Strategies to control necrotic enteritis in broilers

through gut health optimization

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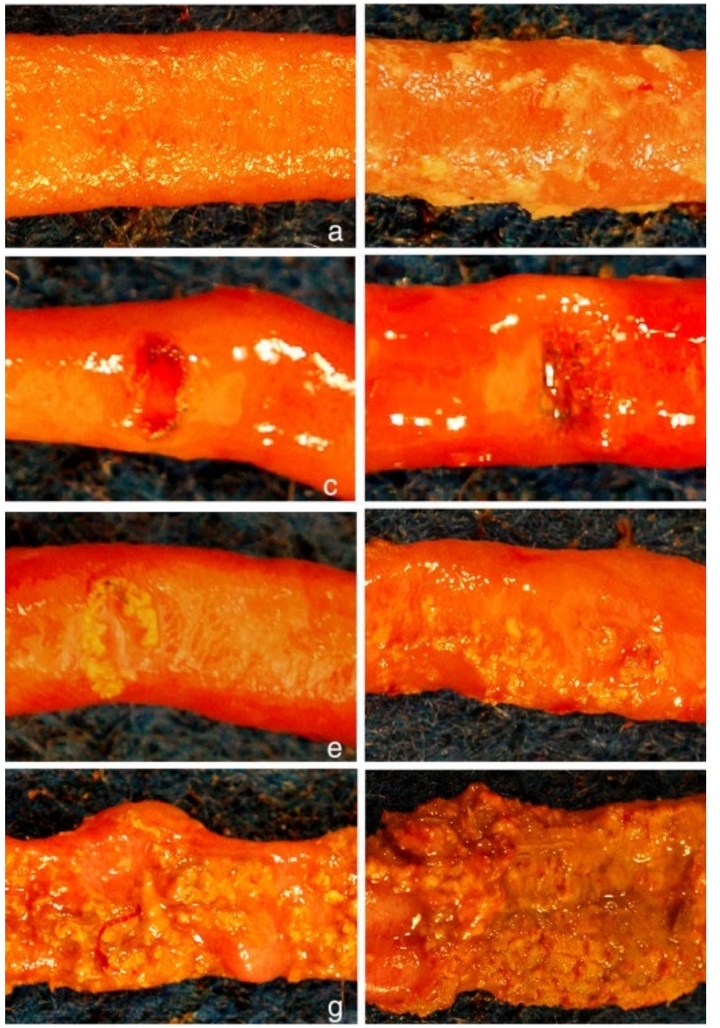
February 2024 - PIP Innovation Showcase

Poultry Necrotic Enteritis

- Enteric disease caused by pathogenic strains of *Clostridium perfringens*, an opportunistic bacterium.
- Predisposing factors: anything that causes intestinal damage or dysbiosis can be a trigger.
- Affects approximately 40% of commercial broiler flocks.
- Typically occurs between 3 and 4 weeks of age.
- Costs $6 billion USD/year for the poultry industry.

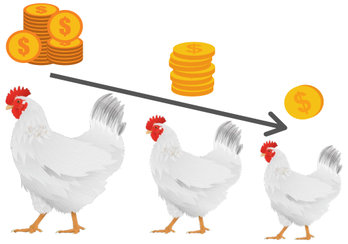


(Shojadoost et al., 2012)

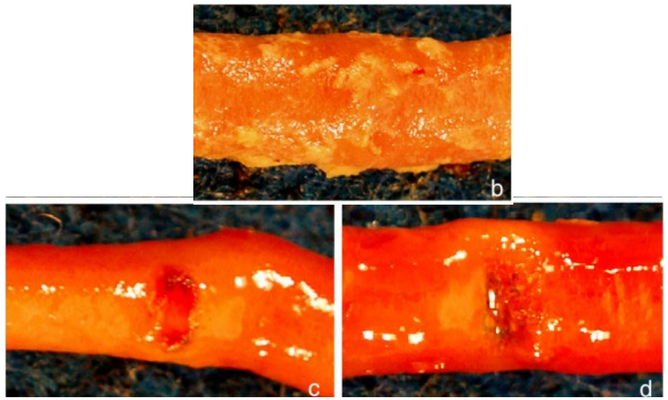
(Timbermont et al., 2011; Wade and Keyburn, 2015)

Necrotic enteritis: Clinical vs subclinical conditions

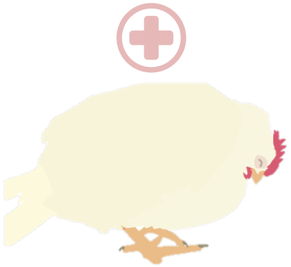
Subclinical



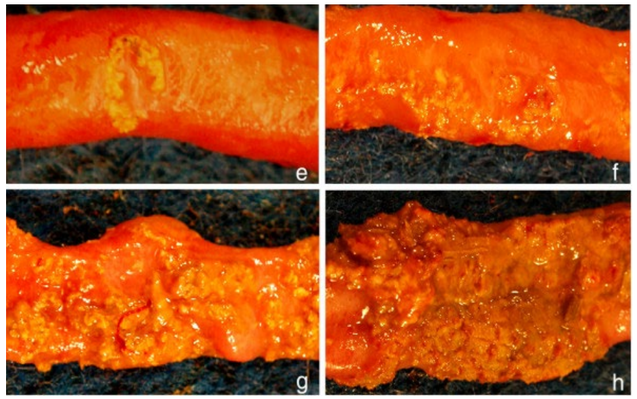
- Silent.
- Chronic damage to the intestinal tissue.
- Decreases growth performance.
- The most responsible for economic losses.



Clinical

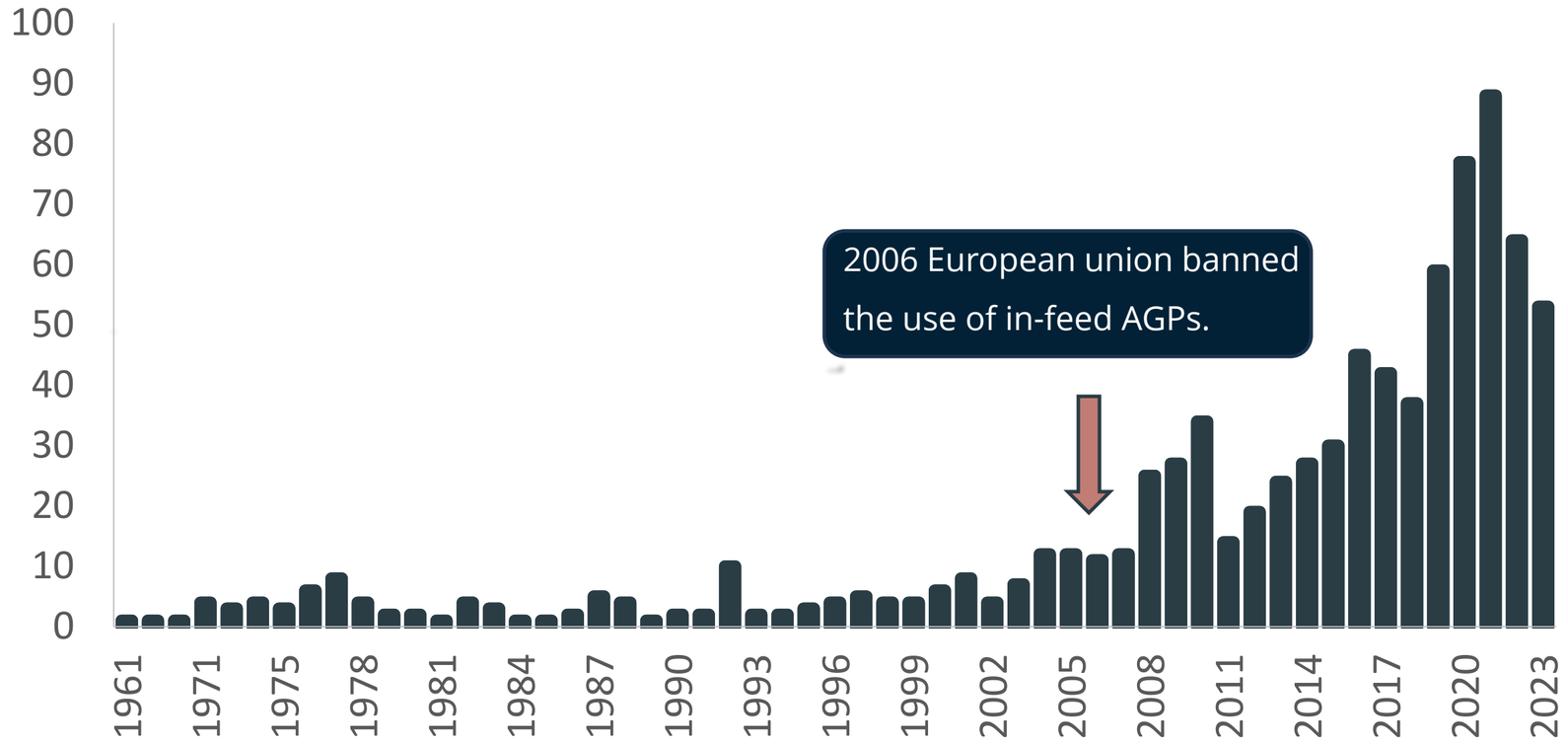


- High mortality (up to 50%).
- Often without premonitory signs.
- Some clinical signs that might be observed are severe depression, diarrhea, ruffled feathers, dehydration, and decrease in feed intake.



