Vaccination as a tool in the battle against Necrotic Enteritis.

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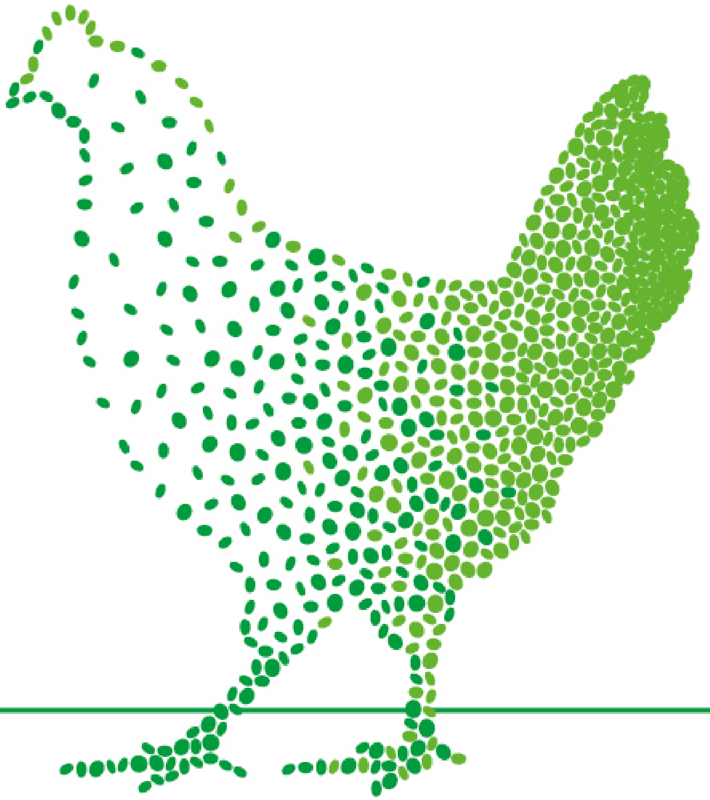
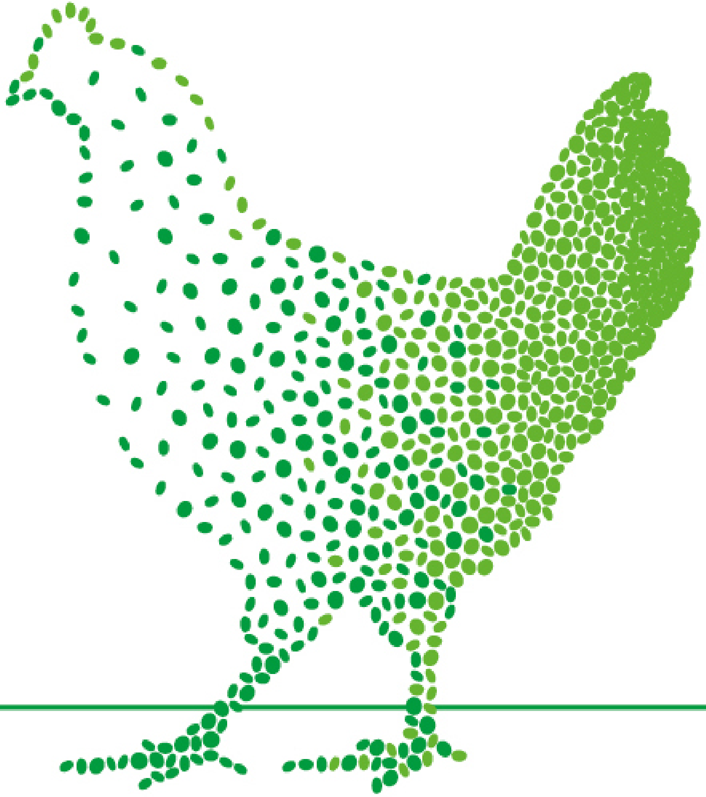
I. What is NE & Clostridium perfringens
II. NE Control Measures
III. Clinical Efficacy
IV. Canadian Field Performance



Summary of presentation

I. NE & Clostridium perfringens



Clostridium perfringens

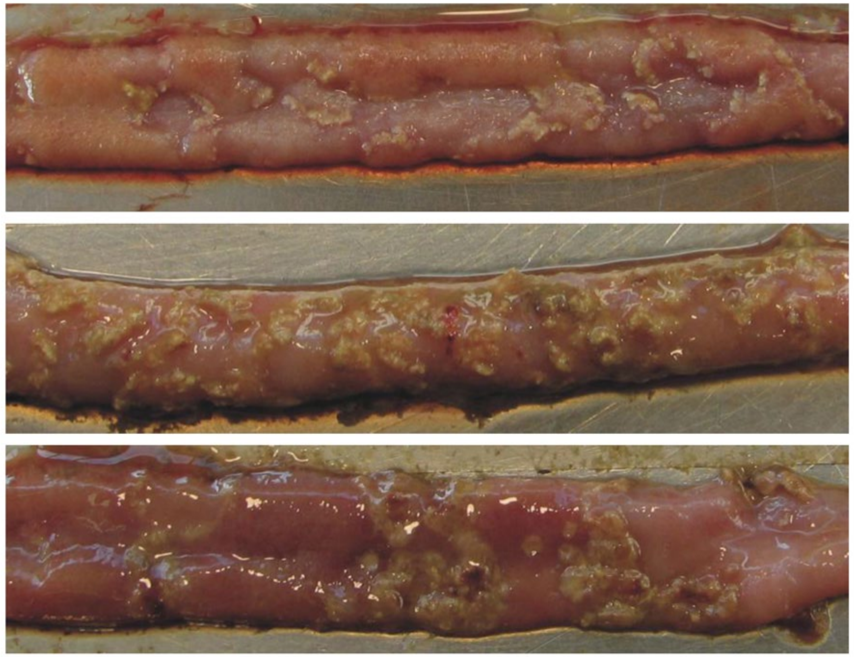
Intestinal damage of all sorts (nutrition, *Eimeria spp.*, stress, etc.)

↓

Clostridium perfringens flourishes and produces virulence factors (toxins)

↓

Toxins cause NE and associated performance loss, mortality



Auxotrophic, Anaerobic
(in need of 16 essential amino acids to stay alive)
Ubiquitous
(soil, dust, feed, intestinal tract and used poultry litter)

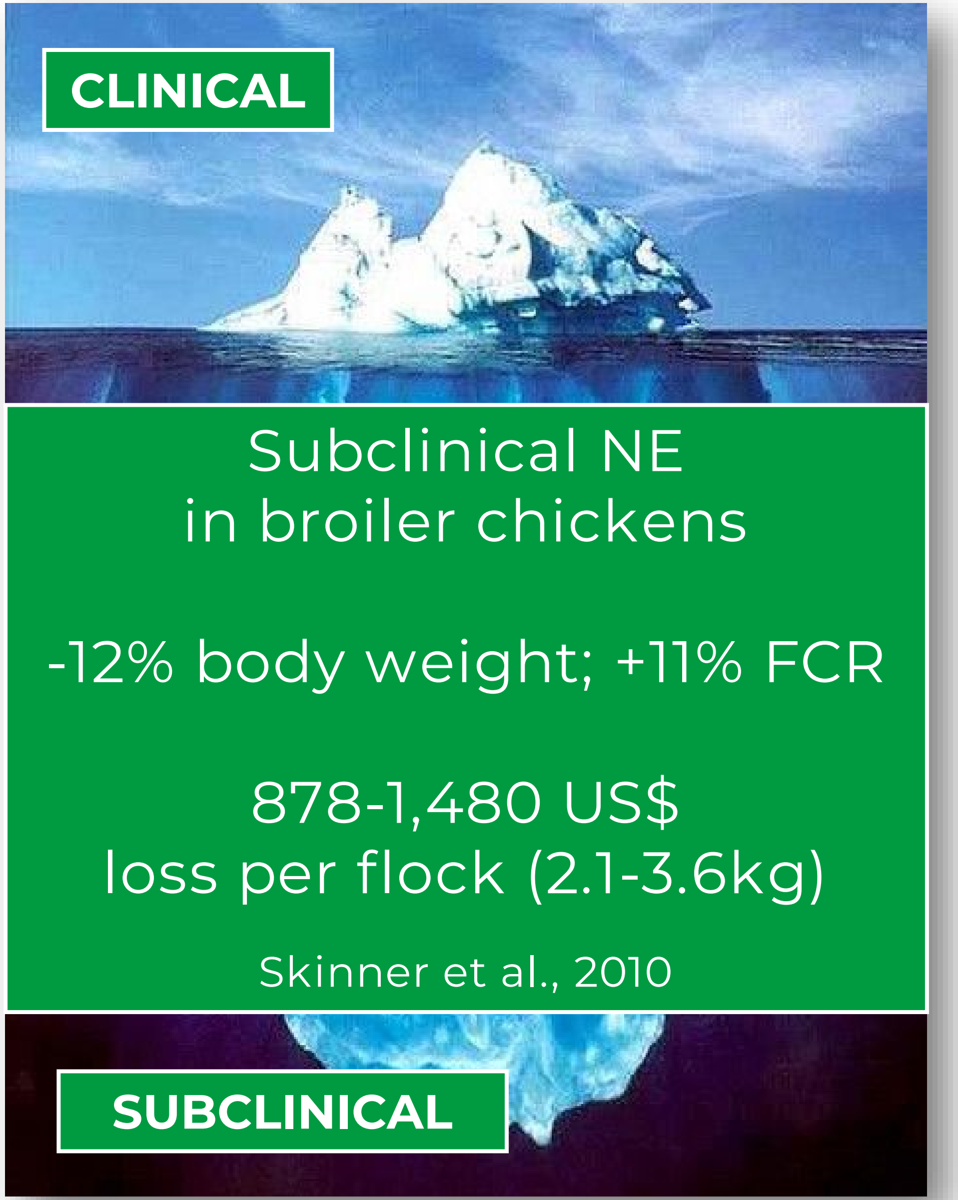
Prof. R. Ducatelle, 2021; Shimizu et al., 2002; Keyburn et al. 2012

