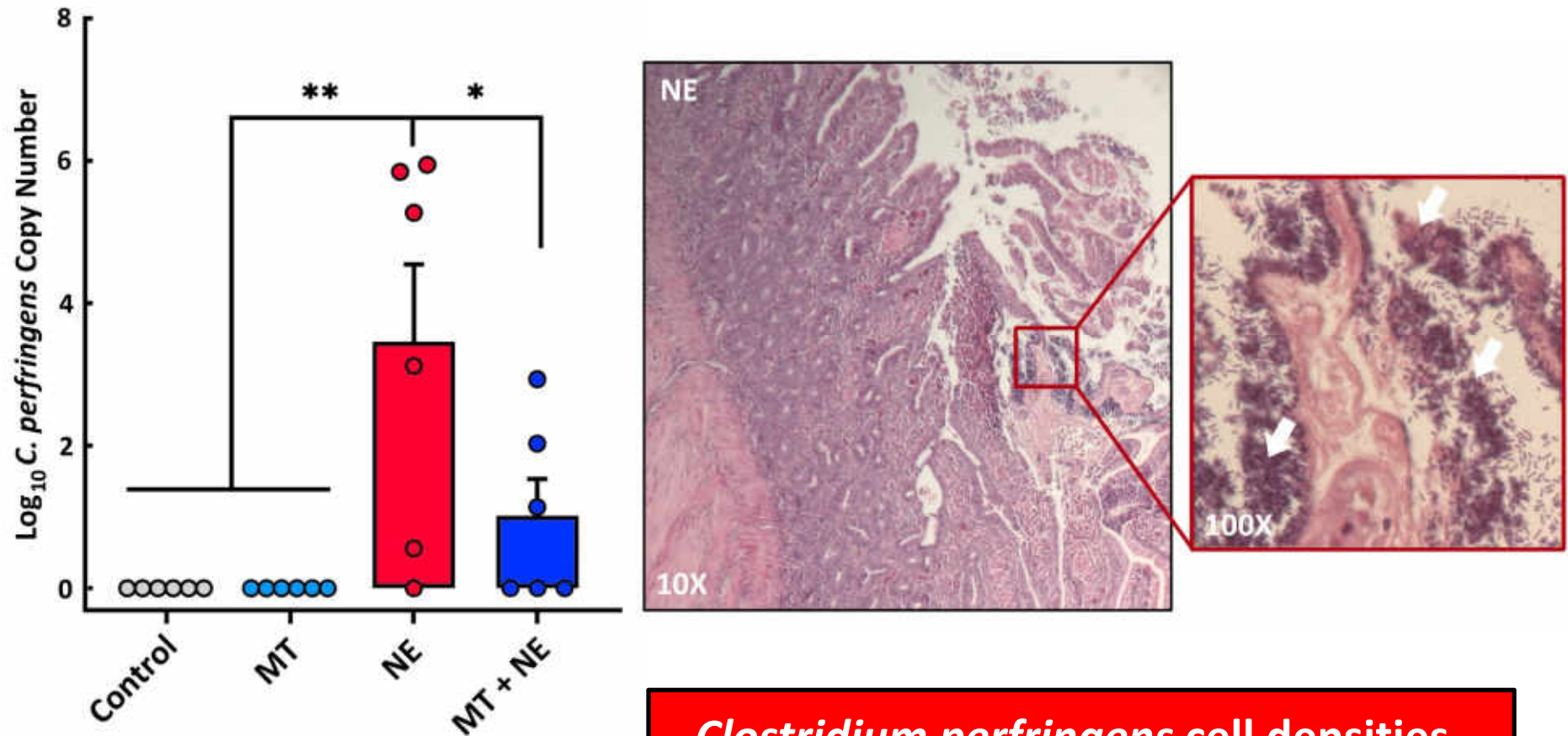
Lesion development



Necrotic lesions were significantly ameliorated in birds administered the microbiota transplant (MT + NE)

**NE = Necrotic enteritis
MT = Microbiota Transplant**

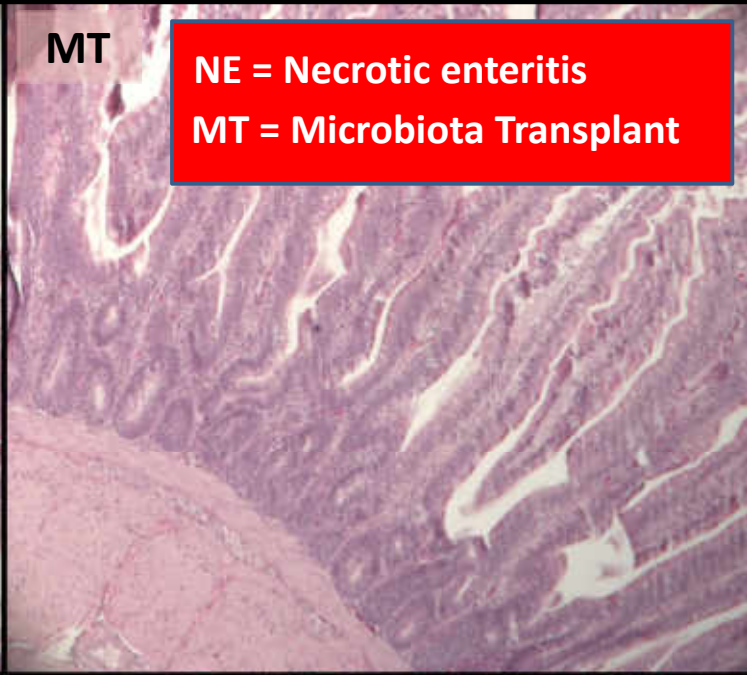
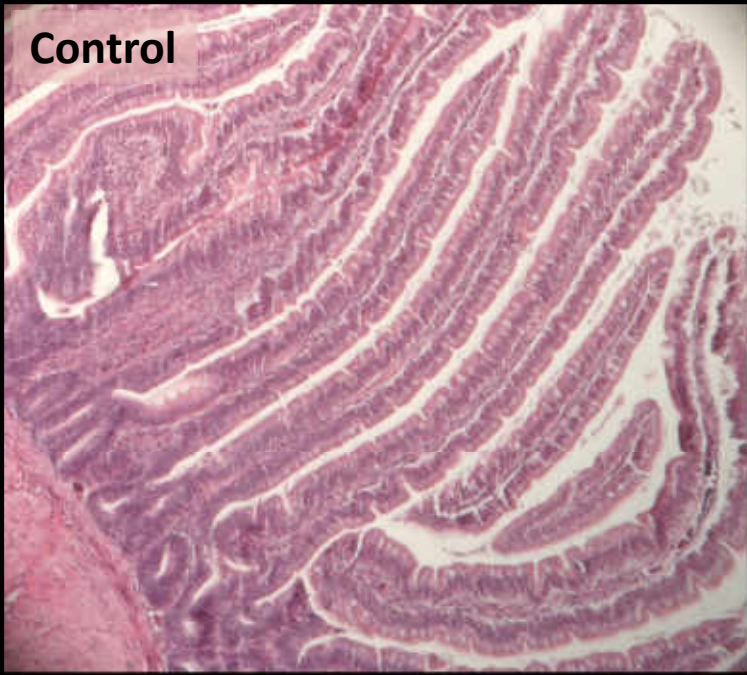
Clostridium perfringens densities