Removal of in-feed antibiotic growth promoters (AGP) increased the incidence of poultry necrotic enteritis

- The number of PubMed citations on “necrotic enteritis and poultry”



Removal of in-feed AGP: Consequences on necrotic enteritis

- In Canada, antibiotics of category I were eliminated in 2014, and those in category II were eliminated in 2018.
- Elimination of antibiotics of category III is still in debate, but we need to be prepared for the consequences!

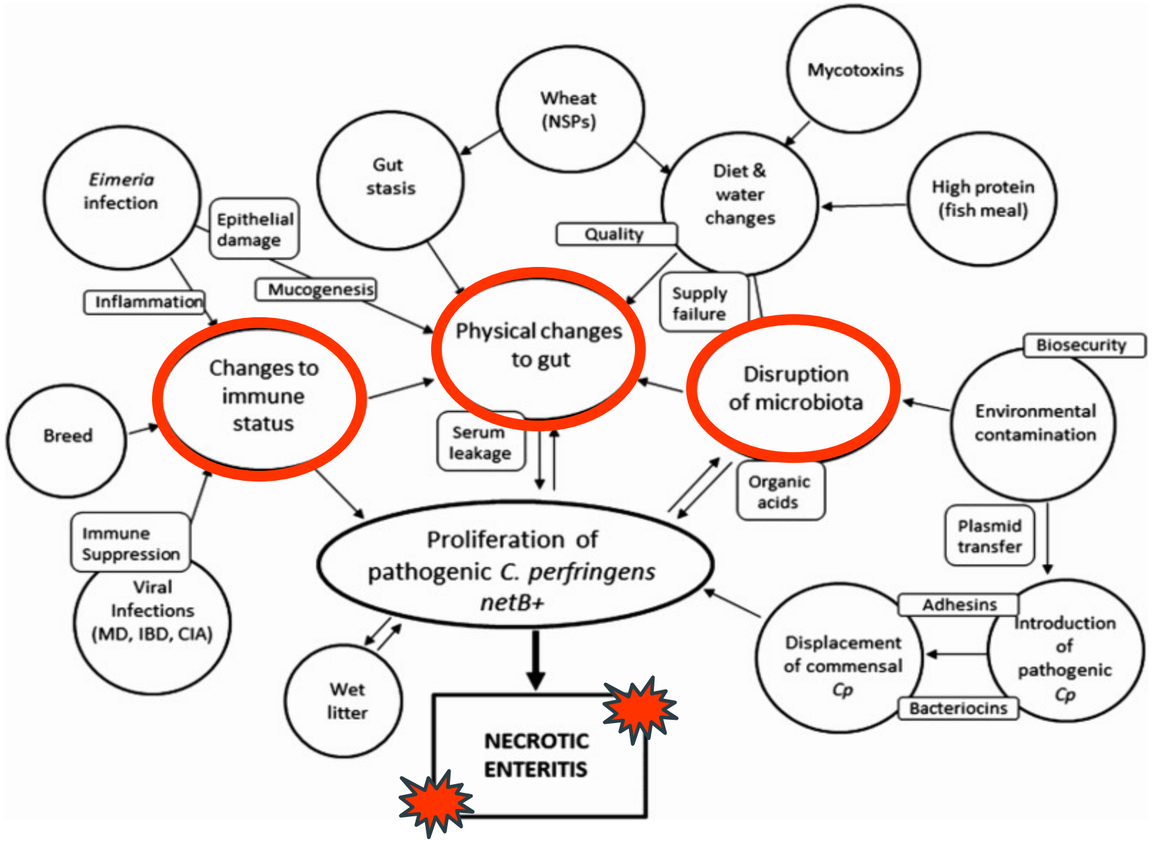
Category	Category Criteria	Antimicrobial Family
I - Very High Importance	Essential for serious human infections and limited or no alternatives available	Cephalosporins
		Fluoroquinolones
II – High Importance	Essential for treating serious human infections and few alternatives available	Aminoglycosides
		Lincosamides
		Macrolides
		Penicillins
		Streptogramins
		Diaminopyrimidines
III - Medium Importance	Important for treating human infections and alternatives generally available	Bacitracins
		Sulphonamides
		Tetracyclines
IV – Low Importance	Not used for humans	Flavophospholipids
		Ionophores
Uncategorized		Orthosomycin

Category II: Lincomycin
Virginiamycin
Avoparcin

Category III: Bacitracin
Oxytetracycline

Necrotic enteritis: Multifactorial and complex disease

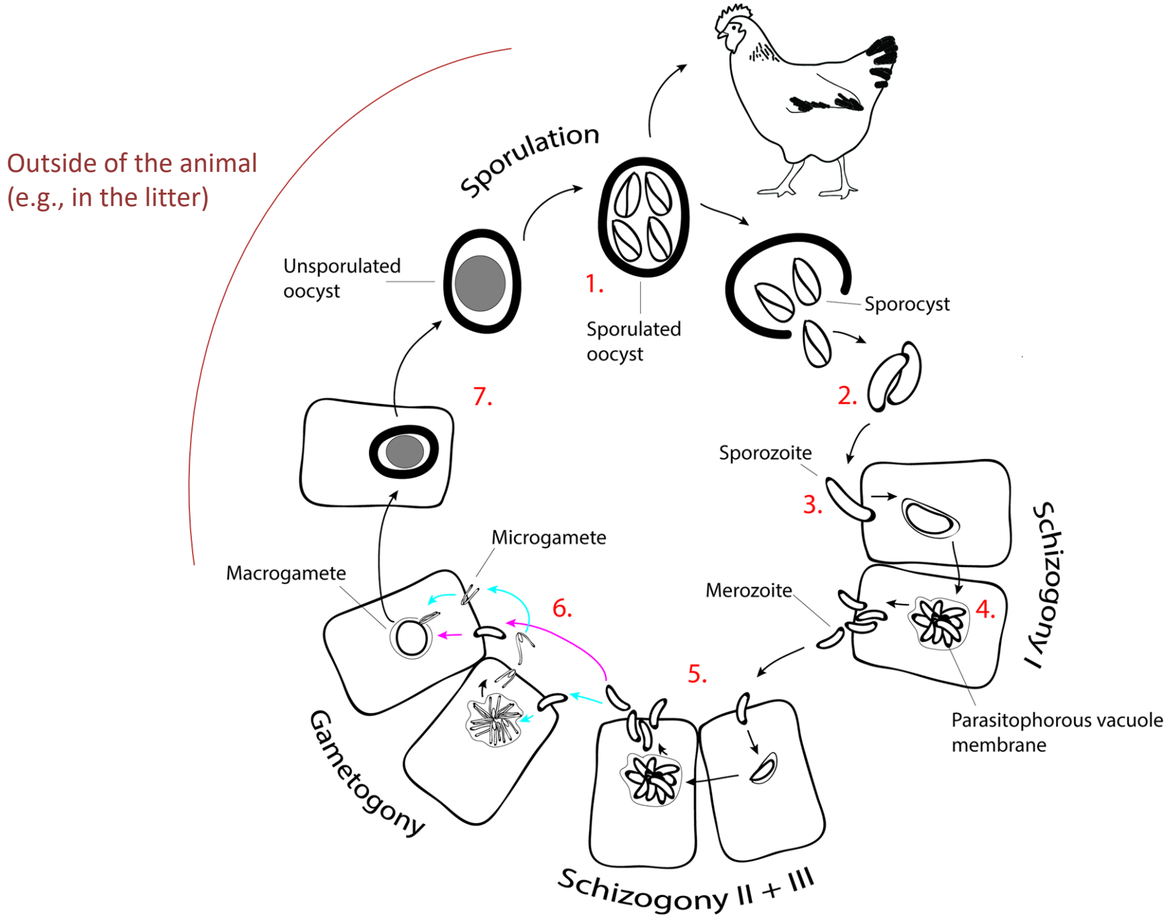
- Several predisposing factors are associated with NE development in broilers.
- Every factor that can change the normal physical structure or environment of the GIT or cause immune suppression is a potential NE trigger.



(Moore et al., 2016)

Coccidiosis as an NE predisposing factor

- Coccidiosis is caused by *Eimeria* spp. parasites, and it is the main NE predisposing factor.



- Exposure to extracellular matrix protein
- Protein plasma leakage

Adhesion sites and nutrients rich in amino acids for *C. perfringens* proliferation.

Dietary components as NE predisposing factors

- Ingredients rich in non-starch polysaccharides (e.g., wheat, barley, oats).
- Proteins of low digestibility or from animal sources (e.g., fish meal).
- Excess of dietary nutrients (over the requirements).
- Anti-nutritional factors, mycotoxins, oxidized oil, and others.