Clostridium perfringens
Cause of “NE”

NE has major economical impact to the broiler industry globally

2 presentations:
- Clinical/acute: increased mortality (up to 10-40%)
- Subclinical: poor productivity (reduced growth, reduced feed efficiency)

Jiang et al., 2015



Necrotic Enteritis major virulence factors

“Necrotic enteritis and the subclinical form of *Clostridium perfringens* infection in poultry are majorly caused by ***Clostridium perfringens* type A**”

Van Immerseel et al., “Clostridium perfringens in poultry: an emerging threat for animal and public health”, *Avian Pathology (December 2004) 33(6):537-/549*, 2004

Clostridium perfringens type A produces **toxins** that are associated with NE lesions and disease symptoms.

Songer J.G., “Clostridial enteric diseases of domestic animals”*, Clinical Microbiology Reviews, Apr. 1996, p. 216–234*, 1996

Alpha toxin (membrane active phospholipase)

For many years considered the major toxin associated with NE in broilers

Titball et al., “The *Clostridium perfringens* alpha-toxin”, *Anaerobe* 5:51-64, 1999

+

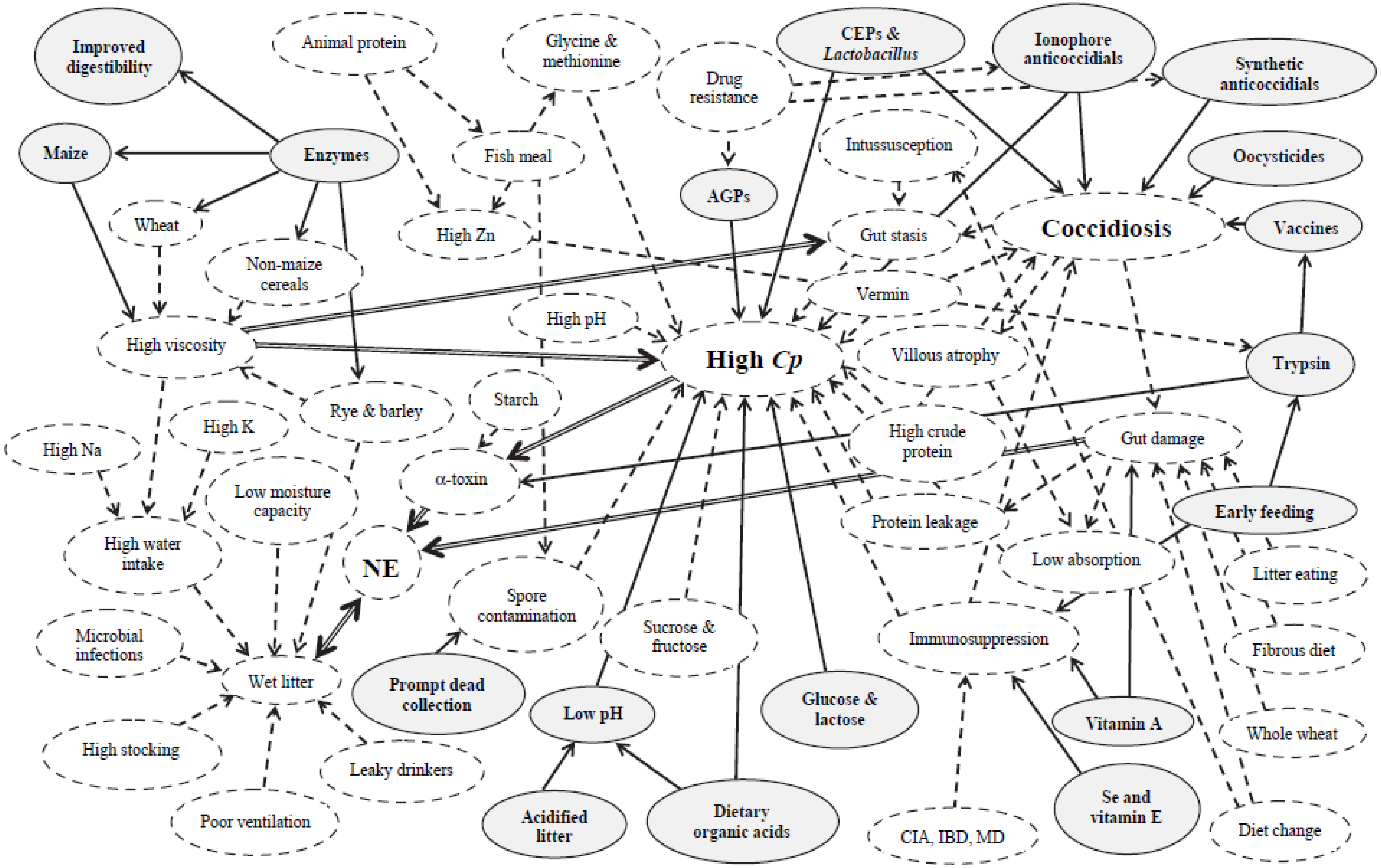
NetB toxin (pore-forming toxin)

More recent studies identified this critical virulence factor for the development of NE in broilers

Lepp et al., “Identification of novel pathogenicity loci in *Clostridium perfringens* strains that cause avian necrotic enteritis”, *Plos One* 5:e10795, 2010

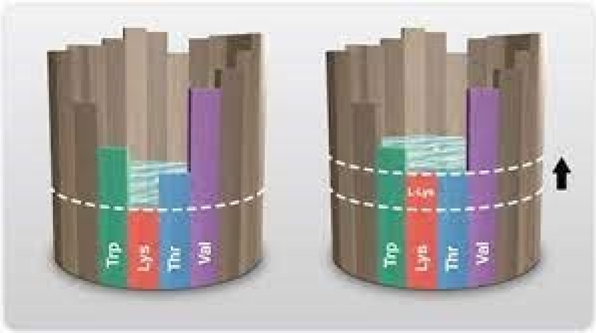
Source: USAHA Poultry Report

Necrotic Enteritis – Complex disease



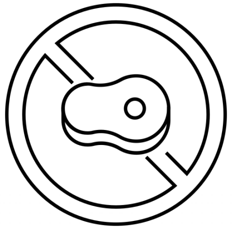
Williams 2005

Formulation tools to help limit NE



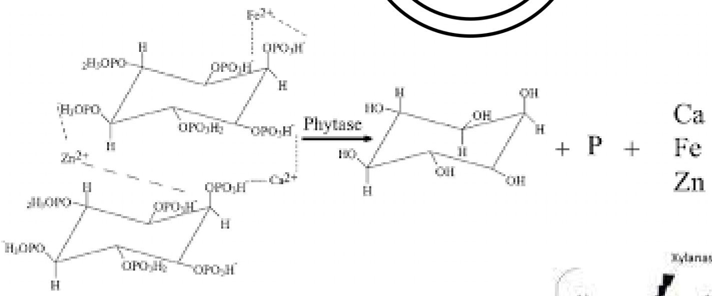
Auxotrophic – require amino acids

- ↓ crude protein (ideal protein balance with synthetic AAs)
- Use more digestible raw materials



