Discovery of PhytoComplexes to Reduce Diarrhea in Young Calves

Dr. Yanming Han

Nutreco Exploration

NutEx

NUTRECO EXPLORATION

- NutEx was created in **July 2021** with the intention of creating **dedicated novel & proprietary ultra-specialties**
- Ultra specialists of **2 programs: Phytotechnology** and **Microtechnology**
- Ultra specialists for **exploration, discovery and development.**

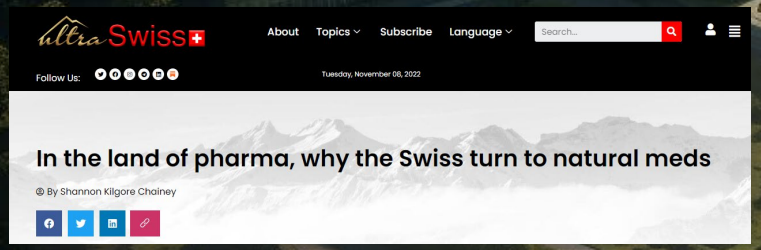
Plant Kingdom

UNLIMITED POSSIBILITIES (>390,000)

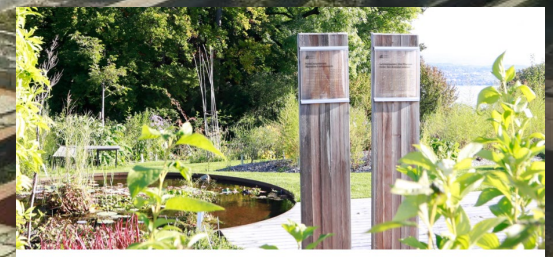
Hosted in “Phyto Valley”

NUTRECO'S
GARDEN OF
THE FUTURE

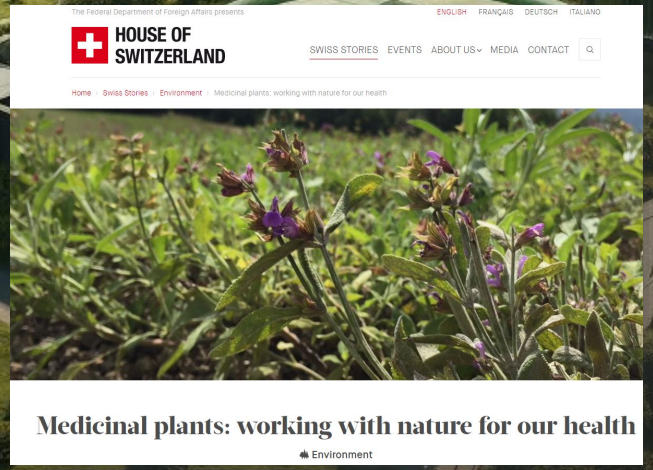
- A strategic location n Thurgau region, Switzerland
- Home of a long tradition in Phytomedicine & Phytopharmacy
- Concentration of +50 companies geared around functional plants

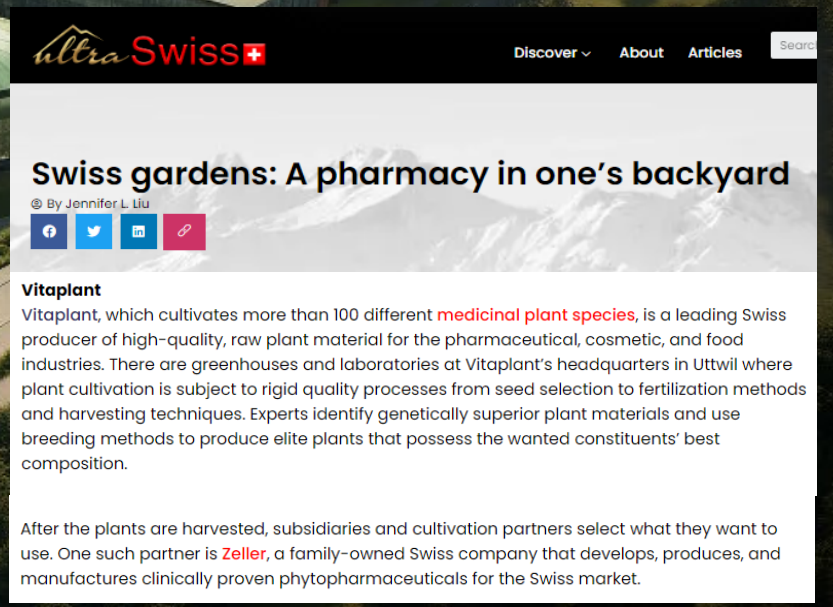


Phytovalley®-Tirol



TCM MEDICINAL PLANT GARDEN





Nutreco’s Garden of the Future

Nutreco’s Center of Excellence for Phytotechnology

ACTIVITY GUIDE

1. Ethnobotany and Screening
2. PhytoComplex analytics
3. Biological modelling
4. Plants propagation
5. Domestication & Breeding
6. Growing & Harvesting
7. Processing
8. Regulatory, patents & cultivars

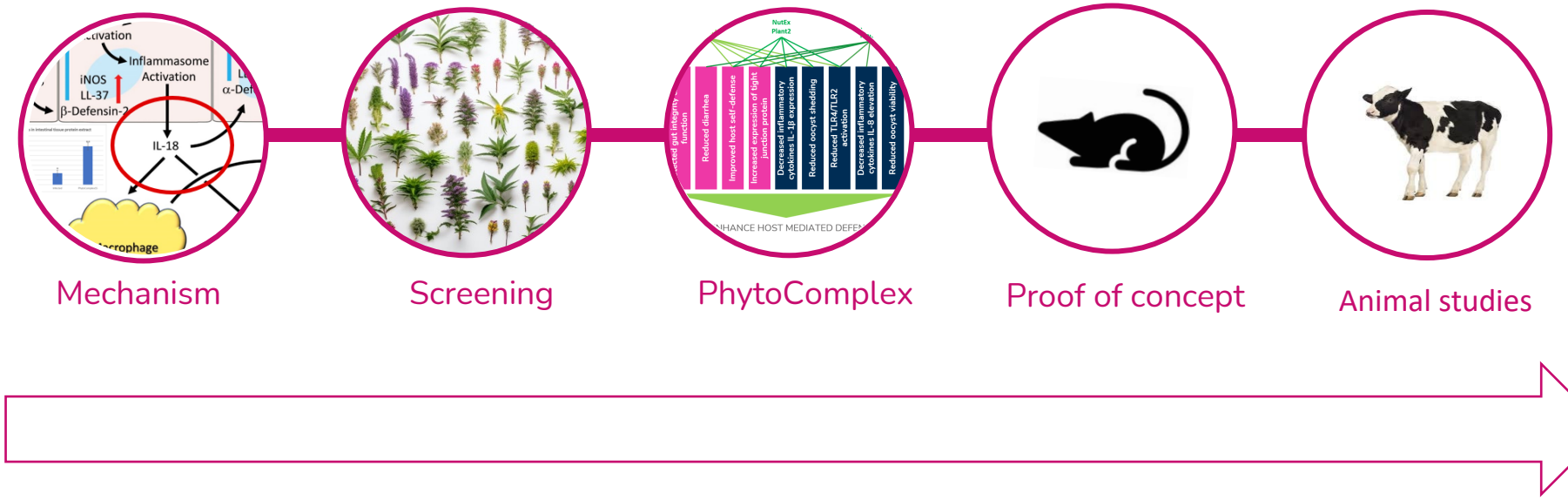
Phytotechnology

A new approach

- Long term commitment to Phytotechnology through Nutreco's Garden of the Future
- Discovery process with targeted screening of nature's potential
- Mode of action: Host-mediated mechanisms leveraging the complexity of plants
- New forms of actives : PhytoComplexes expressing full spectrum of plant compounds
- Production: Cultivation & continuous improvement of our plants embedded in development