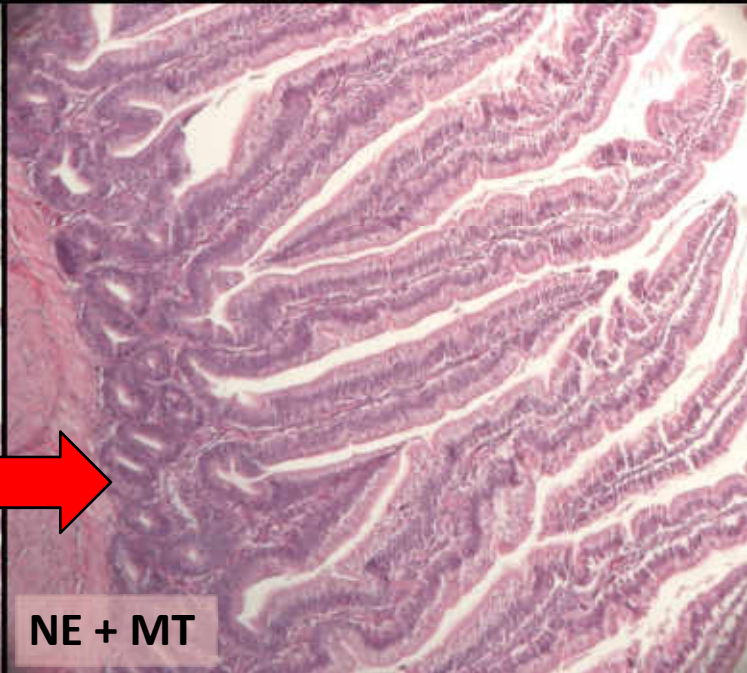
NE = Necrotic enteritis
MT = Microbiota Transplant

Clostridium perfringens cell densities were significantly reduced in birds administered the microbiota transplant (MT + NE)





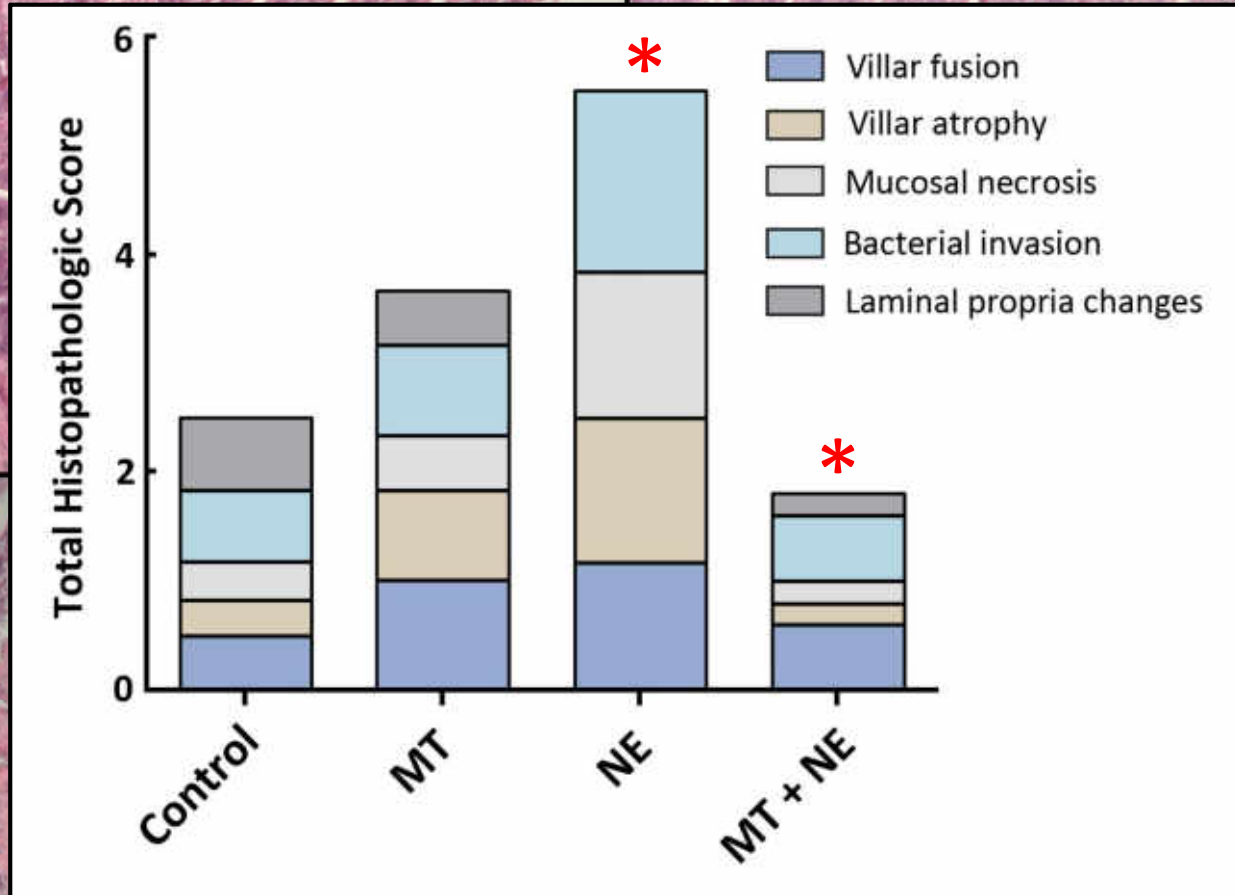
NE = Necrotic enteritis
MT = Microbiota Transplant