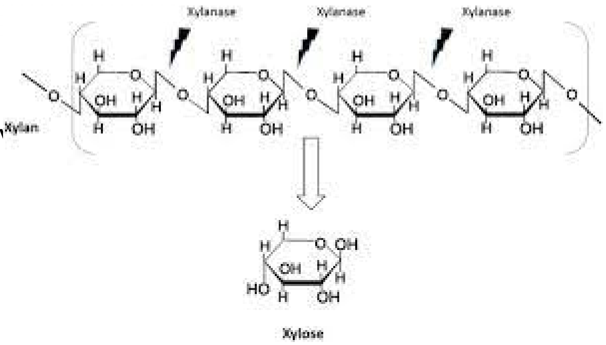
Collagen adherence locus (NetB toxin)

- ↓ use of animal by-products (e.g. fishmeal, meat and bone meal)



Ca-dependent phospholipid binding (alpha-toxin)

- ↓ Ca levels (move to available Ca system)
- Use phytase with Ca/P matrix values



Digesta viscosity/NSPs (increase viscosity, mucus production and bacterial gro

- ↓ wheat, barley and rye inclusion and include NSPase enzyme

Management factors influencing NE

- Eimeria – predisposing factor for NE due to tissue damage
- Physiological stress increases NE risk (Zaystoff et al. 2020)
- Heat stress – weakens tight junctions
- Wet litter – increases risk for Eimeria cycling (esp. in vaccinated flocks)
- Stocking density (high increases risk)
- Litter type (low moisture absorption potential)
- Ventilation (management of temperature and litter moisture)
- Watering lines (leaky nipples increase litter moisture)
- Insect control (darkling beetles as vector)

Conventional NE control (Canada)

- Medicinal
  - Bacitracin (as bacitracin methylenedisalicylate)

Broiler chickens	1. Reduction of early mortality due to diminished feed consumption and chilling. 2. Prevention of necrotic enteritis	0 days	1. BMD 110 G 2. Bacitracin MD

Medications target the bacteria

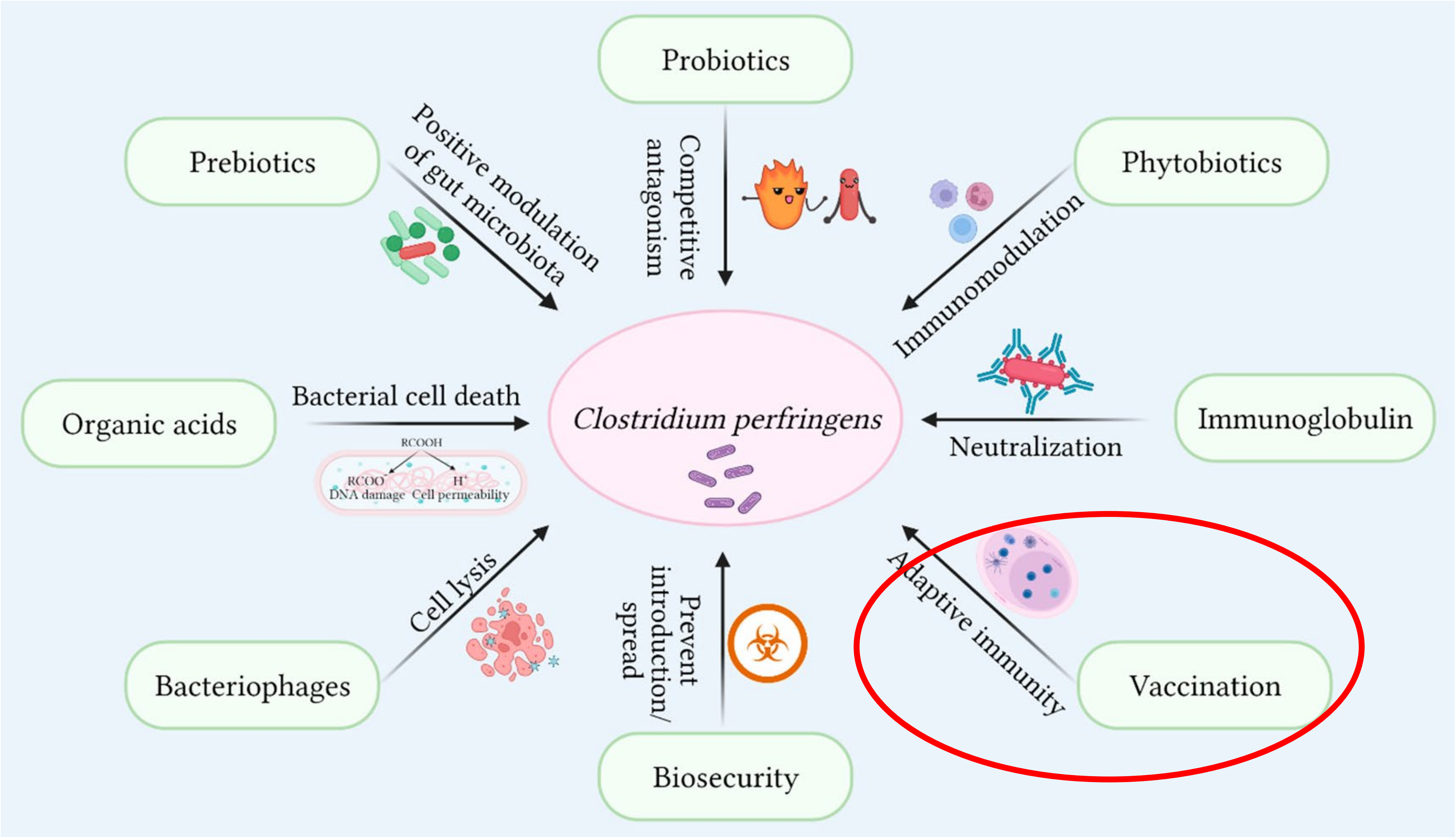
  - Avilamycin

Broiler chickens	1. Prevention of necrotic enteritis	0 days	Surmax Premix

CFC goal to reduce use of preventative Cat. III antibiotics

https://www.chickenfarmers.ca/faq/how-are-antibiotics-categorized-and-why-does-it-matter/