Outline

Discovery and development of a novel PhytoComplex



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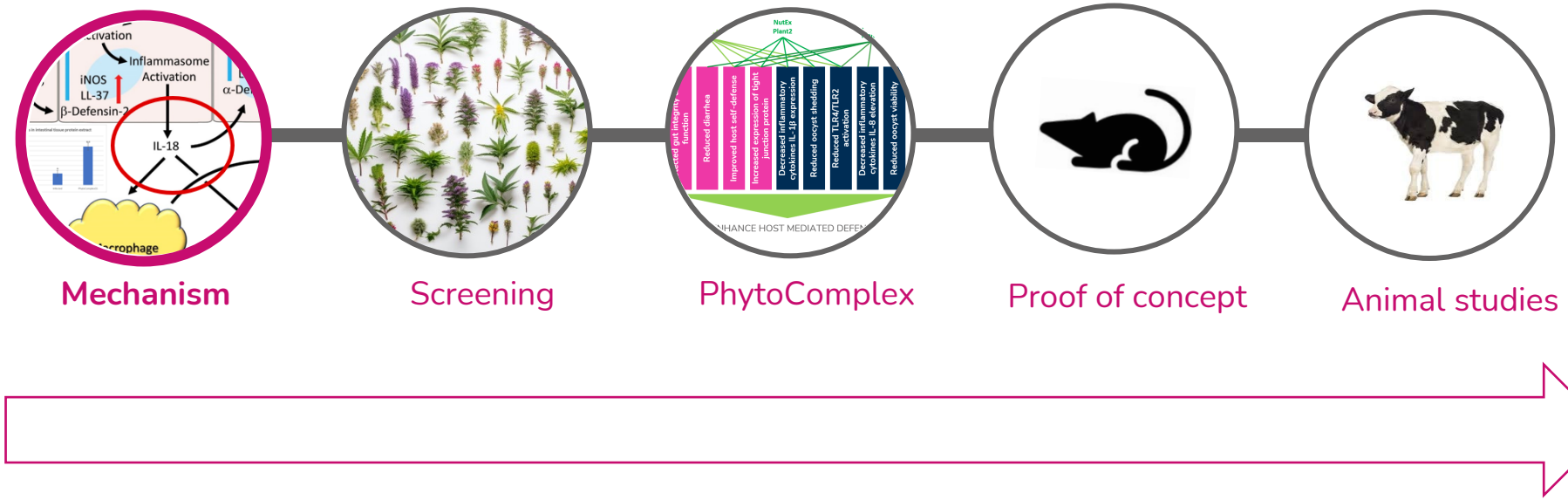
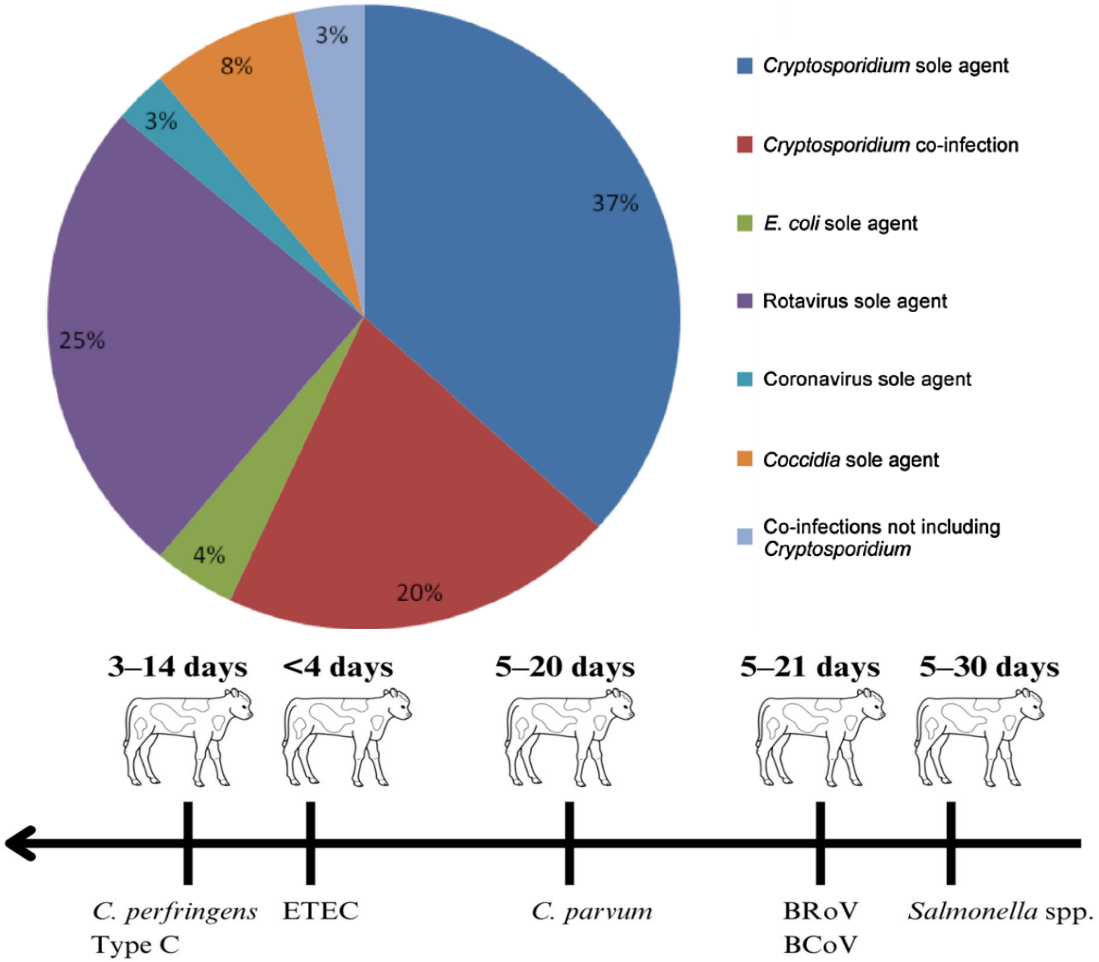


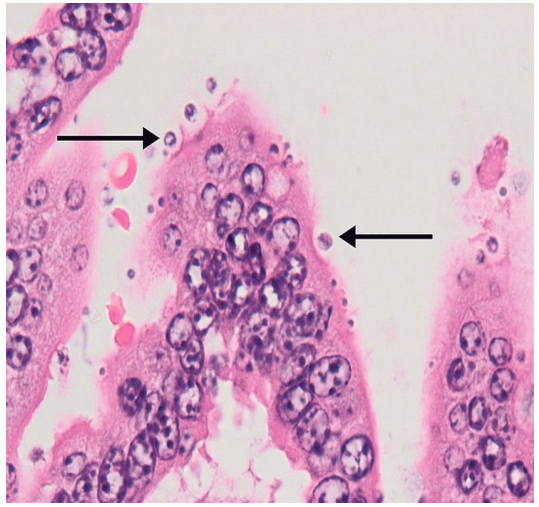
Diarrhea in neonatal calves: a complex challenge