Control

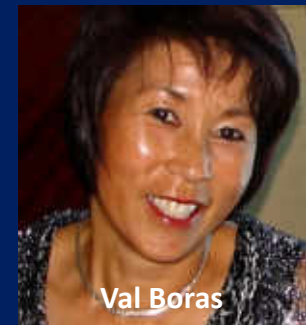
MT

NE = Necrotic enteritis
MT = Microbiota Transplant



NE

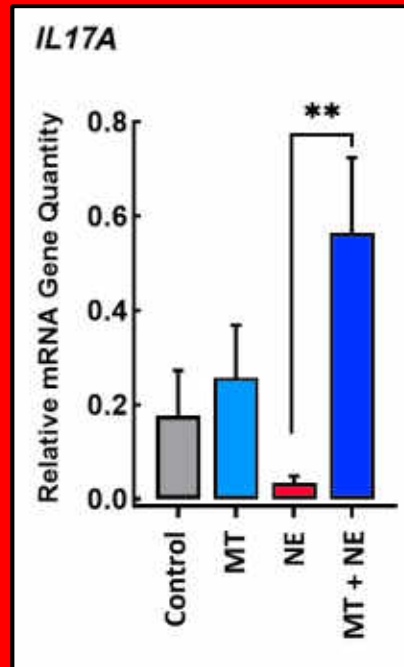
NE + MT



Val Boras

- **Transplantation of intestinal bacteria propagated in an artificial intestine to day-old chicks provided colonization resistance to necrotic enteritis**
- **Via increased microbial diversity and by promoting positive host responses (immunological competence)**

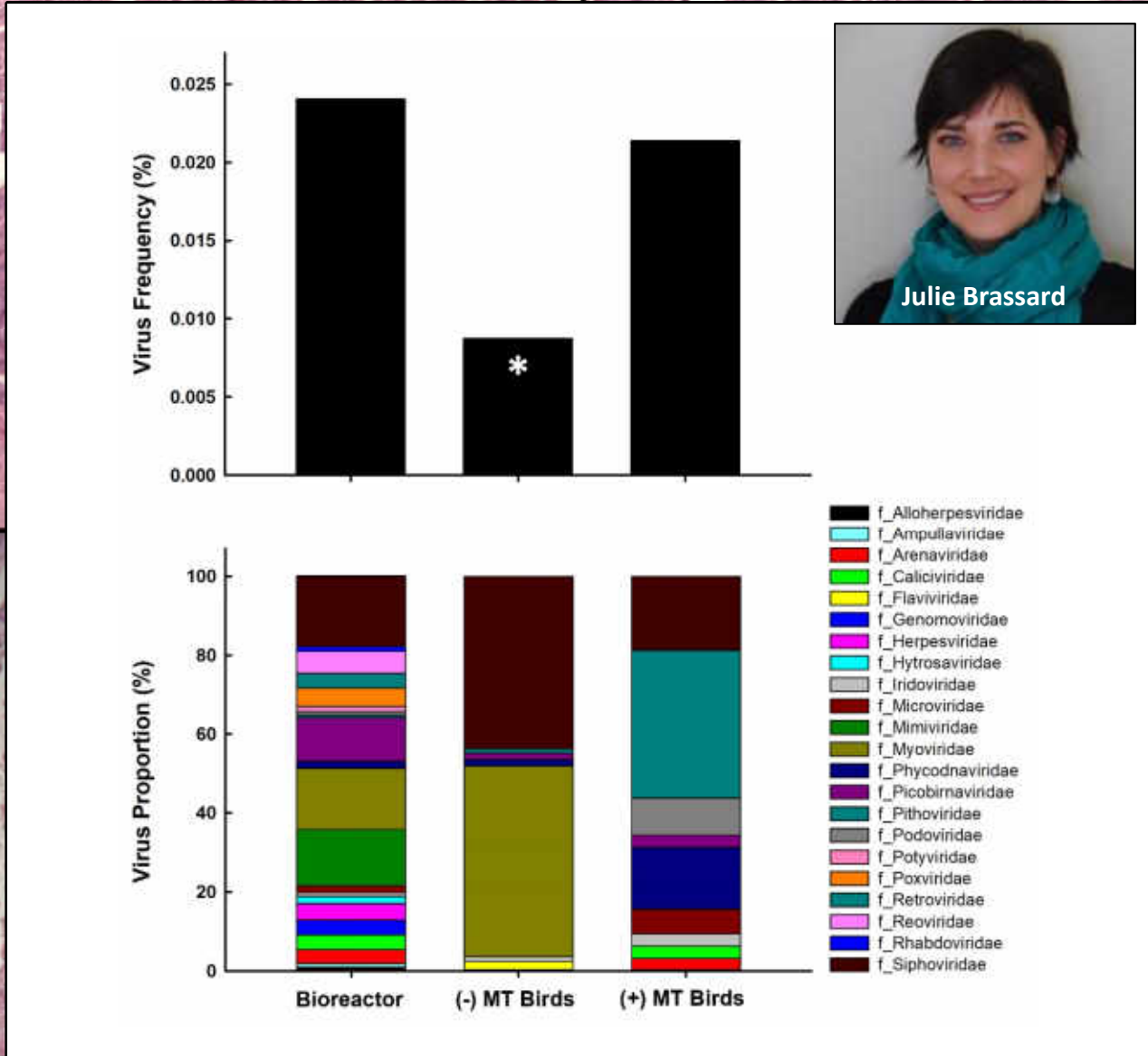
Interleukin-17A (IL-17A) is a key cytokine that links T cell activation to neutrophil mobilization and activation. As such, IL-17A can mediate protective innate immunity to pathogens.