Non-antibiotic NE control strategies



Fathima et al. 2022

Novel Approach: *CLOSTRIDIUM PERFRINGENS* TYPE A VACCINE

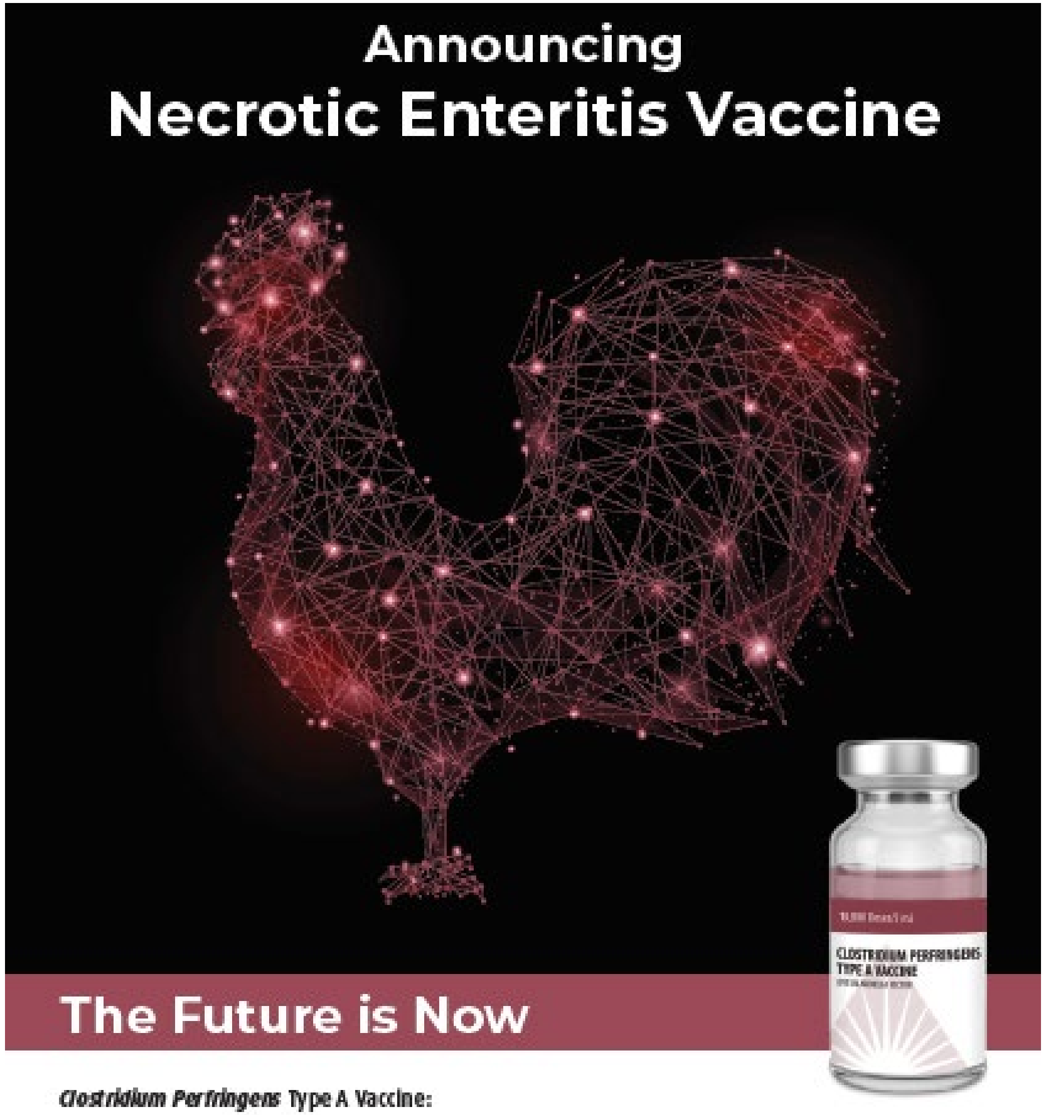
HUVEPHARMA®

CLOSTRIDIUM PERFRINGENS TYPE A VACCINE®, LIVE SALMONELLA VECTOR.

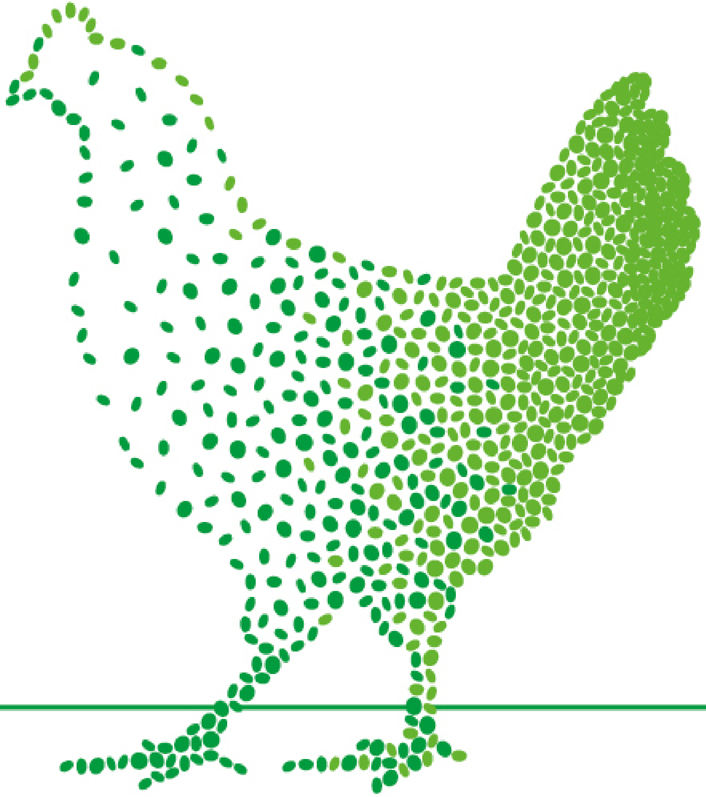
The vaccine is a **R**ecombinant **A**ttenuated **S**almonella **V**accine (RASV) expressing *Clostridium perfringens* genes coding for α-toxin & NetB toxin.

The vaccine has been shown to be effective for the vaccination of healthy chickens day of age (and older) against necrotic enteritis due to *Clostridium perfringens* type A.

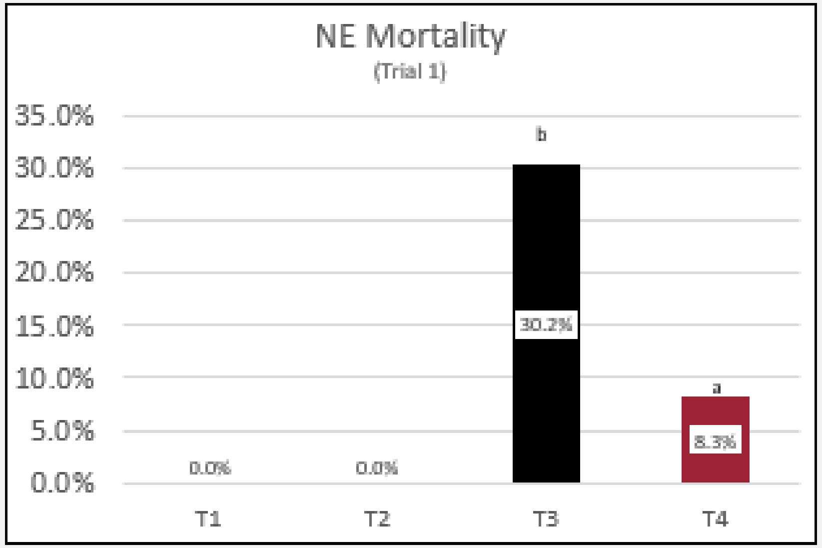
Gives protection against NE by stimulating mucosal antibodies (IgM, IgA, & IgY) against 2 major toxins: α toxin and

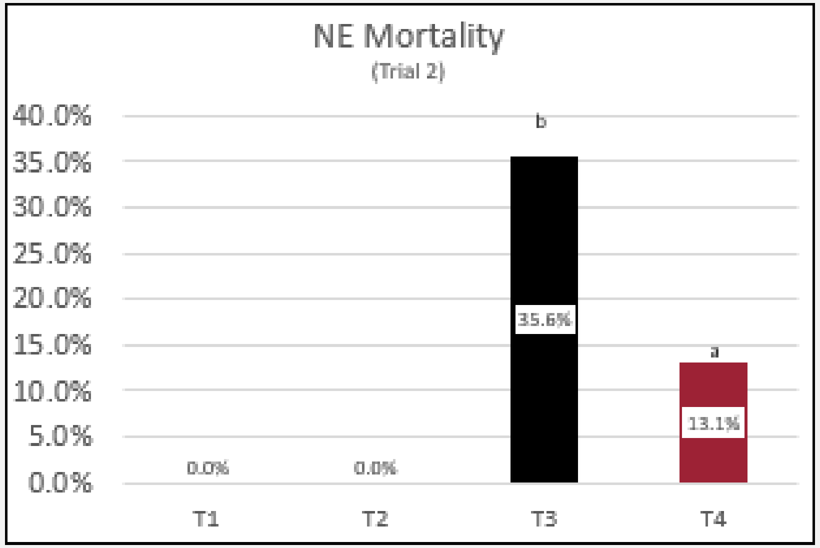


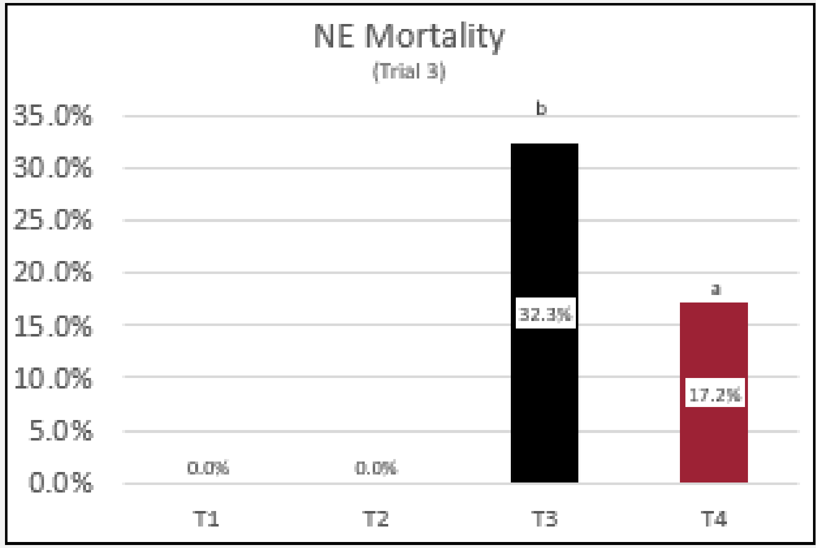
III. Efficacy – Clostridium perfringens Type A vaccine