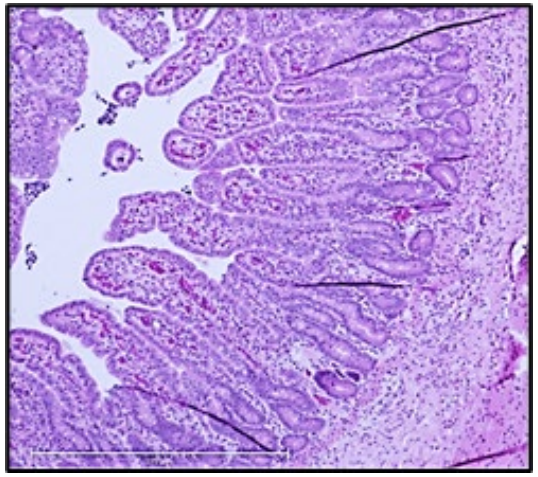


- Important cause of morbidity and mortality
- Affecting 60-100% farms
- Complex causes: main pathogens involved
  - Parasite (Cryptosporidium, Eimeria)
  - Virus (rotavirus, coronavirus)
  - Bacteria (ETEC, Salmonella)
- Cryptosporidiosis as the leading cause of diarrhea (20-80% pre-weaning calves)
- Long-term growth performance loss (>2 months)
- Significant economic loss worldwide (€40/calf)
- No vaccine or effective antimicrobials in calves

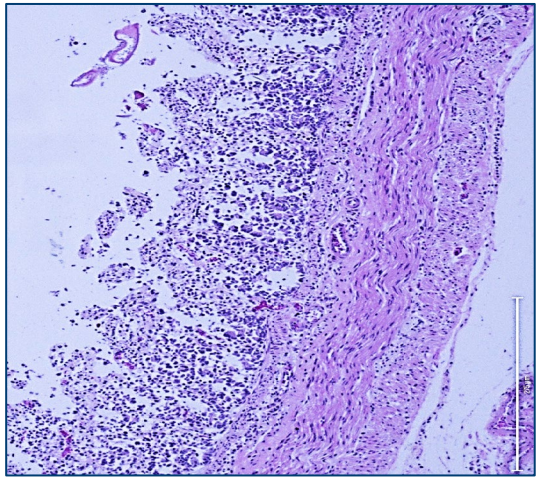
Wiser, 2021; Thomson et al, 2017; Roblin et al, 2023; Jessop et al, 2024

Cryptosporidiosis damages (Intestinal structure & function)





Uninfected



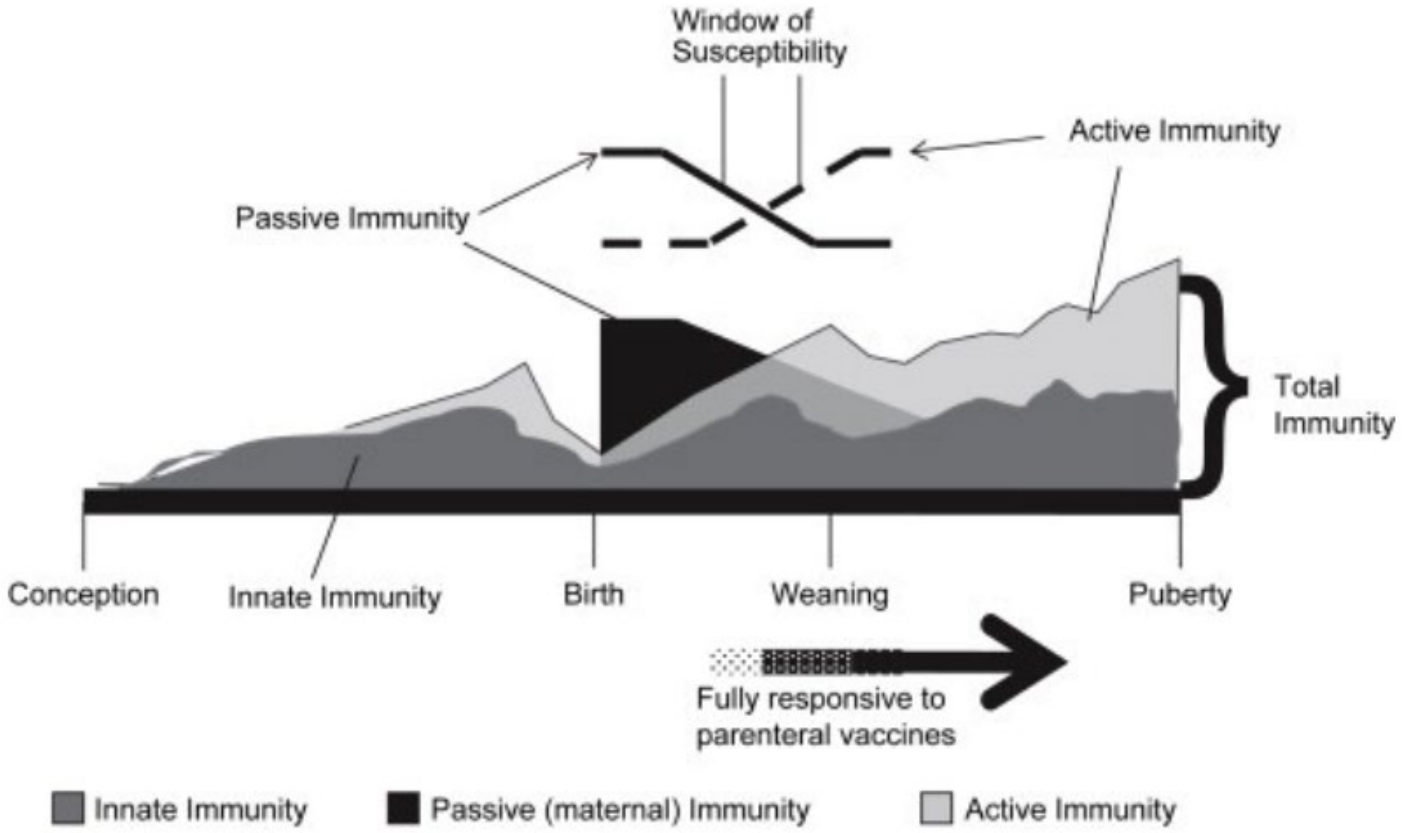
Infected

Intracellular invasion & multiplication

- Breached mucous layer
- Villous atrophy
- Poor nutrient digestion
- Poor nutrient absorption
- Watery diarrhea
- Causing lasting inflammation