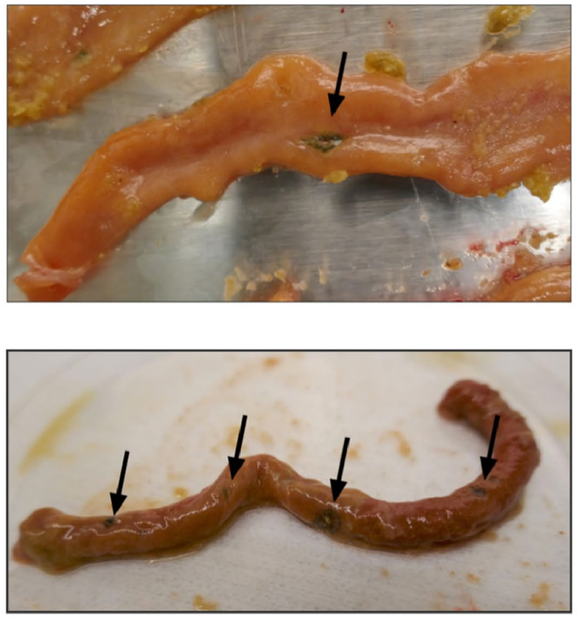
	Incidence[1] of necrotic enteritis lesions		
Diet (different protein sources)	**Duodenum**	**Jejunum**	**Liver**
Potato	33[a]	33[a]	46[a]
Fish	23[ab]	25[ab]	26[b]
Soy	14[b]	17[b]	22[b]
P-value	<0.001	0.005	<0.001

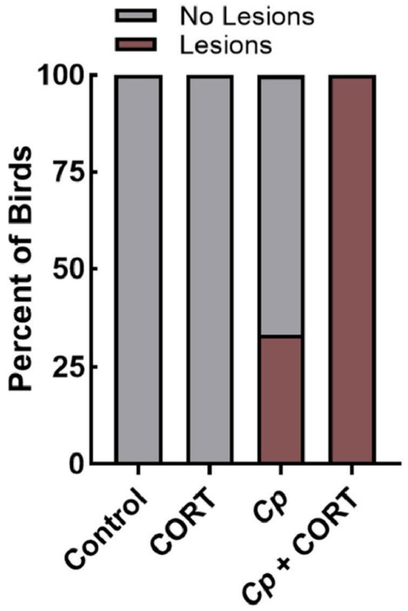
[1] Number of lesion-positive birds out of 48 birds sampled in each treatment.

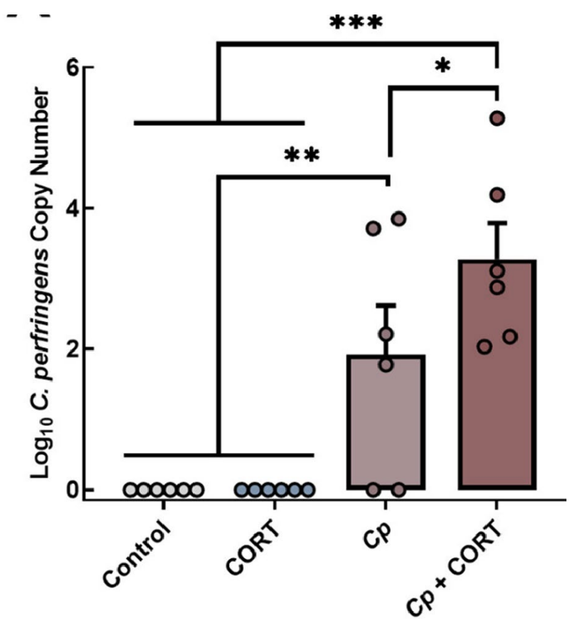
(Adapted from Palliyeguru et al., 2010)

Immune suppression as NE predisposing factors

- Physical and/or physiological stress (e.g., hot/cold stress, high stocking density, sanitary conditions) ➡ Increase plasma corticosterone levels.
- Immunosuppressive diseases (e.g., Marek's , IBD, chicken infectious anemia).



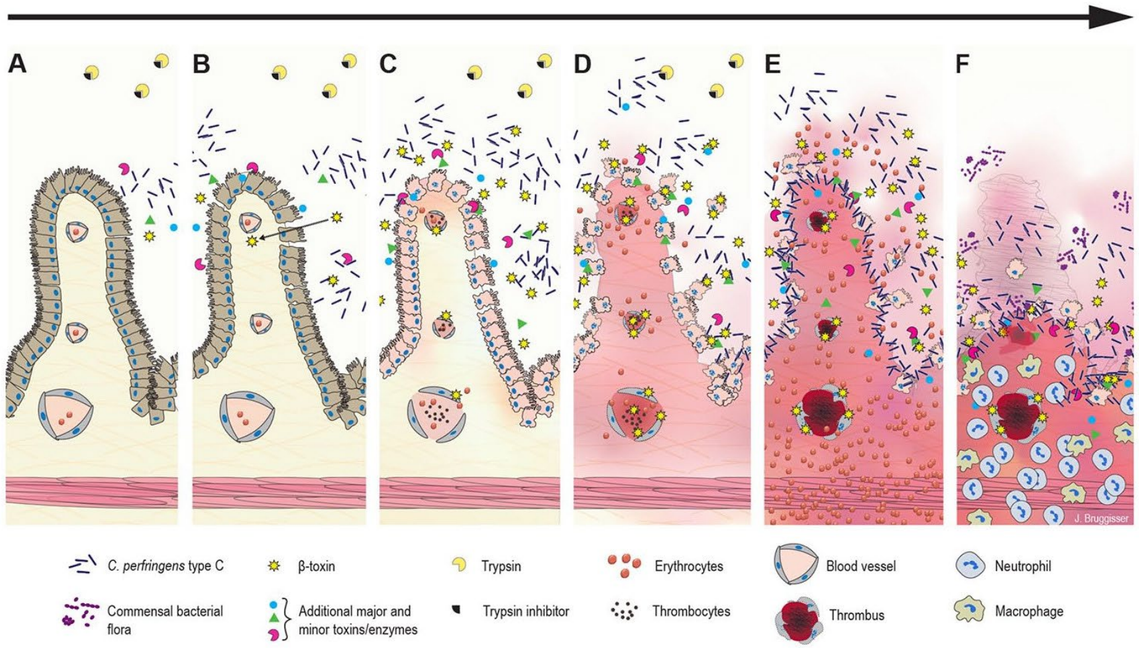




(Zaytsoff et al., 2022)

Why are predisposing factors necessary to cause NE?

- Clostridium perfringens is **opportunistic**.
- Lack of mechanisms to synthesize amino acids and other nutrients.
- When they find an environment rich in nutrients and with plenty of adhesion sites, they proliferate and cause damage to the host.



(Posthaus et al., 2020)

Control of necrotic enteritis in a post-antibiotic era

- Effective management (stocking density, water quality, litter, temperature, ventilation…)
- Biosafety practices
- Nutrition – Use of good quality ingredients and phase-feeding
- Use of feed additives aimed to enhance gut health

There is not only one single solution. It will depend on the necessities of each farm.

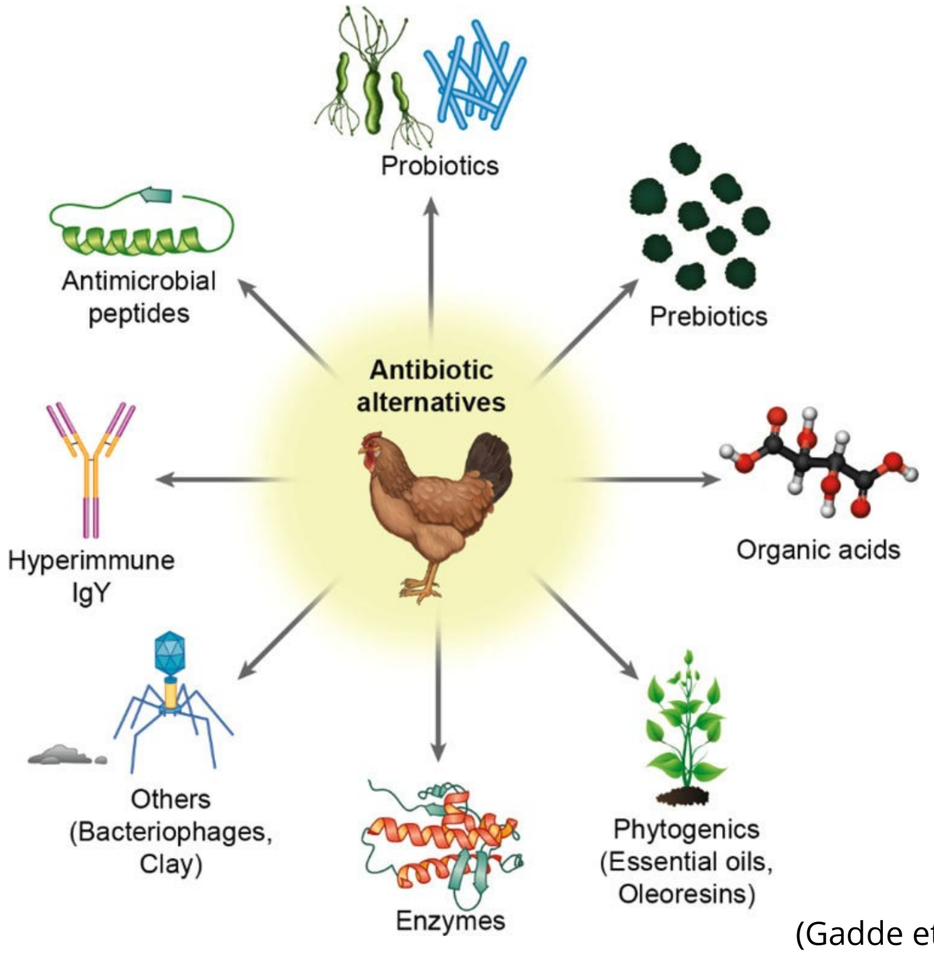
Different farms experience different challenges!