Efficacy: Pivotal Trials





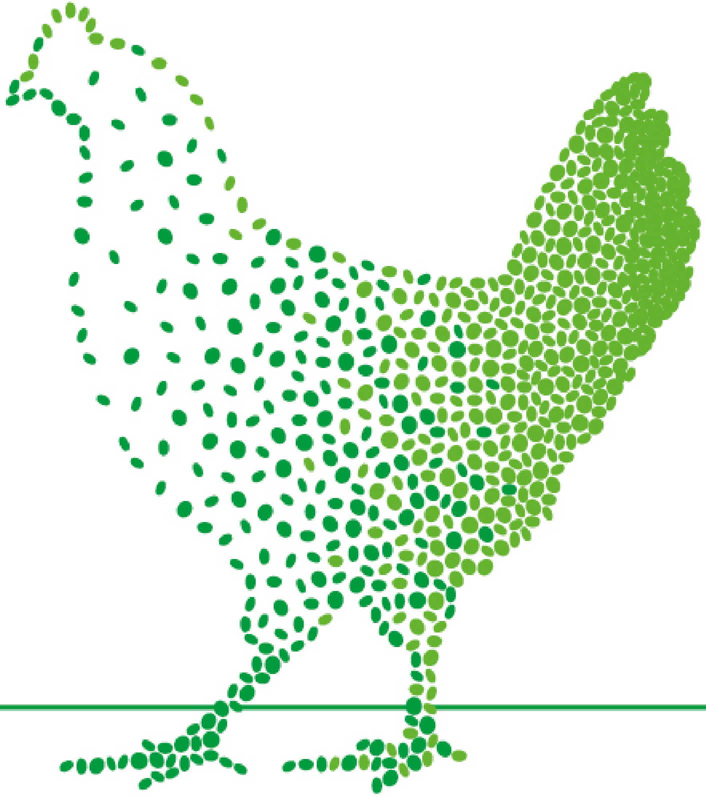


CLOSTRIDIUM PERFRINGENS TYPE A VACCINE reduces mortality due to necrotic enteritis.

Treatment Group	Vaccination	Eimeria Challenge	*C. Perfringens* Challenge
T1	None	None	None
T2	None	*E. maxima* D14	None
T3	Placebo	*E. maxima* D14	D19-21 or D19
T4	Cp-01	*E. maxima* D14	D19-21 or D19

Study Nos. USA-18-01-018 (slide 36-37), CHC-19-019 (for further information see slide 39-40), CHC-19-010-P (slide 42-43), Cp-01 = CLOSTRIDIUM PERFRINGENS TYPE A VACCINE

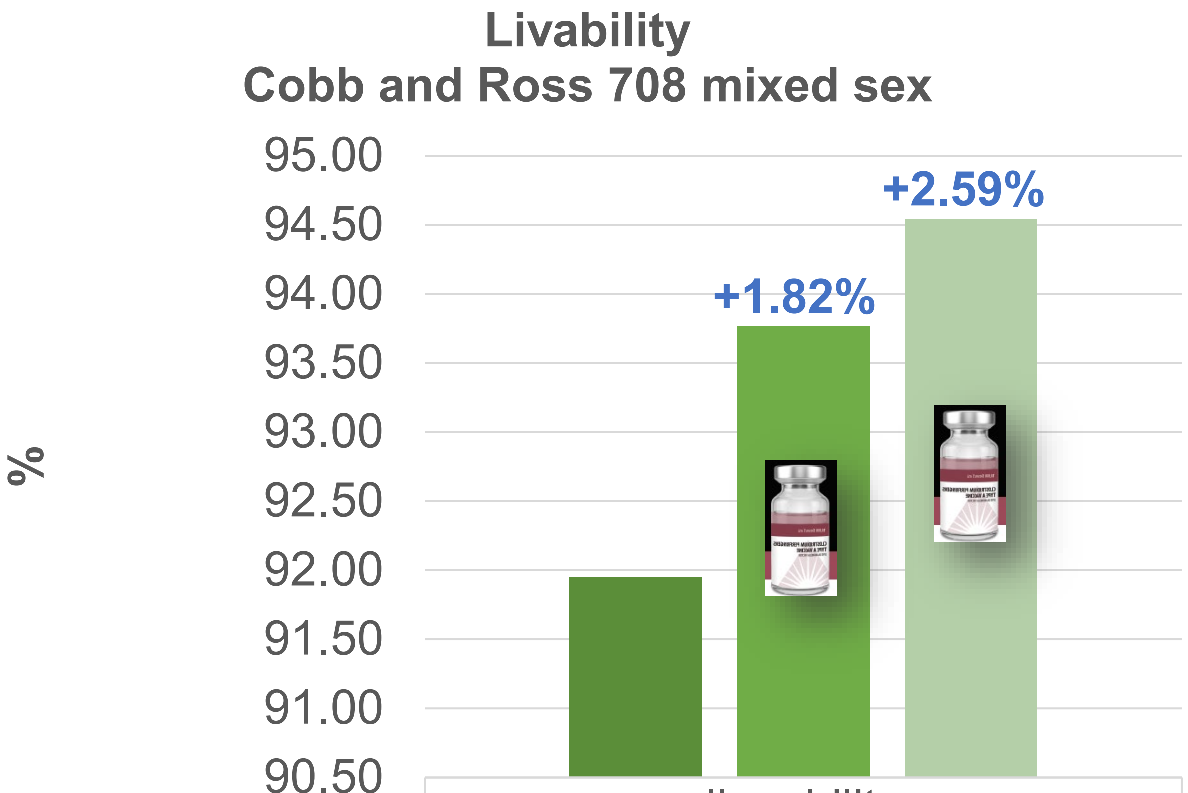
IV. Canadian Field Performance Trials



Birds and treatments (Dec 2021) - ON

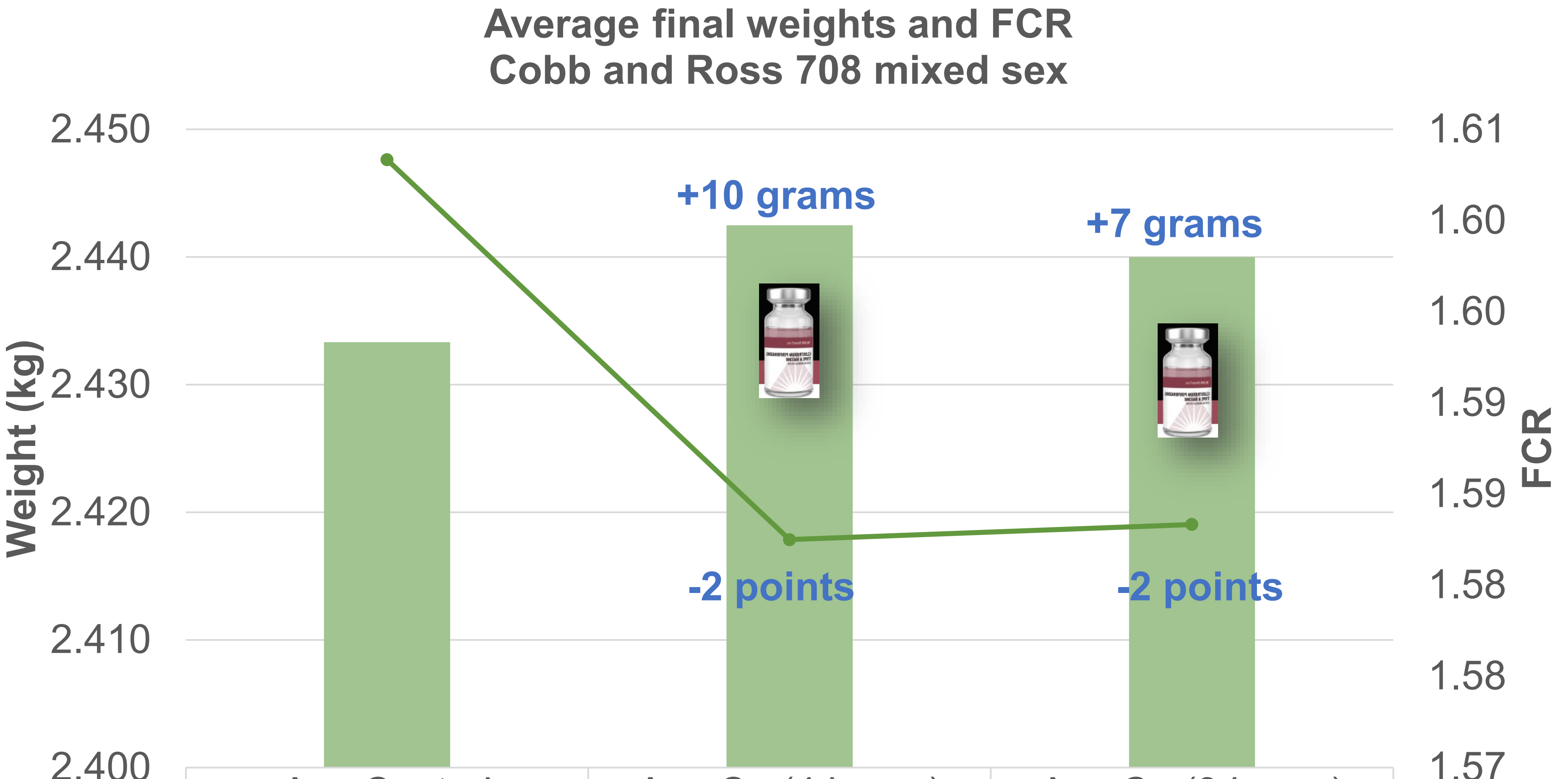
- Cobb mixed sex (33 days) = **total 47,000**
- Ross 708 mixed sex (37, 38 or 40 days) = **total 52,300**
- Control barns: 3, spread over 3 farms
  - Every control barn had a corresponding test barn on the same farm
- Test barns: 4, spread over 4 farms (**no in-feed ABs**)
  - Every test barn had a corresponding control barn on the same farm, and there was one extra test barn on another farm
- All chicks were vaccinated at the hatchery with:
  - Marek's
  - Bronchitis (bivalent, Massachusetts and Connecticut)
  - Coccidiosis
- Treated chicks additionally vaccinated with Clostridium perfringens Type A vaccine (spray at hatchery)