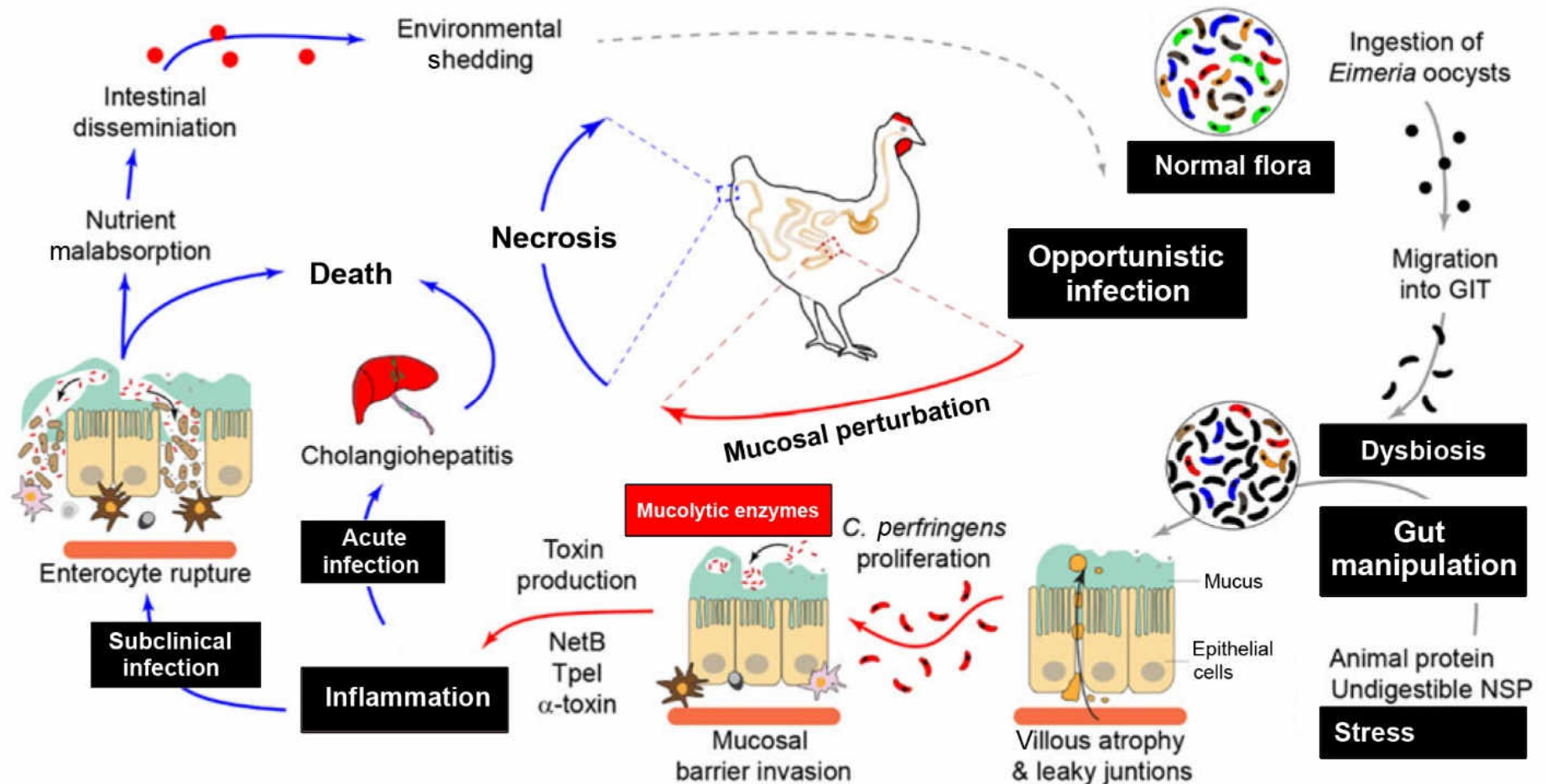
I'm disease free

- **Transplantation of intestinal bacteria propagated in an artificial intestine to day-old chicks provided colonization resistance to necrotic enteritis**
- **Via increased microbial diversity and by promoting positive host responses (immunological competence)**
- **A one-time administration of a microbiota transplant shortly after hatch may provide an effective alternative to antibiotics to mitigate important diseases**
- **Barn clean out ± sanitation solution**
- **Evidence indicated that avian viruses were eliminated during propagation of the microbiota within the artificial intestine**