Witola, W. University of Illinois. University of Calgary. Party et al, 2024

Immature neonatal immune system

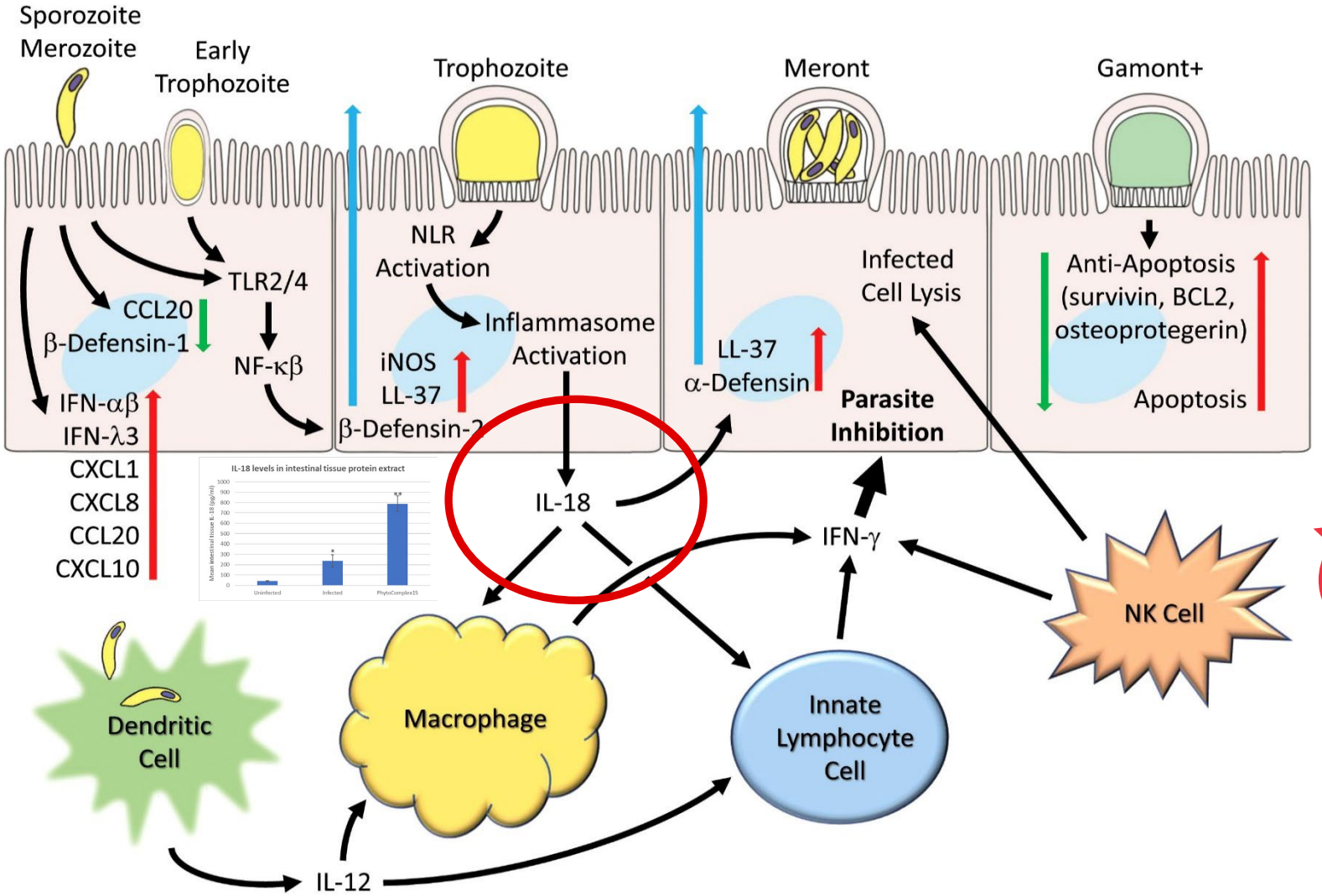


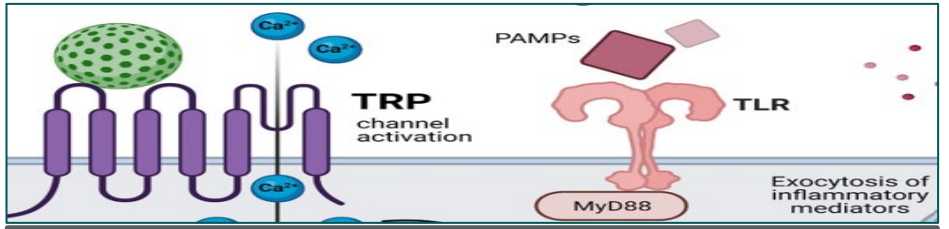
- Neonatal calves are still immuno-deficient
- Cryptosporidiosis activates both innate and adaptive immunity
- Cryptosporidiosis prompted local inflammation
- Cryptosporidiosis associated with long-term elevation of pro-inflammatory cytokines IL1-β, IFN-γ, TNF-α

Chase et al, 2008; El-Deeb et al, 2022

Mechanism

Indication as a start: to promote self-defence of the host





Activation of transient receptor potential (TRP) channels

Modulating Expression of Toll Like Receptor (TLRs)

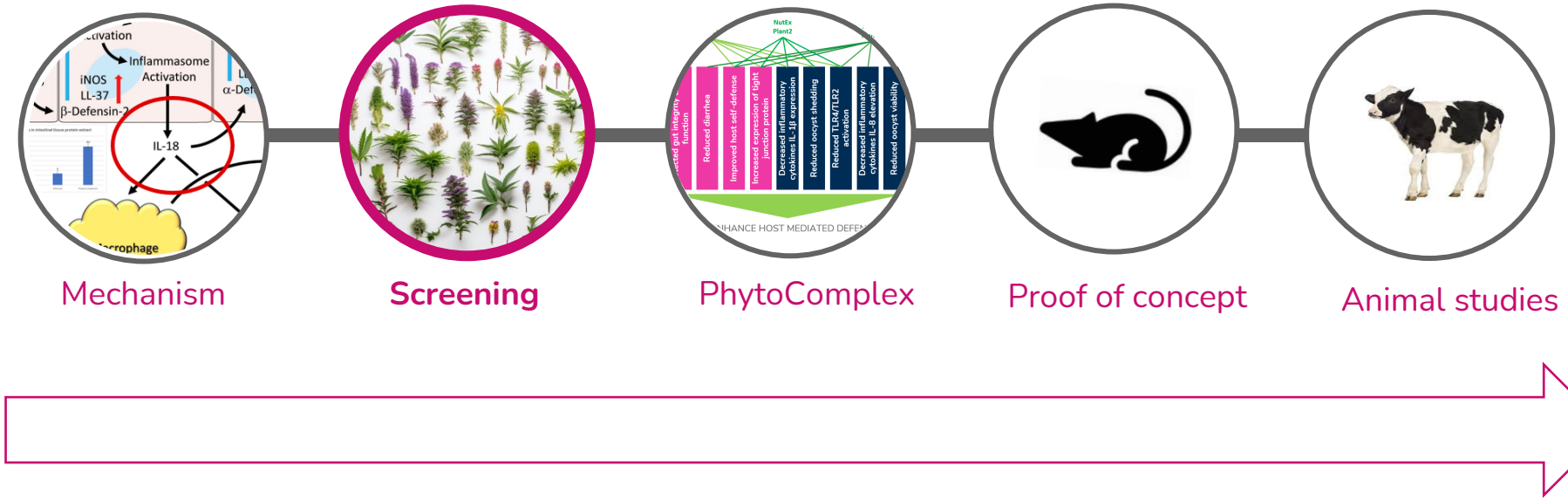
Regulating cytokines and other inflammatory mediators

Actions targeted:
- Reduce IL-1β, IL-8, TNFα, IFN-γ levels in parasitic infection
- Increase IL-18 for host production of antimicrobials

Mead, 2023; Crawford & Kol, 2021

Outline

Discovery and development of a novel PhytoComplex



Screening

Our Exploration Strategy

Old fashioned approach

Isolated single compounds from plants

- Antimicrobial strategy
- Directly targeting pathogens

➢ Limited approach (efficacy, regulatory, cost…)
➢ Not empowering animals

Modern complex strategy

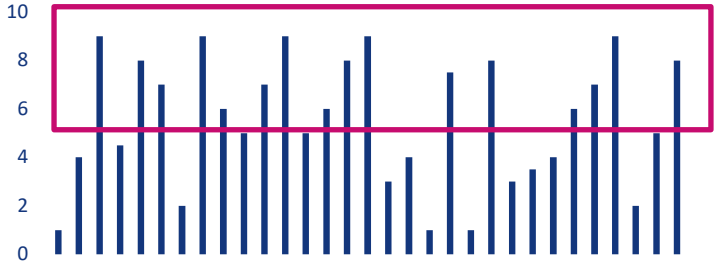
Plant metabolites → PhytoComplex
Complex set of compounds produced to defend against pathogens, predators.