Results average livability of control and treatment (Cp)



	liveability
Avg Control	91.95
Avg Cp (3 barns)	93.77
Avg Cp (4 barns)	94.54

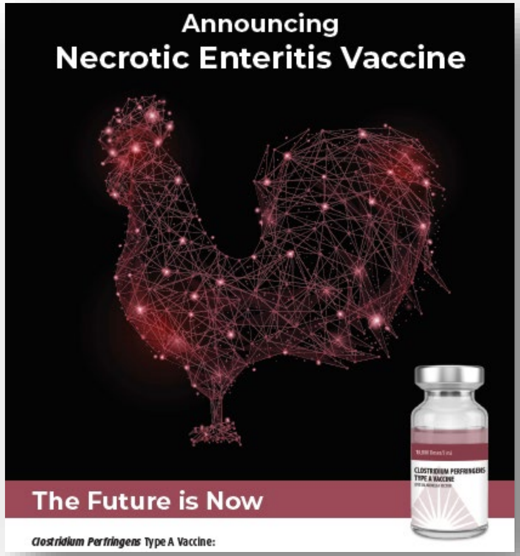
Results final average weights and FCR of control and treatment (Cp)



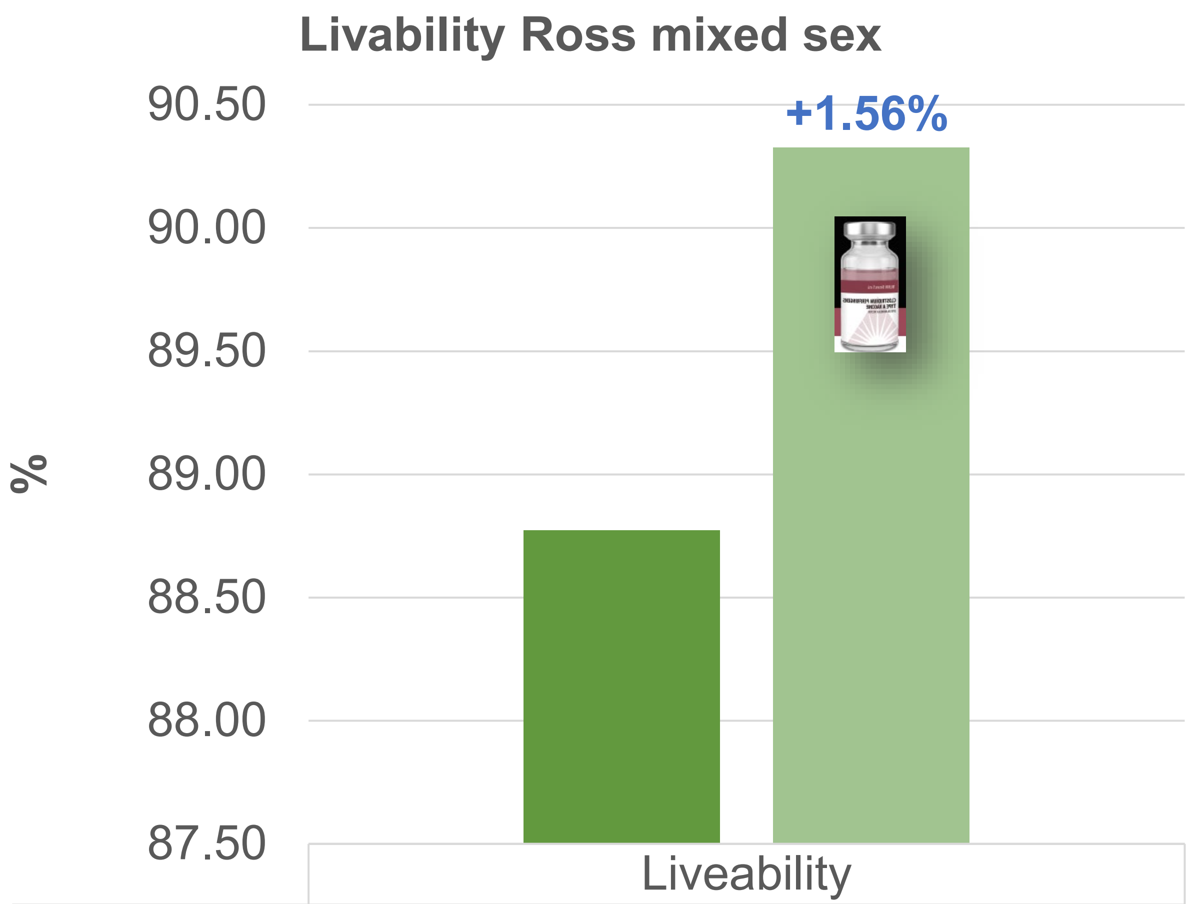
	Avg Control	Avg Cp (4 barns)	Avg Cp (3 barns)
weight	2.433	2.443	2.440
FCR	1.60	1.58	1.58

Birds and treatments (Nov ‘21-Jan ‘22) - BC

- Ross 308 mixed sex (37-47 days)
- Control: 15 sides of a barn (or top/bottom floor)
    - Every “control” had a corresponding “test” in the same barn
    - **Total = 220,697**; average per location = 14,713
- Test barns: 15 sides of a barn (or top/bottom floor) – **no in-feed ABs**
    - Every “test” had a corresponding “control” in the same barn
    - **Total = 217,214**; average per location = 14,481
- Continuous flow system with many challenges like ILT, IBV, which continuously induce elevated mortality
- All chicks were vaccinated at the hatchery with:
    - Marek’s
    - Bronchitis
- Treated chicks were additionally vaccinated with Clostridium perfringens Type A vaccine (spray at hatchery)
- No clinical necrotic enteritis was found