Control of necrotic enteritis in a post-antibiotic era

- Many studies have been conducted to identify functional feed additives or feedstuff that can modulate the immune system and/or the microbiota, providing similar benefits to AGP.

Limitations:

- Lack of knowledge about their mechanisms of action.
- Inconsistent results.
- No single product have been worked as well as AGP.



(Gadde et al., 2017)

Potential AGP replacements that we have been studied at the University of Alberta

- Natural feed additives focusing on the prevention of subclinical necrotic enteritis in broilers through gut health optimization.

Chitosan oligosaccharides extracted from shellfish.

Punicic acid extracted from pomegranate seed oil.

Glucosamine-derived caramels.

Products obtained from the caramelization of glucosamine.

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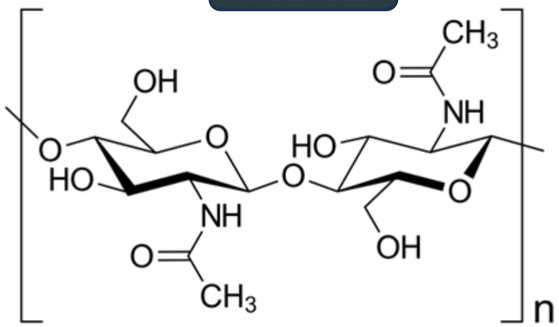
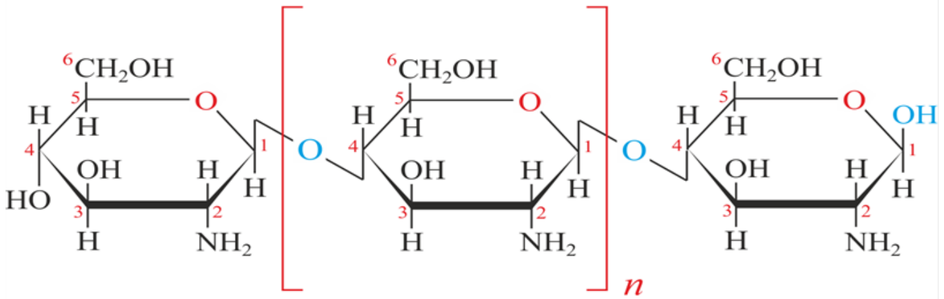
Chitosan oligosaccharides (COS) as a potential AGP replacement

- Natural, positively-charged compounds.
- Obtained from the shells of crustaceans, exoskeleton of insects and fungal cell walls.
- Depending on the molecular weight, they can exert antimicrobial, anti-inflammatory, or prebiotic properties.

Chitin



COS



β-(1,4)-D-glucosamine

(Zhou et al., 2020; Moine et al., 2021)

COS as a potential AGP replacement

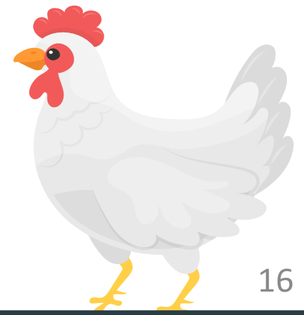
- Study 1 (pilot project): select the optimal COS molecular weight and level of inclusion in the diet able to prevent/mitigate subclinical NE in broilers and keep performance.

Treatments	COS level of inclusion
Positive Control (PC)	0 g/kg
Negative Control (NC)	0 g/kg
COS 220 kDa	0.2, 2 and 5 g/kg
COS 180 kDa	0.2, 2 and 5 g/kg
COS 110 kDa	0.2, 2 and 5 g/kg
COS 95 kDa	0.2, 2 and 5 g/kg
COS 30 kDa	0.2, 2 and 5 g/kg
COS 25 kDa	0.2, 2 and 5 g/kg
COS 17 kDa	0.2, 2 and 5 g/kg
COS 14 kDa	0.2, 2 and 5 g/kg

→ Commercial-type diet + Antibiotic + Coccidiostat

→ Commercial-type diet without any medications

→ COS medium to high molecular weights showed promising to mitigate NE gross lesions in the intestine and maintaining growth performance.



COS as a potential AGP replacement

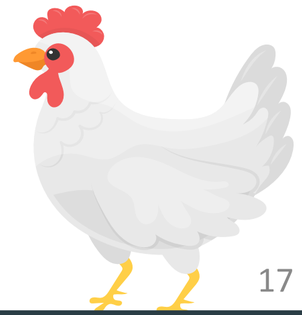
- Study 2 (large-scale experiment): further investigate the most promising treatments selected from study 1 on performance, immunomodulation, and gut health of broilers.

Treatments	Levels of inclusion
Positive Control (PC)	0
Negative Control (NC)	0
COS 180 kDa	0.2, 2 and 5 g/kg
COS 110 kDa	5 g/kg
COS 95 kDa	0.2 and 5 g/kg

→ Commercial-type diet + Antibiotic + Coccidiostat

→ Commercial-type diet without any medications

} Shellfish COS high and medium molecular weight – most promising treatments selected based on Study 1.



Natural subclinical NE infection model

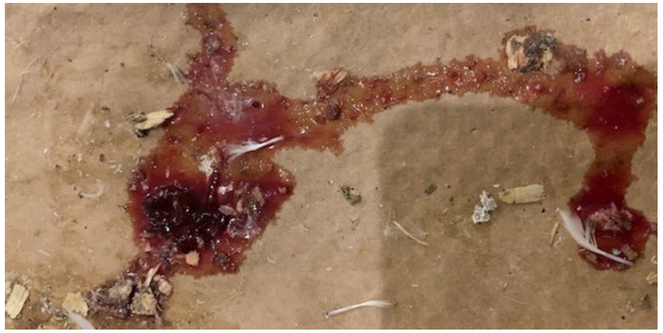
- Use of predisposing factors that stimulate intestinal dysbiosis:

  - 15x coccidiosis vaccine by gavage at 12 d

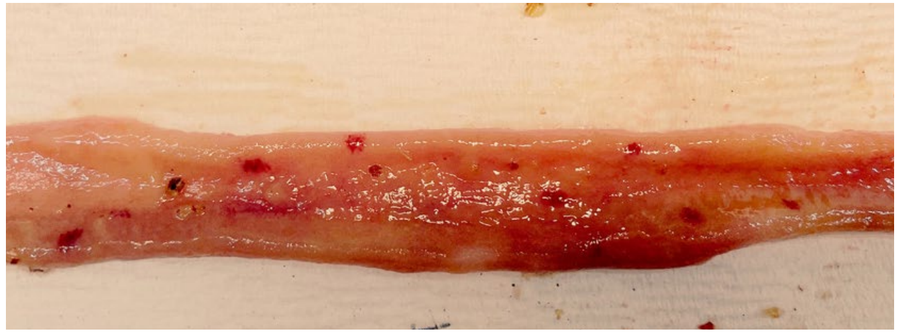
  - 24-hour feed removal at 18 d

- Natural infection with *C. perfringens* present in the barn environment.

15x Coccidiosis vaccine by gavage at 12 d



Bloody feces observed at 18 d



Intestinal lesions observed at 22 d

(He et al., 2022)

COS study: Performance results

Treatments	Inclusion g/kg	After the challenge (10-25 d)			Entire period (0-36 d)		
		BW/bird at 25 d (g)	BWG d/bird (g)	FCR (g/g)	BW/bird at 36 d (g)	BWG d/bird (g)	FCR (g/g)
Positive Control	0	879.96^{ab}	44.41^{a}	1.573^{a}	1792.5	47.1	1.688
Negative Control	0	866.83^{ab}	42.08^{ab}	1.678^{ab}	1756.4	46.35	1.765
COS 95 kDa	0.2	882.85^{ab}		.655^{ab}	1824.3		738
COS 95 kDa	5.0	904.77^{a}	+ 38 g more than NC (P = 0.09)	.642^{ab}	1838.0	+ 81.6 g more than NC (P = 0.08)	741
COS 110 kDa	5.0	880.74^{ab}		.655^{ab}	1791.3		755
COS 180 kDa	0.2	879.81^{ab}	42.75^{ab}	1.612^{ab}	1765.5	46.31	1.748
COS 180 kDa	2.0	872.55^{ab}	41.77^{ab}	1.703^{ab}	1764.3	46.2	1.777
COS 180 kDa	5.0	838.99^{b}	39.82^{b}	1.723^{b}	1733.4	44.38	1.761
SEM		12.18	0.85	0.03	25.43	0.92	0.02
P-value		0.03	0.02	0.05	0.08	0.28	0.34