Focus on: systemic host-mediated mechanism, e.g.,

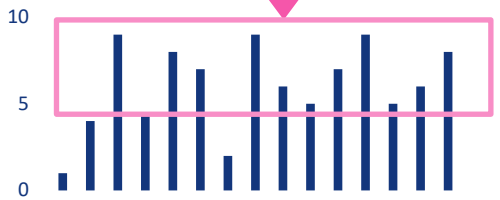
- Modulation of cytokine response and other inflammatory mediators
- Improving intestinal epithelial barrier and mucin production

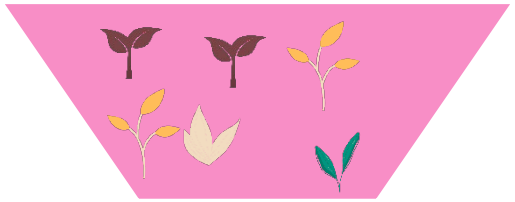
Screening process

Bioassay 1 targeting a mechanism



Bioassay 2

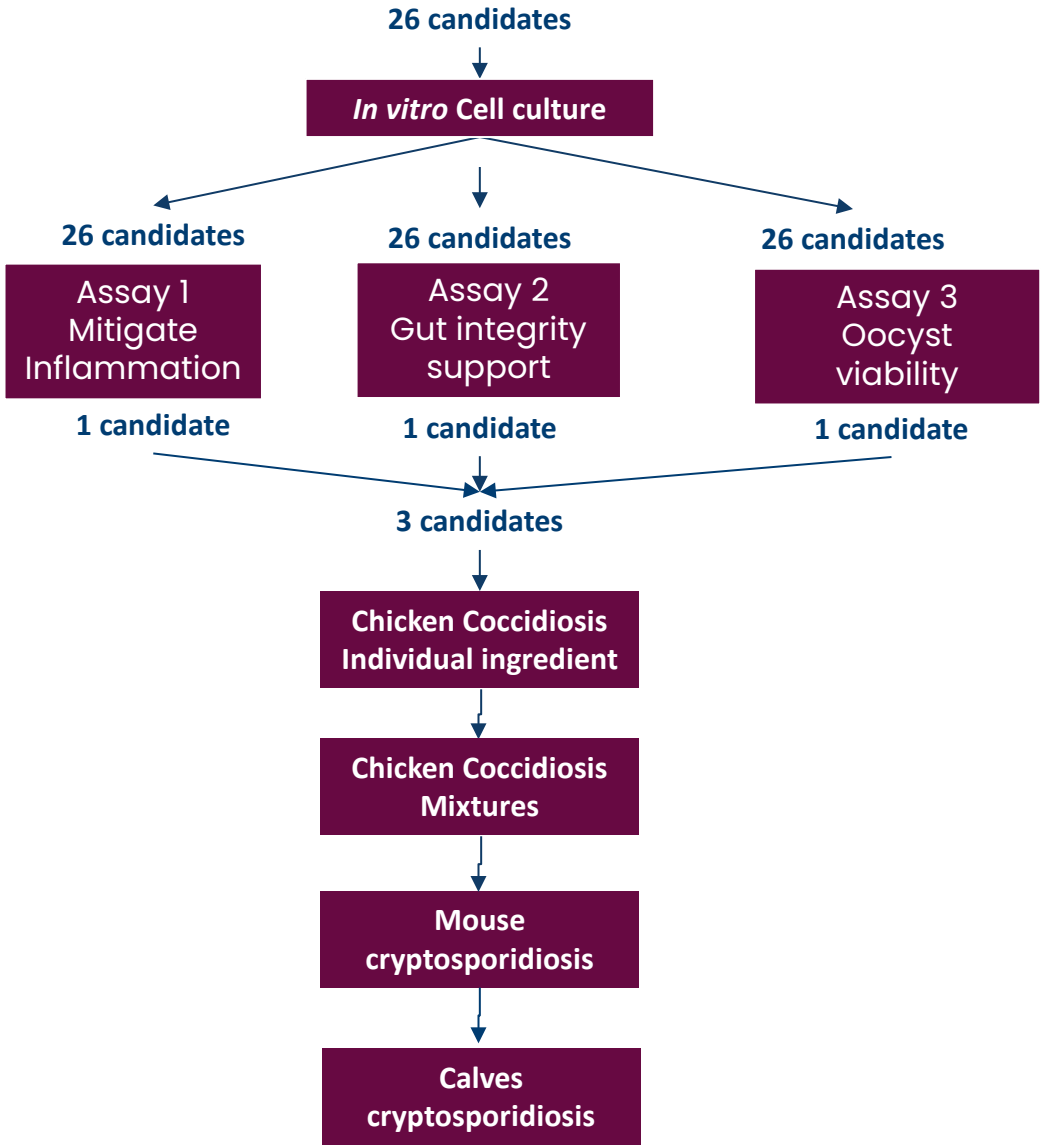




Technical landscaping
- Domestication
- Viability
- Costs
- Production
- Entry barriers/IP

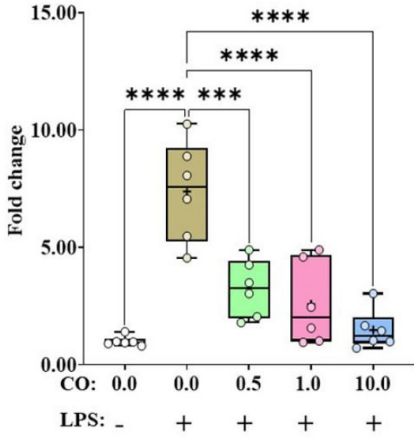
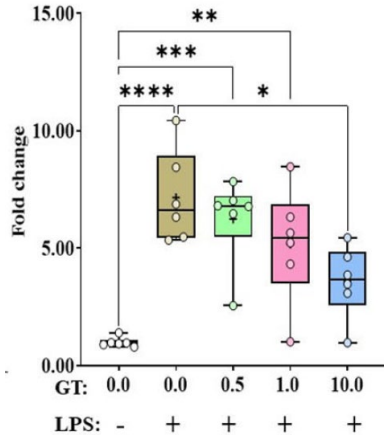
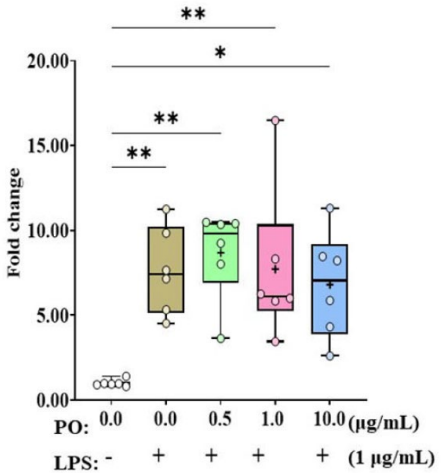
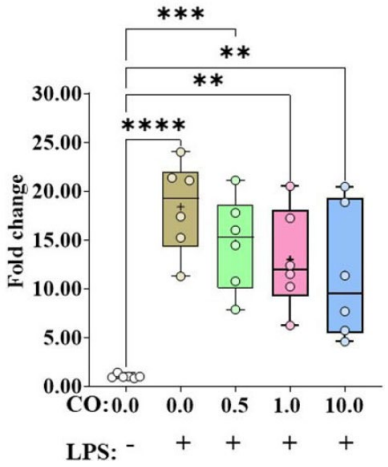
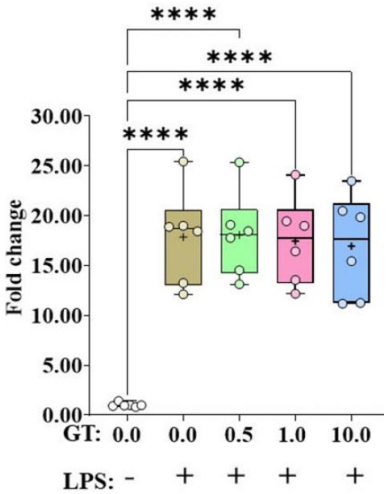
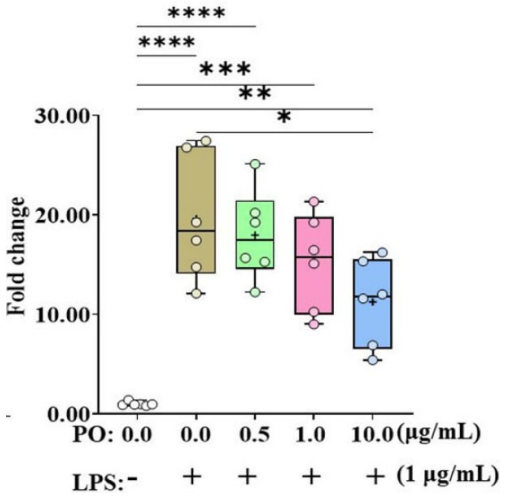
Screening