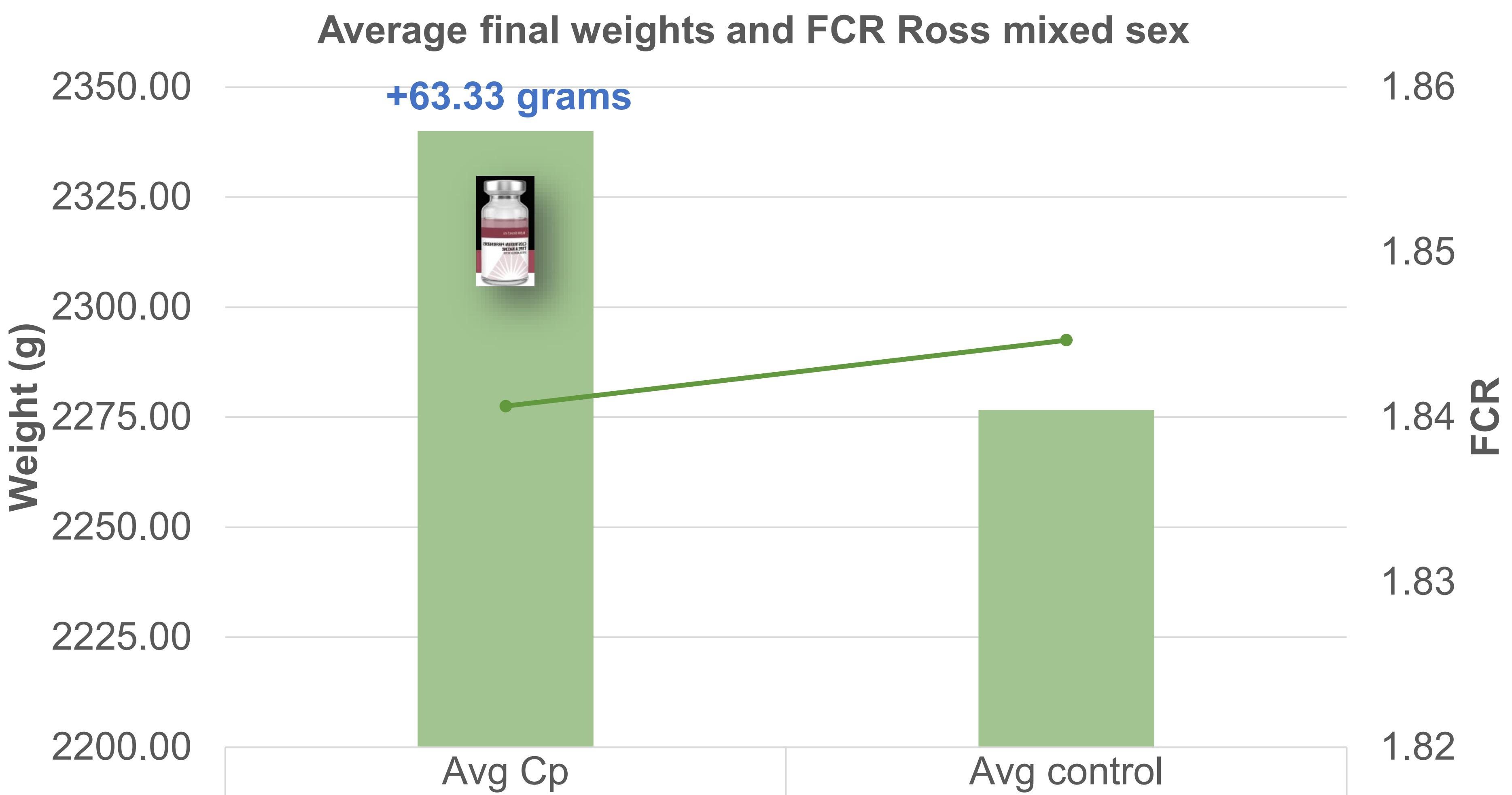


Results average livability of control and treatment (Cp)



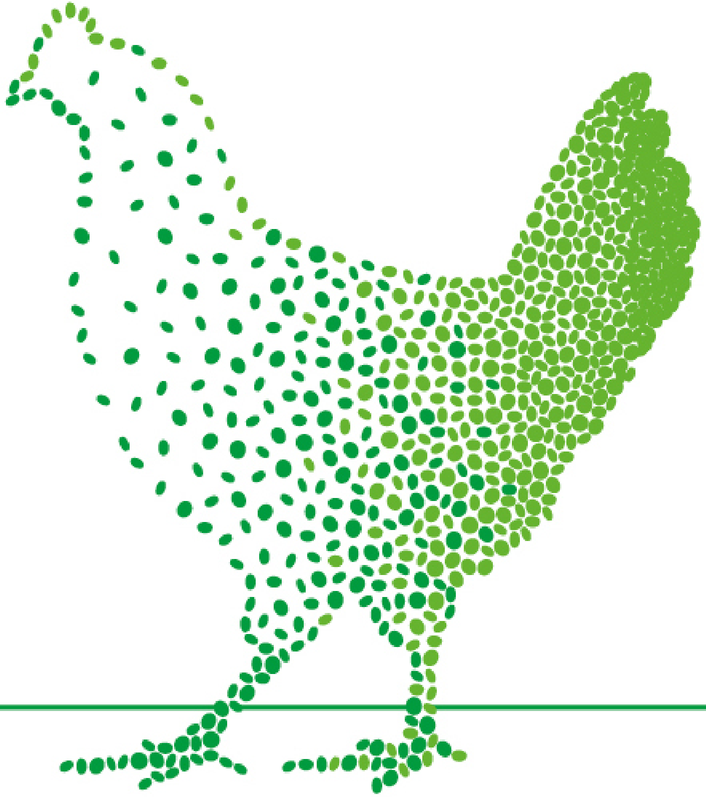
	Liveability
Avg control	88.77
Avg Cp	90.33

Results final average weights and FCR of control and treatment (Cp)



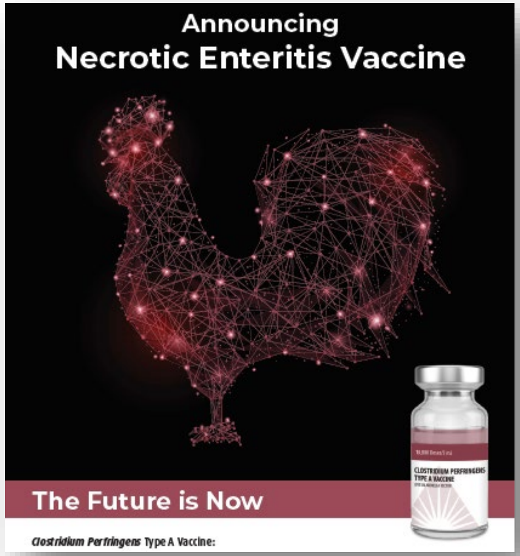
	Avg Cp	Avg control
Weight	2340.00	2276.67
FCR	1.84	1.84

Ontario Field Trial – 2022 Clostridium perfringens Type A vaccine



Birds and treatments (Nov ‘21-Oct ‘22) – ON & AB

- Ross 708 mixed sex (37-43 days)
- Total of 67 RWA flocks in analysis (including 16 as pre-study controls).
    - Data includes 43 vaccinated flocks 1.37M birds) and 24 non-vaccinated (0.86 M birds).
    - Selected farms with history of NE breaks and/or variable performance issues



- Control chicks had standard vaccinations at the hatchery (including flocks using cocci vaccination).
- Treated chicks were additionally vaccinated with Clostridium perfringens Type A vaccine (spray at hatchery)
- All vegetable diets; multiple feed suppliers
    - **No in-feed antibiotics**
    - **Feeds contained variable in-feed AB alternatives (specific to feed supplier) and water acidification on farm.**

Statistical Analysis

Main Effect

- Analysis of influence of Cp vaccine administration – focus of field evaluation

Covariable analyses

- Factors known to affect broiler performance and can influence data interpretation:

1. Days to Market – has significant impacts on market weights and FCR in commercial situations and variable between farms without consistent on-farm controls
2. Pre-trial analysis – influence of "timing" of non-vaccinated flocks to account for seasonality on broiler performance (proxy for quota period)
3. Study geography – not factored as this was unknown, but could account for variations in medication rotation as well as diet formulation (e.g. wheat vs. corn-based).

Ontario Performance Summary

	Control* (n=3)	Vaccinated (n=18)	P-value
Mortality#	3.9	5.3	0.271
FCR$	1.636	1.607 -1.8% (-2.9 pts)	0.402
Market weights%	2.229	2.326 +4.4% (+97g)	0.052

*Control flocks were flocks without history of NE issues, while vaccinated flocks had history of NE-issues

#Data analysis using feed supplier (P=0.074) as covariable; $ quota period (P=0.037), days to market (P=0.008) as covariables; % days to market (P<0.001) as covariable.