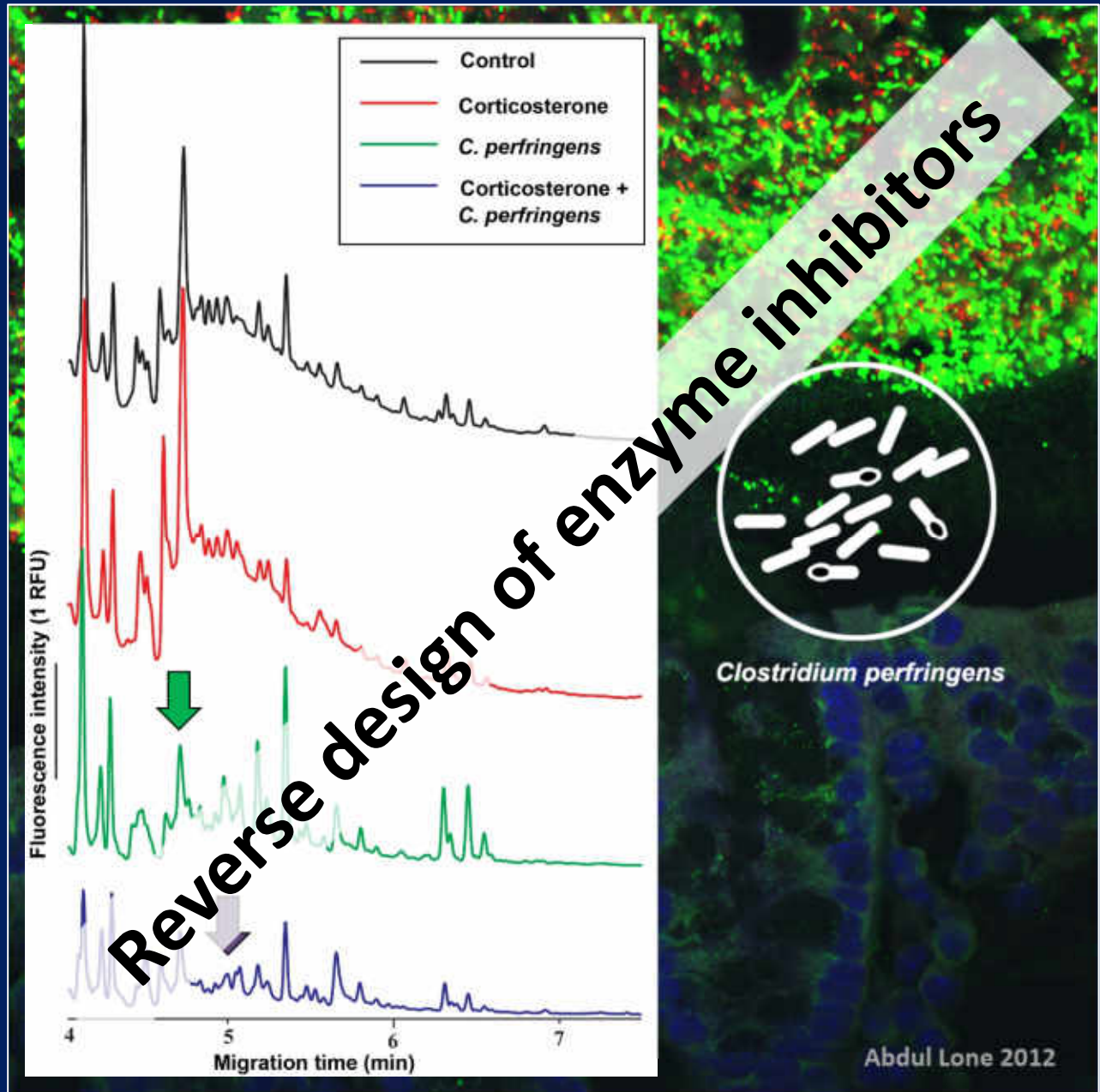
Necrotic enteritis: mucolytic enzymes



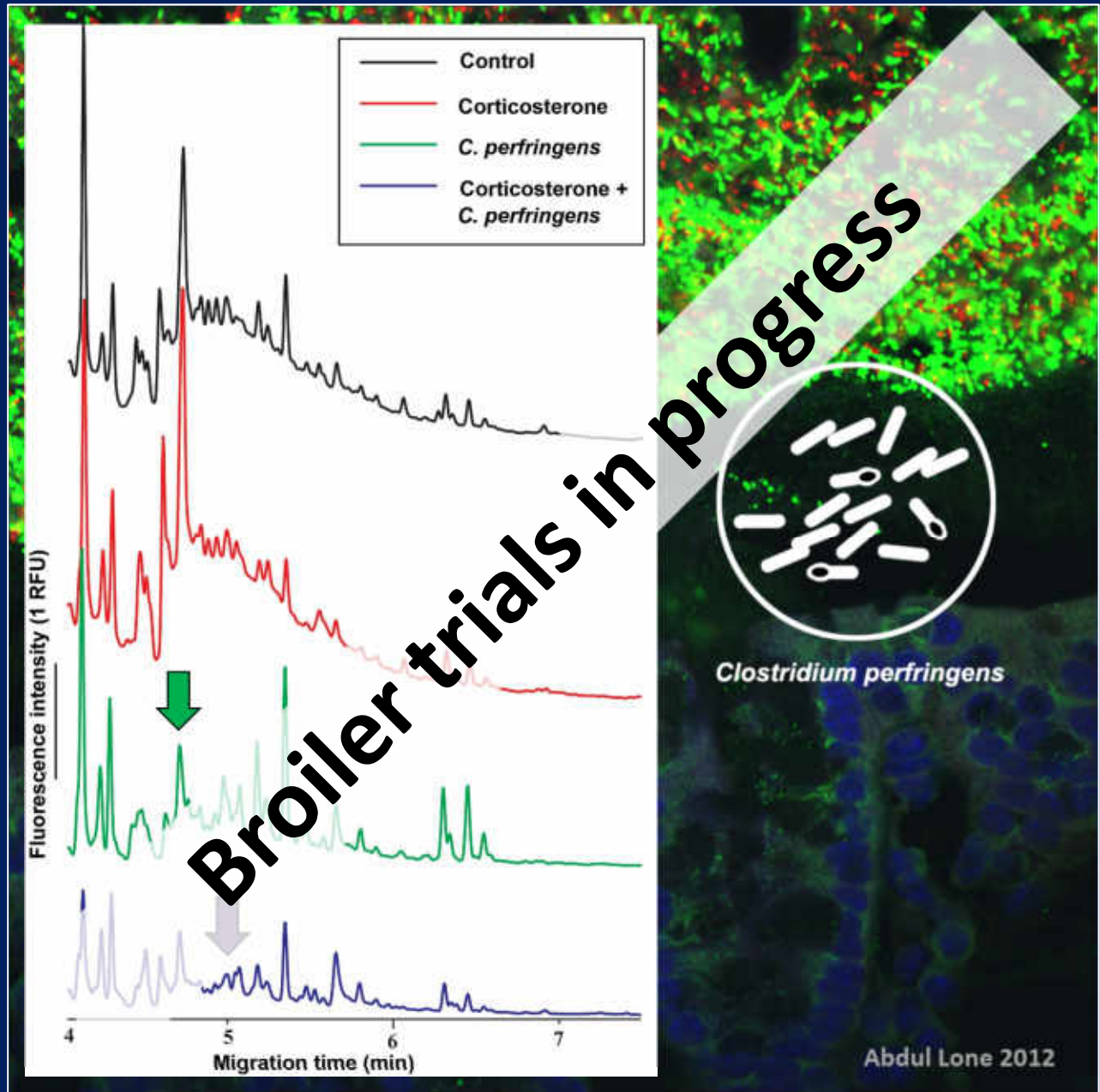
Can mucolytic enzyme inhibitors be an effective control strategy?