COS study: bacterial abundance results (22 d)

Genus	Negative Control		COS 95 kDa		P value	FDR
	Avg relative abundance	SD	Avg relative abundance	SD		
Clostridium sensu stricto 1	0.0013	0.003	0.0005	0.001	< 0.01	0.02
Erysipelatoclostridium	0.046	0.038	0.023	0.022	< 0.01	0.02
Species						
Massiliomicrobiota timonensis	0.024	0.021	0.009	0.010	< 0.01	0.01
Clostridium colinum	0.001	0.002	0	---	< 0.01	< 0.01
Lactobacillus ingluviei	0.004	0.006	0.001	0.003	< 0.01	0.01
Lactobacillus oris	0	---	0.001	0.004	0.01	0.03

P- value for Kruskal-Wallis non-parametric test.
FDR: false discovery rate (adjusted P-value)

COS study: bacterial abundance results (22 d)

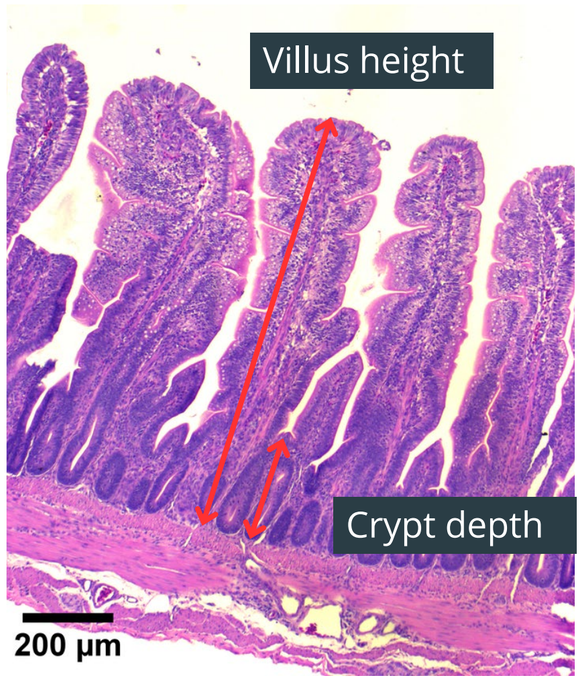
Genus	Positive Control		COS 95 kDa		P-value	FDR
	Avg relative abundance	SD	Avg relative abundance	SD		
Ruminococcus	0.001	0.002	0.0002	0.0005	< 0.01	0.01
Faecalibacterium	0.074	0.086	0.185	0.146	< 0.01	0.01
Species						
Anaerotignum lactatifermentans						
Lactobacillus oris						
Lactobacillus ingluviei						
Eubacterium sp						
Lactobacillus crispatus						

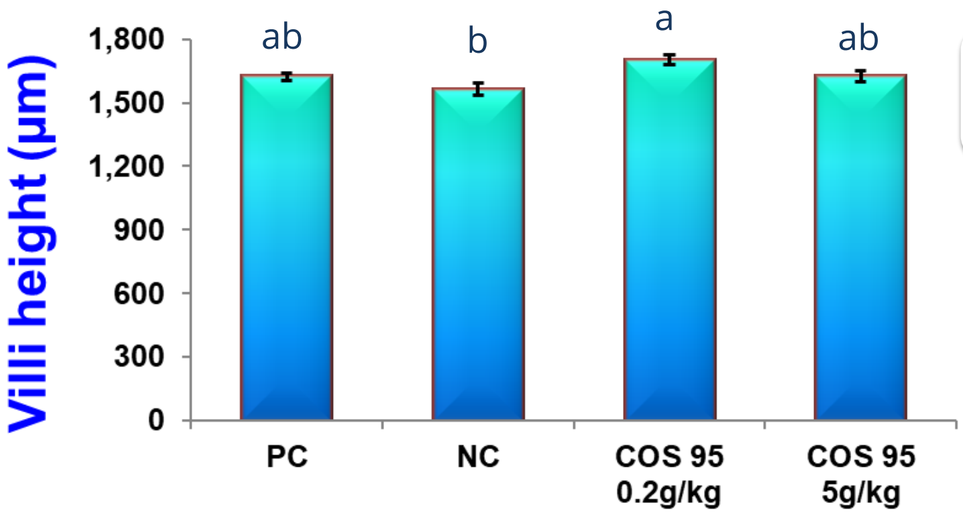
COS supplementation increased the *Lactobacillus* counts in:

- In-vitro experiments (Lee et al., 2002)
- Broiler chickens (Li et al., 2007)
- Weaned pigs (Liu et al., 2008; Yang et al., 2012)
- Humans (Simunek et al., 2012; Mateos-Aparicio et al., 2016)

P- value for Kruskal-Wallis non-parametric test.
FDR: false discovery rate (adjusted P-value)

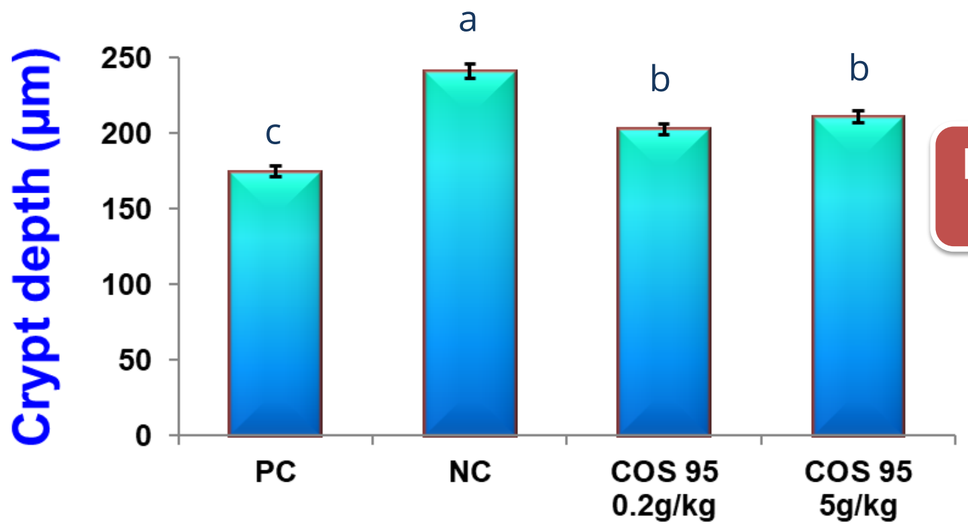
Intestinal morphology results (22 d)