Calf PhytoComplex Discovery Roadmap



Best candidates influencing immune response

Screening

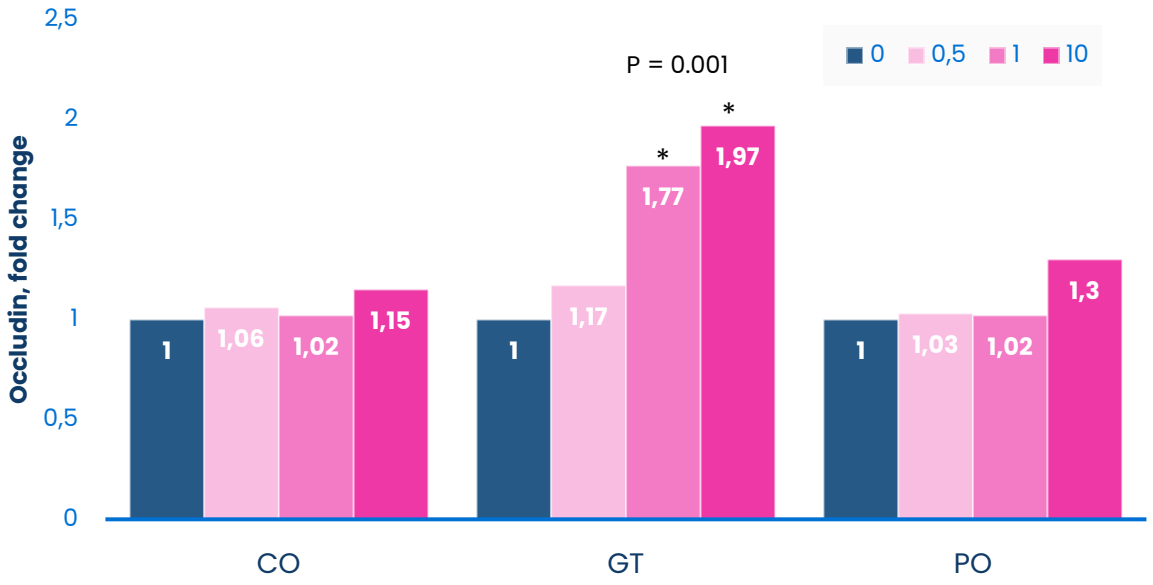
	Plant 1	Plant 2	Plant 3
IL-1β			
IL-8			

Park et al, 2023

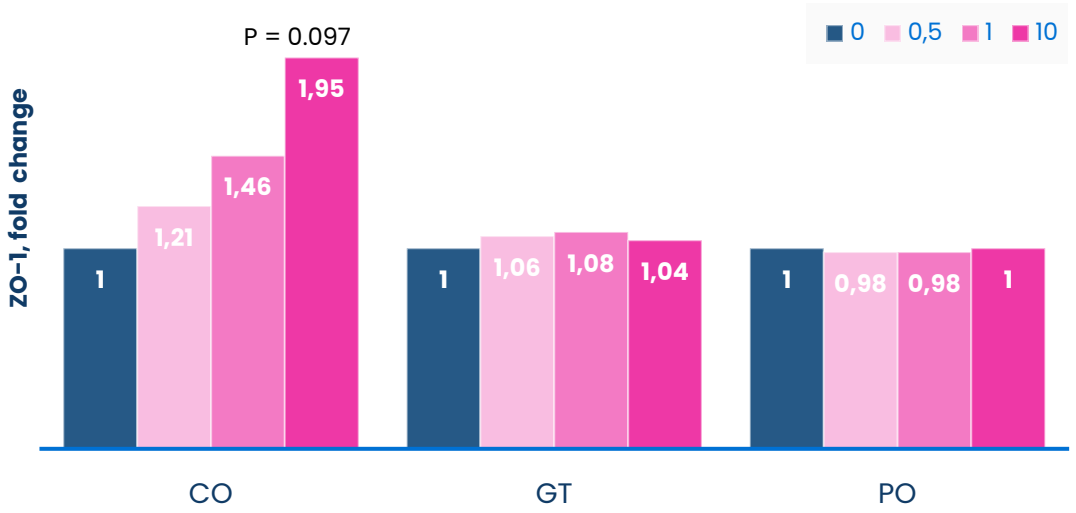
Best candidates for intestinal integrity