First step is to understand the role that mucolytic enzymes play in pathogenesis (glycomics), and use the information to design inhibitors

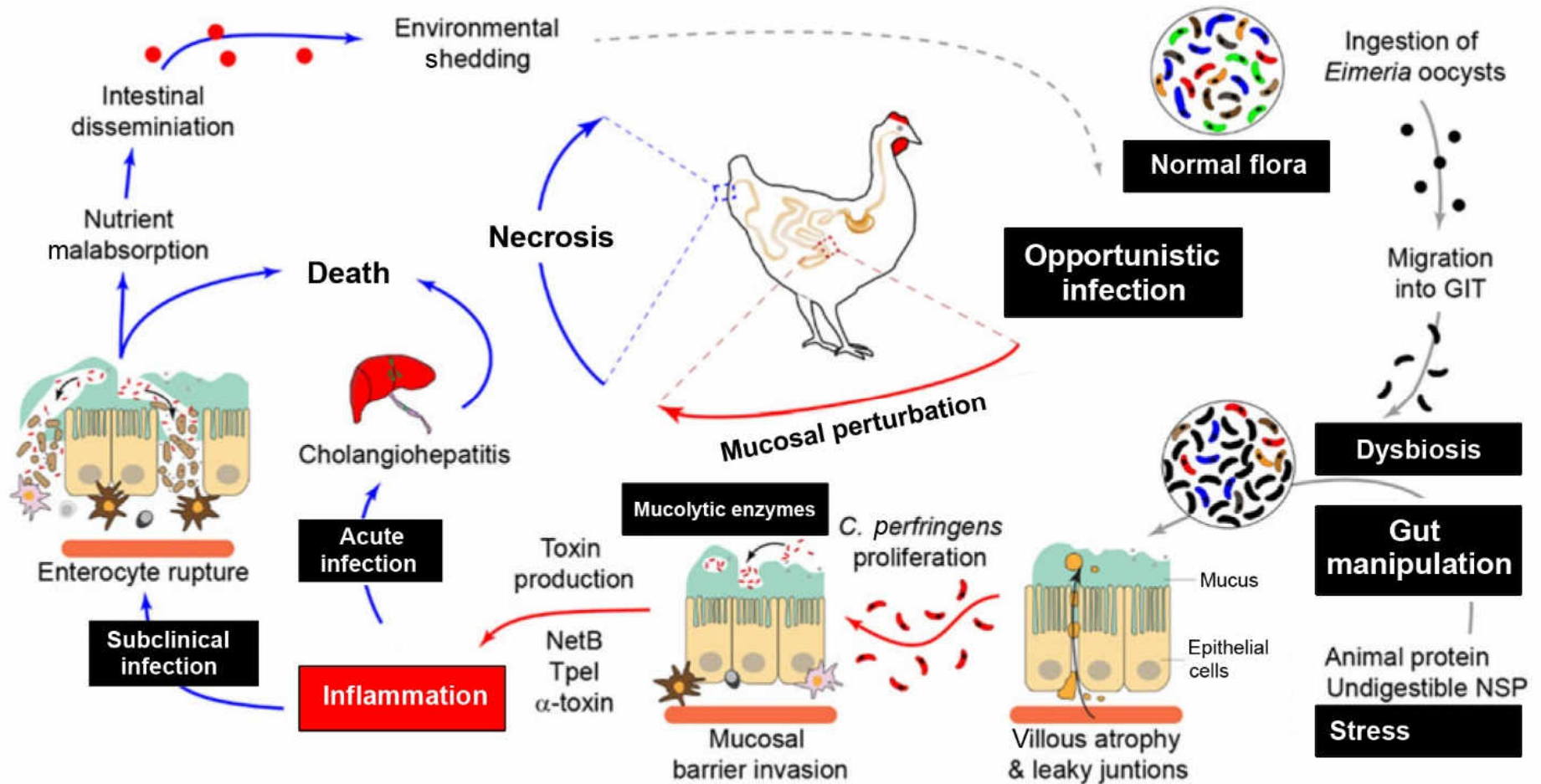
Enzyme inhibitors against *Clostridium perfringens*