NC = Lower area of absorption

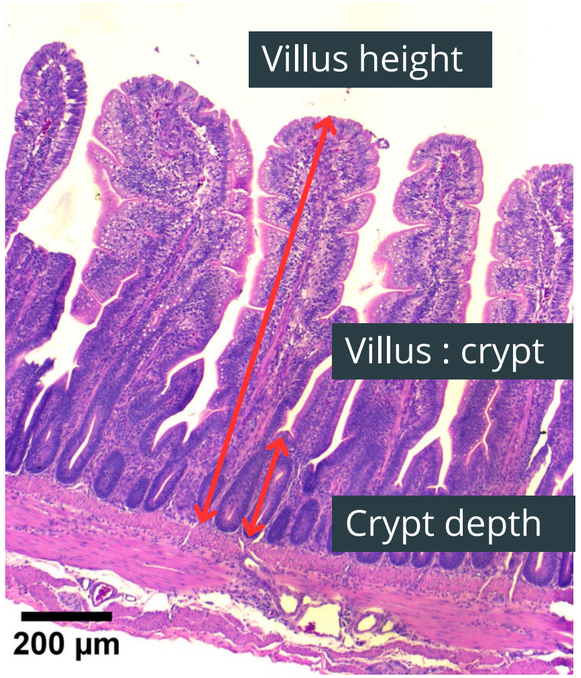
P < 0.01

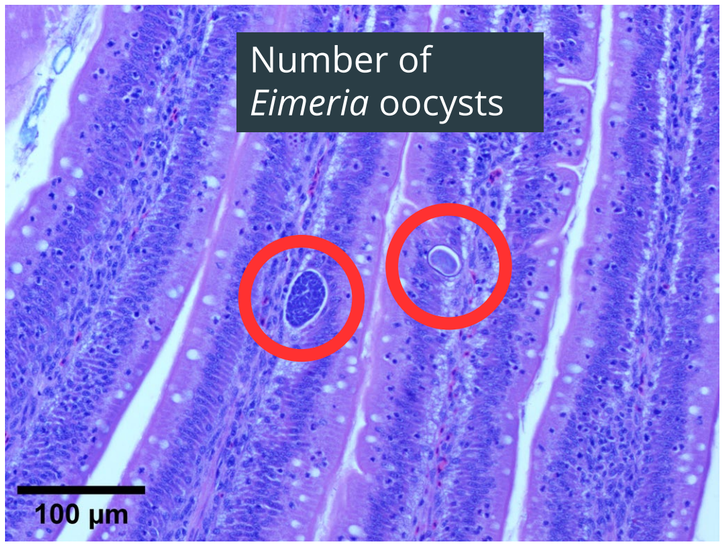


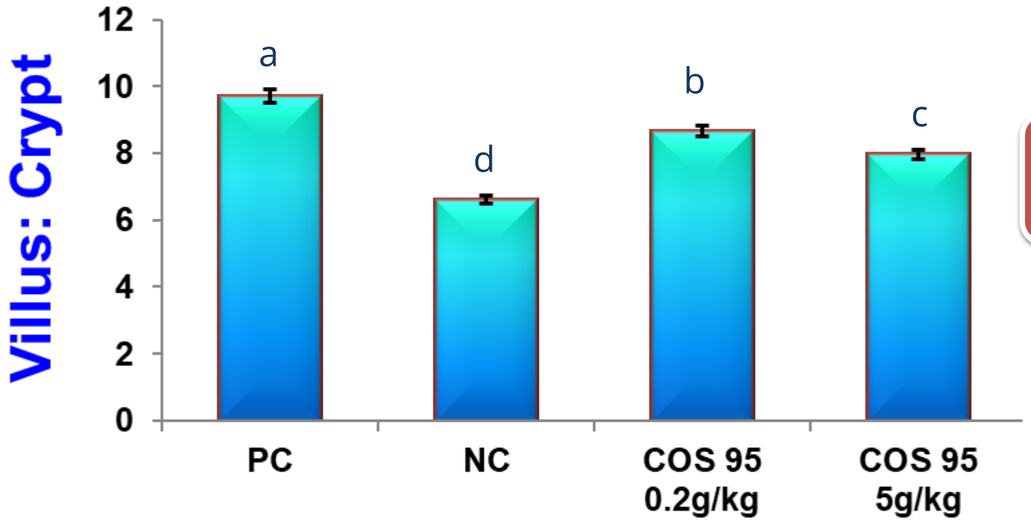
NC = The highest cellular renovation demand

P < 0.01

Intestinal morphology results (22 d)

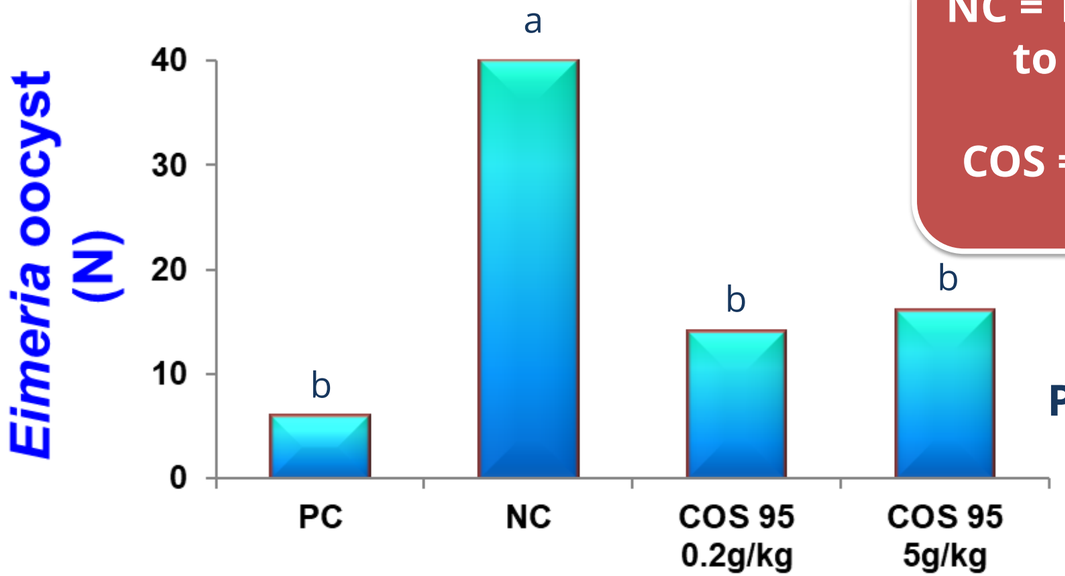






NC = Impaired normal functionality

P < 0.01



NC = The most vulnerable to *Eimeria* infection

COS = Prevented *Eimeria* infection

P < 0.01

Immune biomarkers in the serum (22 d)

Treatment	COS g/kg	Concentration (pg/mL)									
		IFNα	IFNγ	IL-2	IL-10	IL-16	M-CSF	MIP-1β	MIP-3α	RANTES	VEGF
Positive Control	0	1.53^{b}	44.94	89.15	70.55	51.80^{b}	685.71	10.41	57.30^{b}	2.05	1.52
Negative control	0	6.81^{a}	65.31	59.60	23.15	64.17^{ab}	762.65	14.57	96.02^{a}	8.08	0.43
COS 95 kDa	0.2	4.24^{ab}	49.11	47.67	43.00	84.79^{a}	744.00	14.49	99.82^{a}	6.20	1.31
COS 95 kDa	5.0	1.83^{ab}	40.14	87.60	18.58	61.30^{ab}	566.48	12.52	68.67^{ab}	2.37	0.58
SEM		0.75	3.86	9.76	15.1	3.69	46.6	0.73	6.42	1.19	0.41
P-value		0.03	0.10	0.44	0.28	<0.01	0.46	0.14	0.05	0.20	0.74

IFNα (interferon alpha), IFNγ (interferon gamma), IL-2 (interleukin 2), IL-10 (interleukin 10), IL-16 (interleukin 16), M-CSF (macrophage colony-stimulating factor), MIP-1β (macrophage inflammatory protein-1 beta), MIP-3α (macrophage inflammatory protein-3 alpha), RANTES (regulated on activation, normal T cell expressed and secreted), VEGF (vascular endothelial growth factor).

Immune biomarkers in the serum (22 d)

Treatment	COS g/kg	Pro/anti-inflammatory				Pro-inflammatory			Pro-inflammatory		
		IFNα	IFNγ	IL-2	IL-10	IL-16	M-CSF	MIP-1β	MIP-3α	RANTES	VEGF
Positive Control	0										1.52
Negative control	0										0.43
COS 95 kDa	0.2										1.31
COS 95 kDa	5.0										0.58
SEM		**0.75**	3.86	9.76	15.1	**3.69**	46.6	0.73	**6.42**	1.19	0.41
P-value		**0.03**	0.10	0.44	0.28	**<0.01**	0.46	0.14	**0.05**	0.20	0.74

- **Stimulating pro-inflammatory responses is not always a bad thing!**
- **Taking into consideration the entire context, COS may have stimulated a vigorous immune response in order to reestablish homeostasis.**

IFNα (interferon alpha), IFNγ (interferon gamma), IL-2 (interleukin 2), IL-10 (interleukin 10), IL-16 (interleukin 16), M-CSF (macrophage colony-stimulating factor), MIP-1β (macrophage inflammatory protein-1 beta), MIP-3α (macrophage inflammatory protein-3 alpha), RANTES (regulated on activation, normal T cell expressed and secreted), VEGF (vascular endothelial growth factor).

What we have concluded so far...

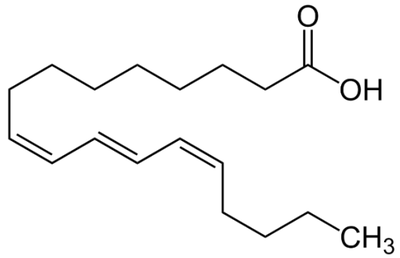
- COS 95 kDa tended to enhance performance and increased the abundance of beneficial bacteria in the ceca compared to NC birds.
- COS 95 kDa were able to mitigate the challenge effects and keep the intestinal morphology integrity at the same level as the AGP.
- COS 95 kDa stimulated a vigorous immune response, which may help to accelerate homeostasis restoration.
- COS 95 kDa may be part of a strategy to replace in-feed AGP in broilers; however, further mechanistic studies are required.

Punicic acid as a potential AGP replacement

- An unusual long chain polyunsaturated fatty acid (18:3; n-5).
- Pomegranate seed oil is the most abundant natural source of punicic acid (about 50 to 80%).
- It has gained wide attention for its range of beneficial bioactivities, including anti-diabetes, anti-obesity, antioxidant, and anti-inflammatory properties.

Pomegranate

Pomegranate seed oil



C18:3-9 *cis*, 11 *trans*, 13 *cis*

Punicic acid

(Aruna et al., 2016; Shabbir et al., 2017; Holic et al., 2018)

Punicic acid as a potential AGP replacement

- Objective: Evaluate the effects of pomegranate seed oil, high in punicic acid, as a potential AGP replacer on performance and necrotic enteritis lesion scores in broiler subjected to subclinical necrotic enteritis challenge.