Economics – Ave Cdn Performance*

	Control - non-challenged flocks		CP-vaccinated flocks
Customer:			
# Placed:	30,000		30,000
Age:	38.3	days	38.8
# Shipped:	28,410		28,821
Live Weight Shipped	63,326	kg	65,655
Average Weight:	2.23	kg	2.28
ADG:	58.2	g/day	58.7
# Condemns:	0		0
Condemned as %	0.00	%	0.00
Total Mortality as %	5.30	%	3.93
Paid Weight Shipped:	63,326	kg	65,655
Chick Cost	0.97	cents	1.01
Live Price:	2.20	$/kg	2.20
	Feed Info		**Feed Info**
Feed Consumed:	109.40	mt	110.40
Total Feed Cost:	65,639.59	$	66,238.41
Average Feed Cost:	600.00	$/mt	600.00
Feed Cost/kg Gain:	1.037	$/kg Ship	1.009
Feed Conversion:	1.636		1.615
EPI:	337		349
	Income Info		**Income Info**
Gross Payable	139,316.96	$	144,441.73
*Feed Expense:	65,639.59	$	66,238.41
Chick Expense:	29,142.00	$	30,192.00
Revenue after Feed & Chick:	44,535.37	$	48,011.32
Revenue/kg Paid:	0.703	$/kg Paid	0.731
Benefit	3,475.96		
ROI	3.310		

Relative **% improvements***:

- FCR reduced by 2.1 pts

***Improvement of 1.26%**

- BW increased by 50g

***Improvement of 2.2%**

- Mortality reduced by 1.37%

***Improvement of 25.8%**

* Based on average of 4 field studies

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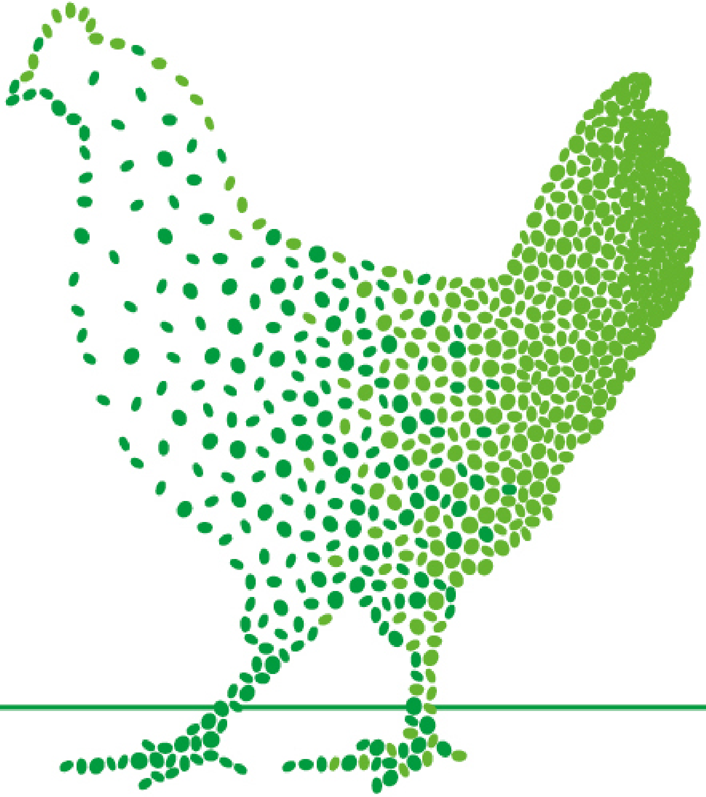
Ontario Field Trial – Clostridium perfringens Type A vaccine



Birds and treatments (Dec ‘21-Jan ‘23)

- Mixed sex broilers (33-37 days)
- Total of 79 flocks in analysis with 3 different feed programs
  - RWA program with in-feed solution (n=23)
  - RWA program without in-feed solution (n=44)
  - Conventional program (n=12)
- Control chicks had standard vaccinations at the hatchery.
- Treated chicks were additionally vaccinated with Clostridium perfringens Type A vaccine (spray at hatchery)
- Multiple feed suppliers (4)

Statistical Analysis

Main Effect

- Analysis of influence of Cp vaccine administration – focus of field evaluation
- Feed program (and interaction with Cp vaccine status)

Covariable analyses

- Factors known to affect broiler performance and can influence data interpretation:

1. Days to Market – to account for impacts on market weights and FCR in commercial situations (variable between farms without consistent on-farm control flocks)
2. Quota period– to account for influence of “timing” of non-vaccinated flocks to account for seasonality on broiler performance
3. Feed Supplier - to account for variations in diet formulation differences (macro-nutrient density), as well as in-feed antibiotic-alternative technologies.

Interaction effects - Market weights

RWA program (including in-feed solution)

	Control (n=13)	Vaccinated (n=10)
Market weights	2.222	2.171

+21 g bwt

RWA program (excluding in-feed solution)

	Control (n=22)	Vaccinated (n=22)
Market weights	2.243	2.243

Calculated based on ADG * days to market; Analysis using Vaccine status as main effect and feed supplier (P=0.759), days to market (P=0.272), and quota period (P=0.244) as covariables.

Interaction effects - Feed Conversion Ratio (FCR)

RWA program (including in-feed solution)

	Control (n=8)	Vaccinated (n=11)
FCR	1.558	1.569

+6.4 pts FCR

RWA program (excluding in-feed solution)

	Control (n=19)	Vaccinated (n=19)
FCR	1.734	1.622

-11.2 pts FCR

Analysis using Vaccine status as main effect and feed supplier (P=0.761), days to market (P=0.309, and quota period (P=0.147) as covariables.

Interaction effects - Mortality, % (total, D0-market)

RWA program (including in-feed solution)

	Control (n=13)	Vaccinated (n=10)
Mortality, %	7.4	7.9

-1.4 pts mortality

RWA program (excluding in-feed solution)

	Control (n=22)	Vaccinated (n=22)
Mortality, %	6.6	6.0

-0.6 pts mortality

Analysis using Vaccine status as main effect and feed supplier (P=0.120), days to market (P=0.098), and quota period (P=0.625) as covariables.

Economics - RWA programs

	RWA - with in feed solution		RWA - non in-feed solution, CP-vaccinated
Customer:			
# Placed:	30,000		30,000
Age:	35	days	34.5
# Shipped:	27,780		28,200
Live Weight Shipped	61,727	kg	63,253
Average Weight:	2.22	kg	2.24
ADG:	63.5	g/day	65.0
# Condemns:	625.05		485.04
Condemned as %	2.25	%	1.72
Total Mortality as %	7.40	%	6.00
Paid Weight Shipped:	60,338	kg	62,165
Chick Cost	0.97	cents	1.01
Live Price:	2.20	$/kg	2.20
	Feed Info		**Feed Info**
Feed Consumed:	103.86	mt	109.14
Total Feed Cost:	57,120.95	$	58,937.97
Average Feed Cost:	550.00	$/mt	540.00
Feed Cost/kg Gain:	0.947	$/kg Ship	0.948
Feed Conversion:	1.558		1.622
EPI:	377		377
	Income Info		**Income Info**
Gross Payable	132,744.26	$	136,762.24
*Feed Expense:	57,120.95	$	58,937.97
Chick Expense:	29,142.00	$	30,192.00
Revenue after Feed & Chick:	46,481.30	$	47,632.28
Revenue/kg Paid:	0.770	$/kg Paid	0.766
Benefit	1,150.97		
ROI	1.096		

Assumes $10/MT in-feed solution cost

ROI of 1.1:1

Producer value of $7480/year

Economics - without in-feed solution

	RWA program wihtout in-feed solution			CP-vaccinated RWA program wihtout in-feed solution
Customer:				
# Placed:	30,000			30,000
Age:	34.5	days		34.4
# Shipped:	28,020			28,200
Live Weight Shipped	62,849	kg		63,253
Average Weight:	2.24	kg		2.24
ADG:	65.0	g/day		65.2
# Condemns:	451.122			479.40
Condemned as %	1.61	%		1.70
Total Mortality as %	6.60	%		6.00
Paid Weight Shipped:	61,837	kg		62,177
Chick Cost	0.97	cents		1.01
Live Price:	2.20	$/kg		2.20
	Feed Info			**Feed Info**
Feed Consumed:	116.68	mt		109.14
Total Feed Cost:	64,174.47	$		60,029.41
Average Feed Cost:	550.00	$/mt		550.00
Feed Cost/kg Gain:	1.038	$/kg Ship		0.965
Feed Conversion:	1.734			1.622
EPI:	350			378
	Income Info			**Income Info**
Gross Payable	136,041.39	$		136,790.07
*Feed Expense:	64,174.47	$		60,029.41
Chick Expense:	29,142.00	$		30,192.00
Revenue after Feed & Chick:	42,724.91	$		46,568.66
Revenue/kg Paid:	0.691	$/kg Paid		0.749
Benefit	3,843.75			
ROI	3.661			

ROI of 3.6:1

Producer value of $24980/year

Take home messages

- NE is re-emerging as major disease concern.
- Antibiotic-free production systems have eliminated traditional NE control protocols.
    - Chicken Farmers of Canada are working to reduce the use of antibiotics on broiler farms, including those that prevent NE.

Clostridium perfringens Type A Vaccine

- Cost-effective preventive solution to mitigate sub clinical NE issues under commercial conditions
- Only vaccine stimulating an immune response to both alpha and NetB toxins.

A pleasure presenting to you!

Greg.page@huvepharma.ca

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Shaping livestock solutions