Enzyme inhibitors against *Clostridium perfringens*



Necrotic enteritis: inflammation amelioration



Inflammation is metabolically costing to birds

Inflammation can be painful thereby adversely affecting production

Targeting inflammation as an alternative to targeting individual pathogens

Inflammation amelioration

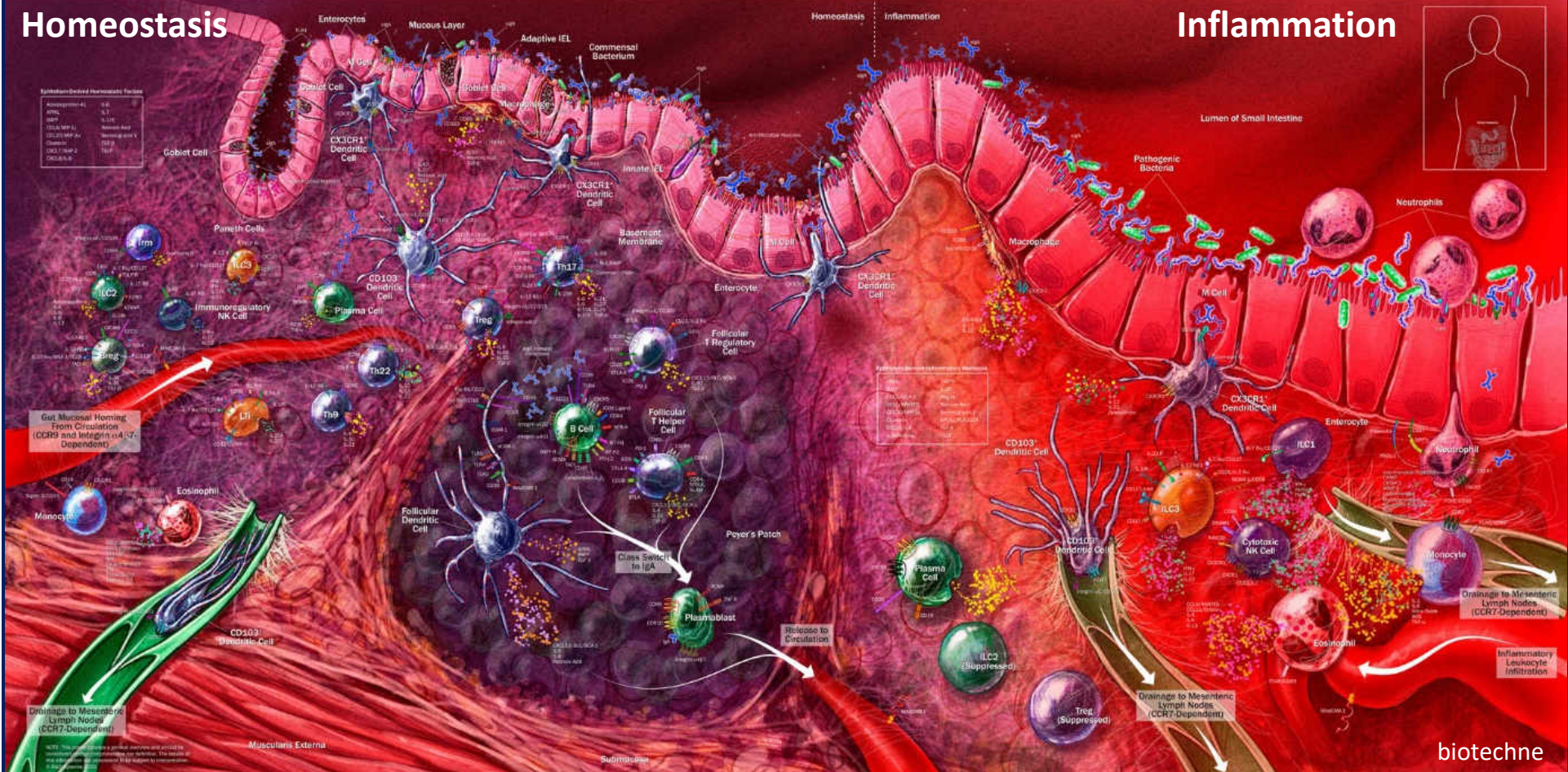
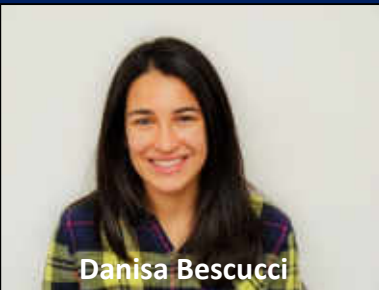


Image showing an inflamed intestine vs an intestine in homeostasis
 Activation of pro-inflammatory molecules and immune cell trafficking
 Redness (rubor), heat (calor), swelling (tumor), and pain (dolor)

Inflammation amelioration

- Microbiota transplantation
- Delivery of anti-inflammatory molecules
- Development of novel and targeted delivery technologies
- Production compatible strategies
- Necrotic enteritis and salmonellosis – current disease models
- Alternatives to antimicrobial growth promoters (immunomodulation hypothesis)
- Targeting inflammation has the potential advantage of being effective against a variety of diseases (opposed to pathogen-specific strategies)