Treatments	Punicic acid Inclusion (%)
Positive Control (PC)	0
Negative Control (NC)	0
NC + Punicic acid	0.1%
NC + Punicic acid	0.25%
NC + Punicic acid	0.5%
NC + Punicic acid	1%
NC + Punicic acid	1.5%
NC + Punicic acid	2%

NC + Pomegranate oil added to a final PA concentration of 0,1; 0.25; 0.5; 1; 1.5 and 2% of the feed

Natural subclinical NE infection model

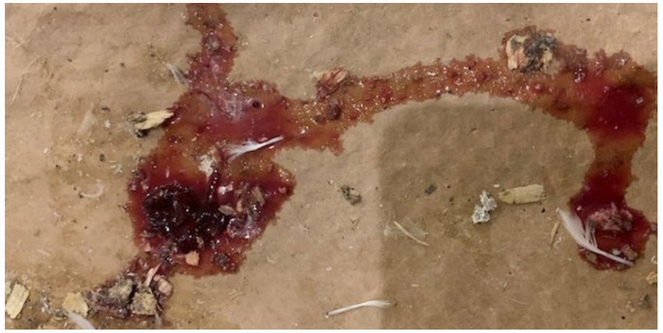
- Use of predisposing factors that stimulate intestinal dysbiosis:

  - 15x coccidiosis vaccine by gavage at 12 d

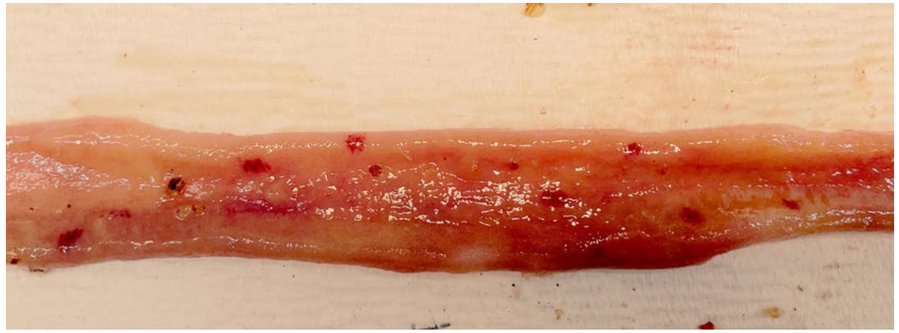
  - 24-hour feed removal at 18 d

- Natural infection with *C. perfringens* present in the barn environment.

15x Coccidiosis vaccine by gavage at 12 d



Bloody feces observed at 18 d

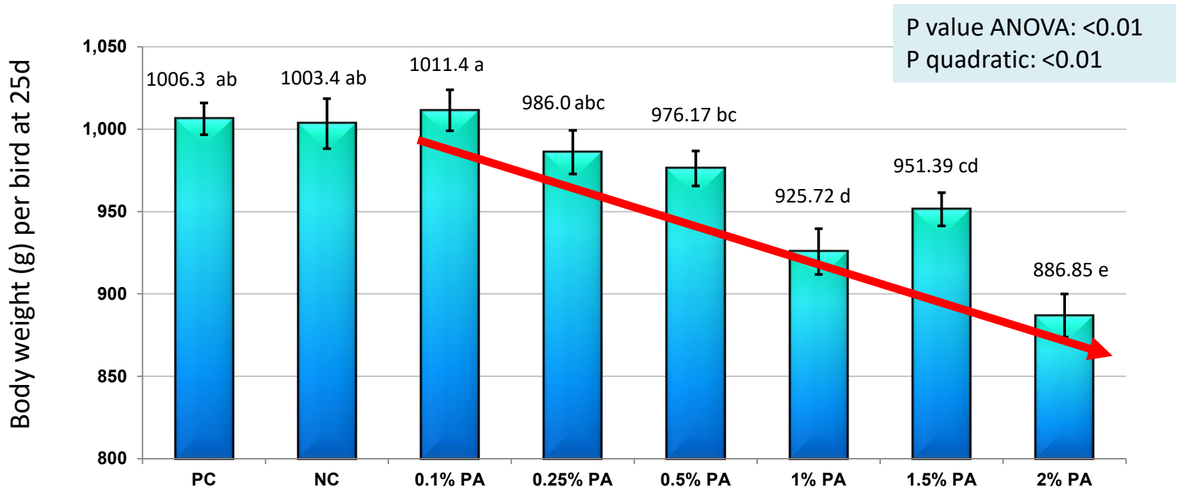


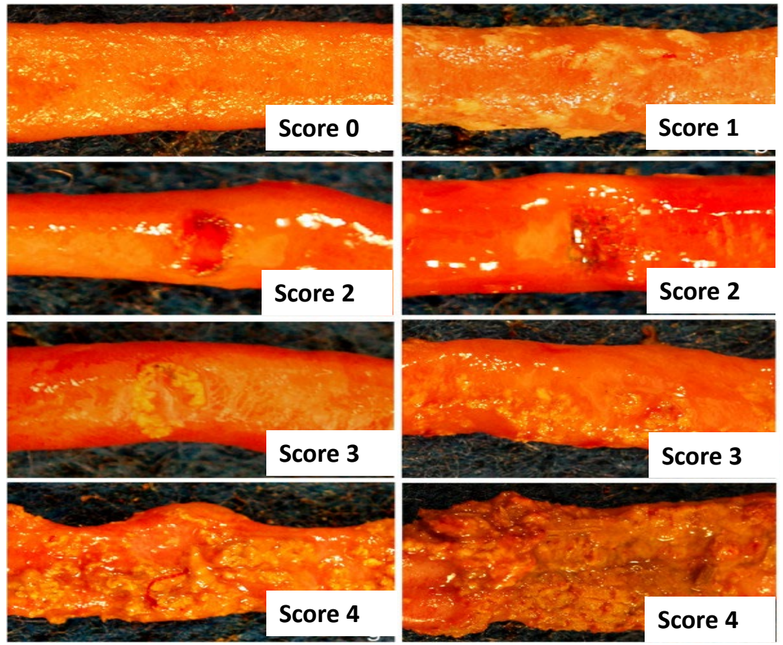
Intestinal lesions observed at 22 d

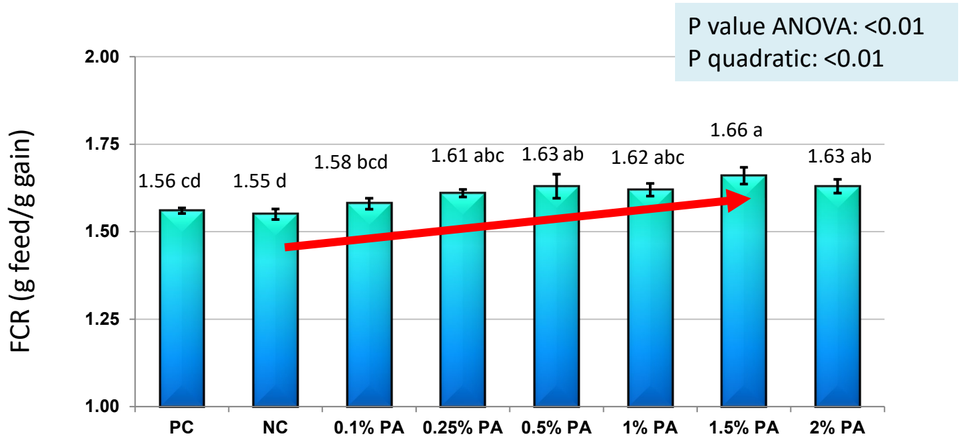
(He et al., 2022)

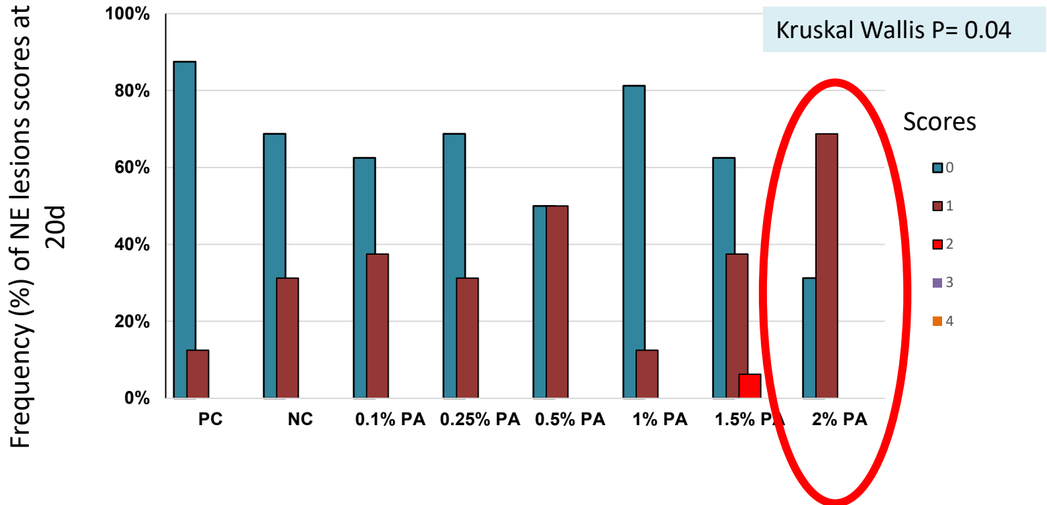
Punicic acid study: Performance and NE lesions results

- Grower phase (period of the challenge application)









What we have concluded so far…

- Punicic acid (provided through pomegranate seed oil) did not maintain performance or protect the gut health of broilers challenged with subclinical NE.
- Reductions in feed intake associated with the pomegranate seed oil properties were likely the main responsible for these results.
- Bakery yeast enriched with punicic acid is being developed at U of A as a new source of punicic acid to be tested in broiler diets as a potential AGP replacement in the control of NE.