Screening

Occludin expression



ZO-1 expression



Individual candidates showing different impact on tight junctions

Park et al, 2023

Outline

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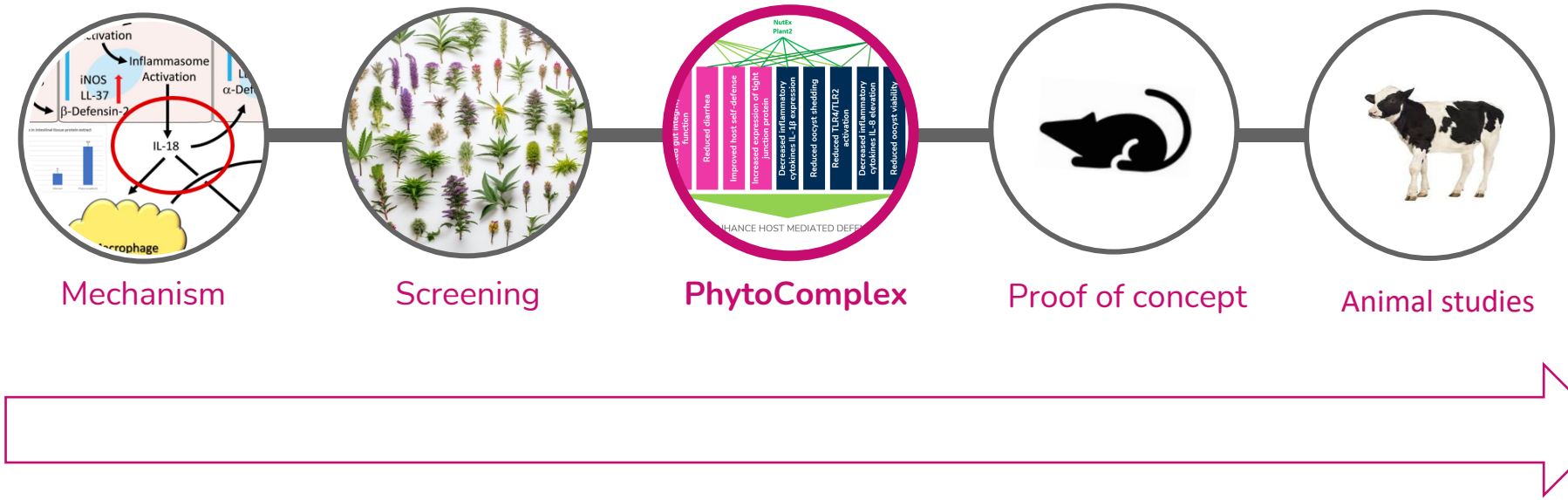
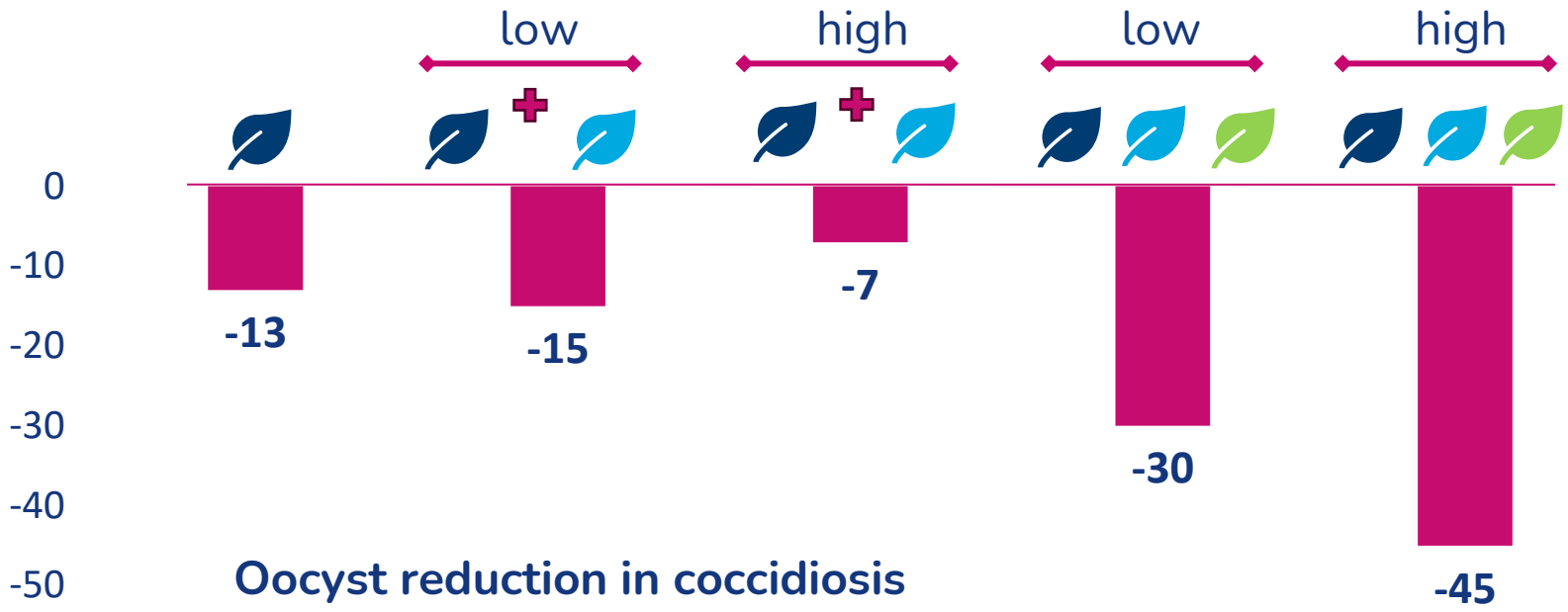


Synergy of plants – Ratio optimization

Ingredient	Dose (ppm)	Oocyst decrease (% relative to PC)
PO	100	-13
CO & PO	100	-15
	200	-7
CO & PO & GT	100	-30
	200	-45



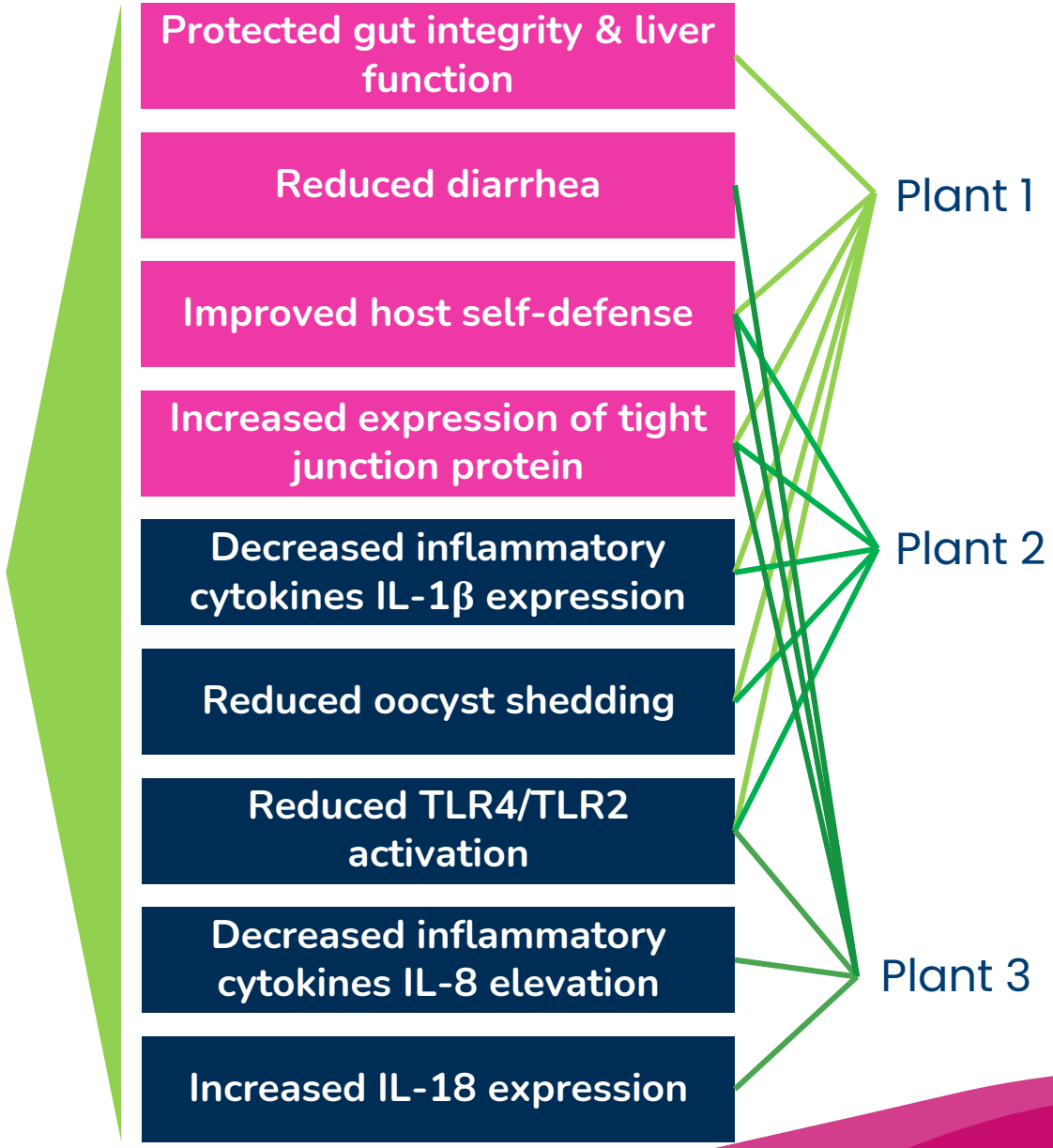
Oocyst reduction in coccidiosis challenged broiler chickens