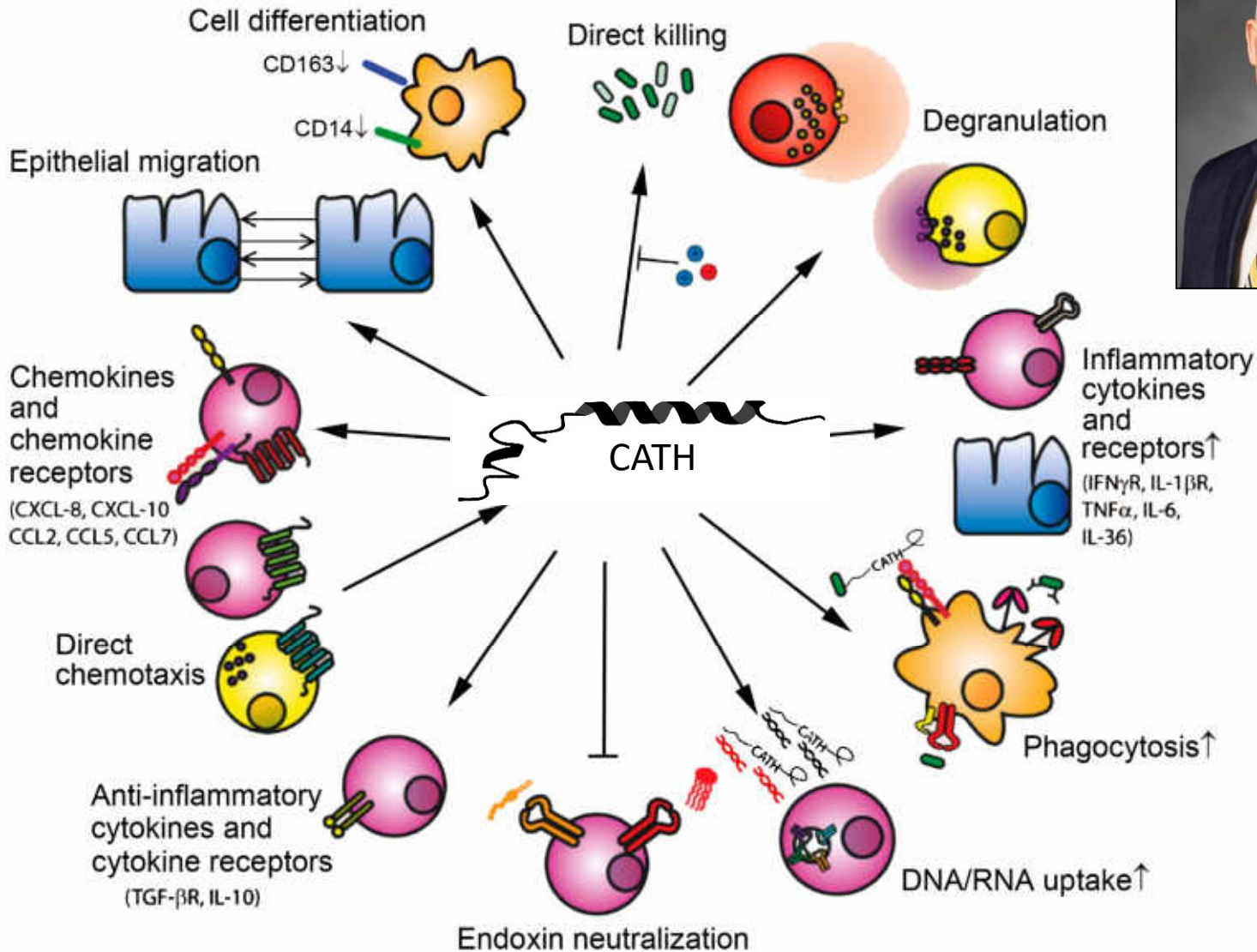


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Delivery of host defense molecules

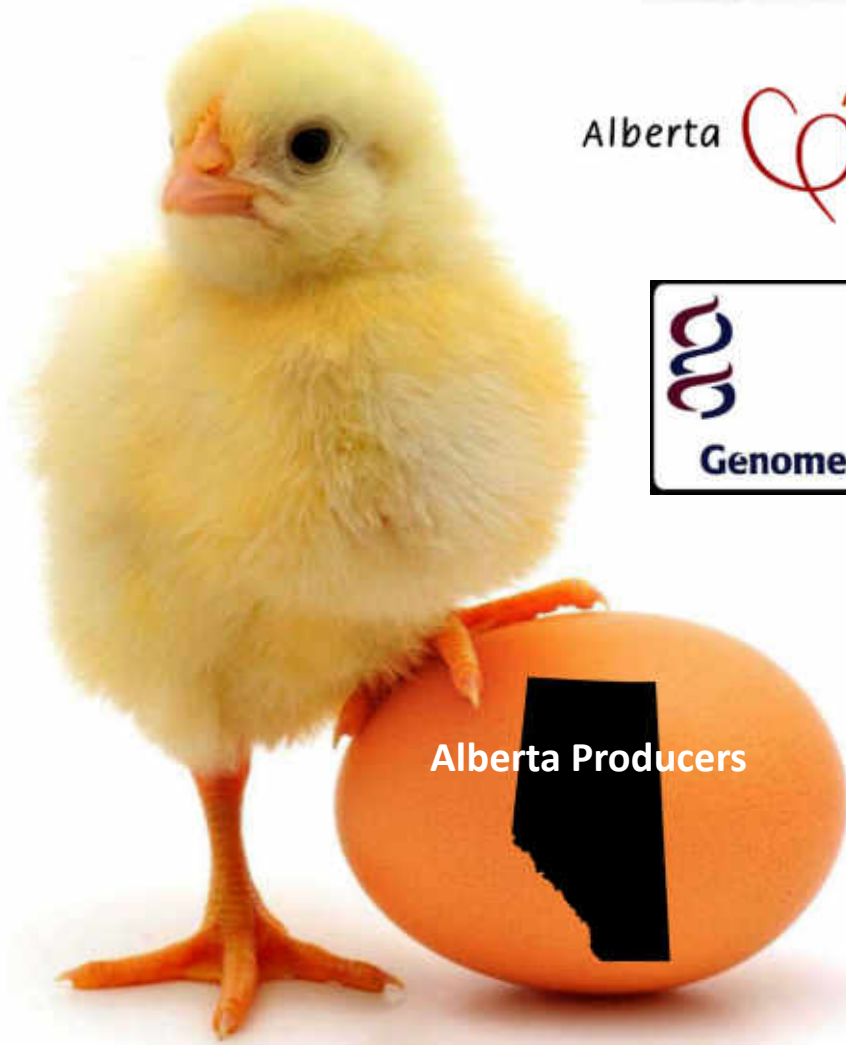


Innovation achievement strategy

- Employment of a multidisciplinary approach
- Utilization of metabolomics to identify relevant biomarkers of bird health, and to develop a diagnostic tool for use on farm
- Goal is to progress to on-farm evaluations in 2022-2023
- Strategies to ameliorate inflammation as an alternative to targeting individual pathogens
- Enhancement of host defense mechanisms
- Rationale-based development of technologies to manage disease (e.g. enzyme inhibitors for necrotic enteritis)
- Development of sector-compatible strategies
- One time microbiota transplant example
- Effective alternatives to antibiotics



Research support



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