Glucosamine caramels as potential AGP replacement

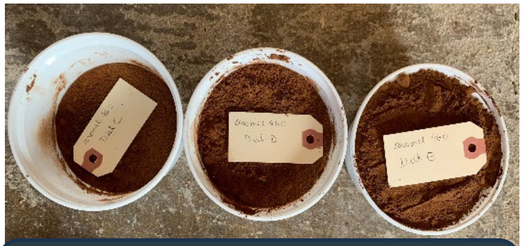
- Glucosamine is an amino sugar commonly used to prevent or relieve osteoarthritis and articular joint disease.

- It can exert anti-inflammatory properties and prevent cartilage degradation.

- Under mild temperatures, glucosamine can self-condensate and generate new compounds with stronger anti-inflammatory properties and prebiotic functions.

Glucosamine + heat

Glucosamine caramel produced at 50 °C

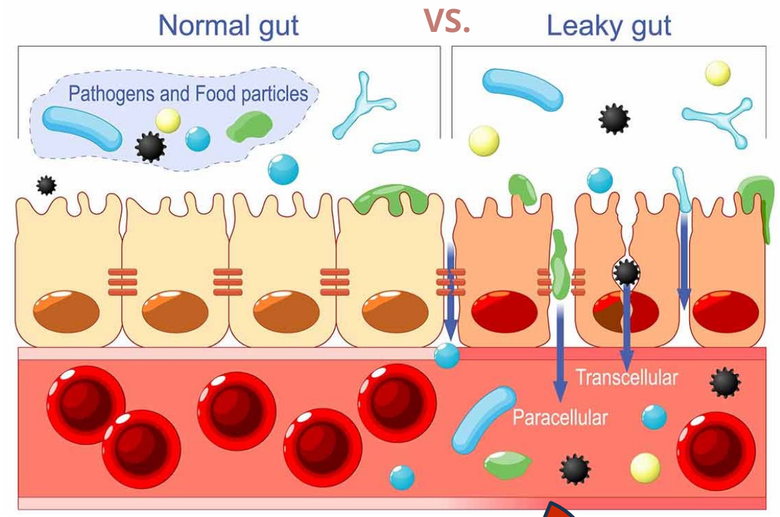


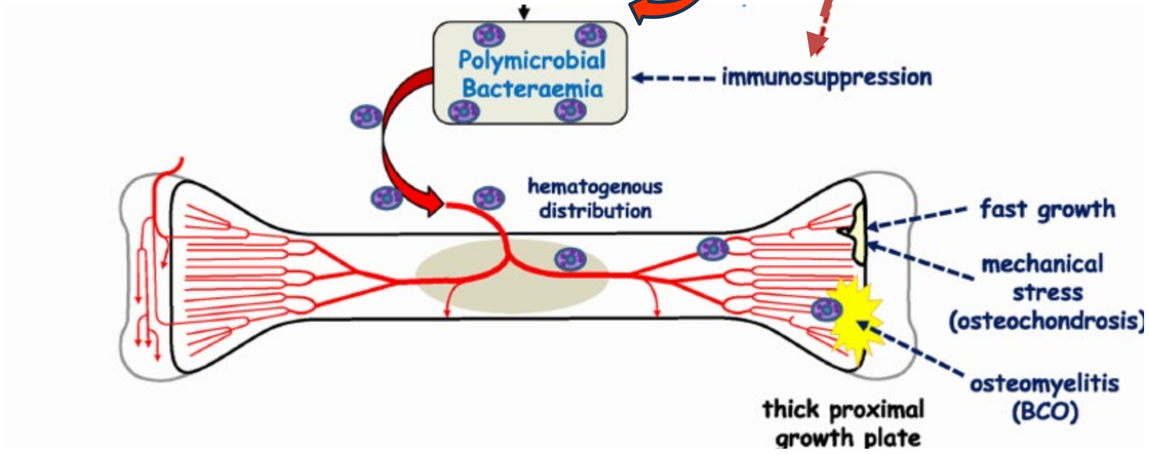
Glucosamine caramel produced at 90 °C

(Aruna et al., 2016; Shabbir et al., 2017; Holic et al., 2018)

Glucosamine study: Problem characterization

Rapid growth, high densities and low activity





(Wideman, 2015)

Glucosamine-derived caramels study

- Objective: Evaluate the potential of glucosamine-derived caramels to prevent tibial and femoral lesions caused by bacterial translocation from the lumen to the joints.

Treatments	Levels of inclusion in the diet
Commercial-type diet (Control)	0
Control + glucosamine (GlcN)	0.24%
Brown Caramel produced at 90°C (BC)	0.08, 0.16 and 0.24%
BC + Caramelized fructose (BC+F)	0.08, 0.16 and 0.24%
Light Caramel produced at 50°C (LC)	0.08, 0.16 and 0.24%
LC + Caramelized fructose (LC+F)	0.08, 0.16 and 0.24%

Glucosamine-derived caramels study: Performance results

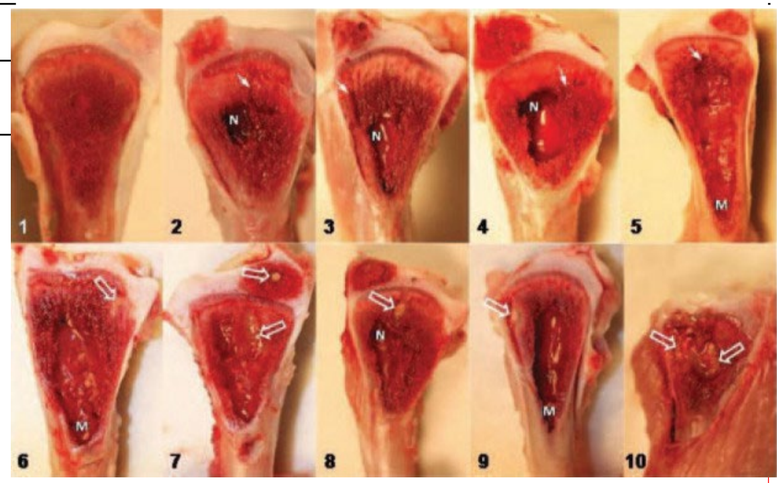
Treatment	Inclusion (%)	BW at 38 d(g)	BWG (0-38 d) day/bird (g)	FI (0-38 d) day/bird (g)	FCR (0-38 d) (g/g)
Control Diet	0	1910.30^{abcd}	45.67^{abcd}	71.53	1.569
Control+GlcN	0.24	1981.50^{ab}	47.40^{ab}	70.54	1.493
Brown caramel	0.08	1976.30^{abc}	47.27^{abc}	72.47	1.531
	0.16	1823.00^{cd}	43.55^{cd}		
	0.24	1996.10^{ab}	47.77^{ab}		
BC + Fructose	0.08	1913.50^{abcd}	45.76^{abcd}		
	0.16	1823.10^{cd}	43.54^{cd}		
	0.24	1985.00^{ab}	47.48^{ab}		
LC + Fructose	0.08	1797.30^{d}	42.91^{d}		
	0.16	1961.00^{abc}	46.89^{abc}		
	0.24	1938.60^{abcd}	46.33^{abcd}		
Light Caramel	0.08	1885.10^{bcd}	45.06^{bcd}		
	0.16	2043.50^{a}	48.91^{a}		
	0.24		48.00^{ab}		
SEM			1.34	2.28	0.03
P-value			0.04	0.34	0.90

+133.2 g heavier than the Control

In a pairwise comparison with the Control, Light Caramel included at 0.16% tended to increase the BW and BWG of broilers from 0 to 38 d (P = 0.08).

Glucosamine-derived caramels study: Bone lesion results

Treatments	Dietary inclusion (%)
Control Diet	---
Control+GlcN	0.24
Brown caramel	0.08
	0.16
	0.24
BC + Fructose	0.08
	0.16
	0.24
LC + Fructose	0.08
	0.16
	0.24
Light caramel	0.08
	0.16
	0.24
SEM	
P-value[7]	



Treatments with lower tibial lesions than the Control in a pairwise comparison (P= 0.02 and 0.03, respectively).

What we have concluded so far…

- Glucosamine-derived caramels produced at mild temperatures are safe to be used in broiler diets.
- Light caramels (produced at 50°C) rich in anti-inflammatory compounds demonstrated the potential to improve broiler performance and ameliorate femoral and tibial lesions caused by translocated bacteria.
- The next step will be the investigation of the caramels' effects on gut health for a more accurate conclusion about the products.

Future perspectives on NE control

Due to the complexity and multifactorial aspects of the disease, we still have a lot to understand about its pathogenesis.

Consistent success in antibiotic-free production will depend on a combination of products with different mechanisms of action, **plus** effective management and biosafety practices.

“No size fits all.” Each farm and flock are different. We need to investigate each case and develop customized solutions.

Ally science and technology - Use of technologies and AI to help us make early decisions on farms.

Thank you!

Questions?