Unpublished data, Nutreco

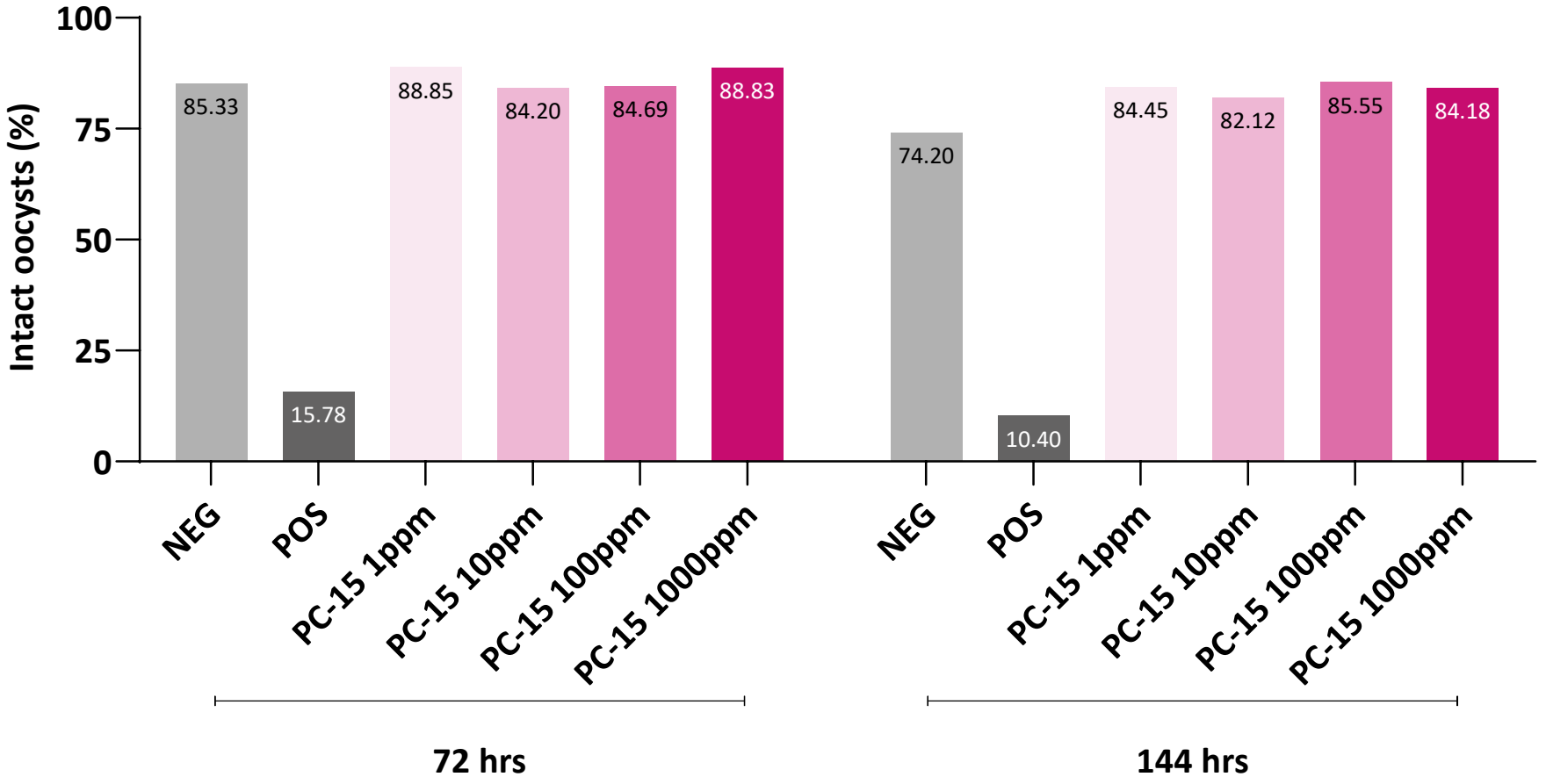
PhytoComplex-15: multi-functional host mediated targets

PhytoComplex

PhytoComplex-15 specifically targets host-mediated anti-inflammatory cytokines, and intestinal tight junction proteins, in alleviating the parasite infection



PhytoComplex-15 *does not kill the pathogen*



Unpublished data, Nutreco

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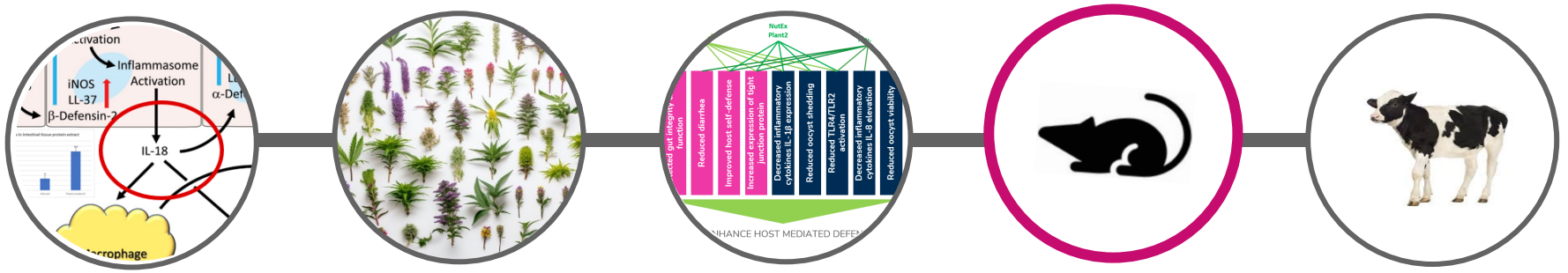
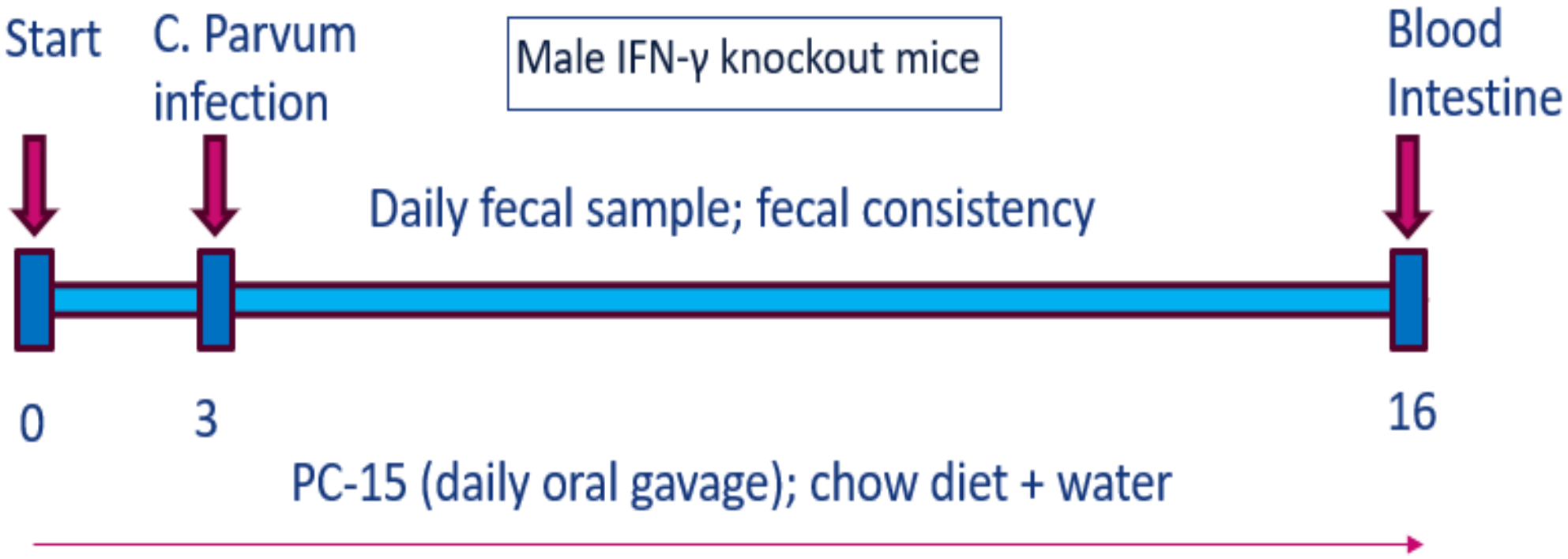


Efficacy of PC15 in *C. parvum* challenged mice

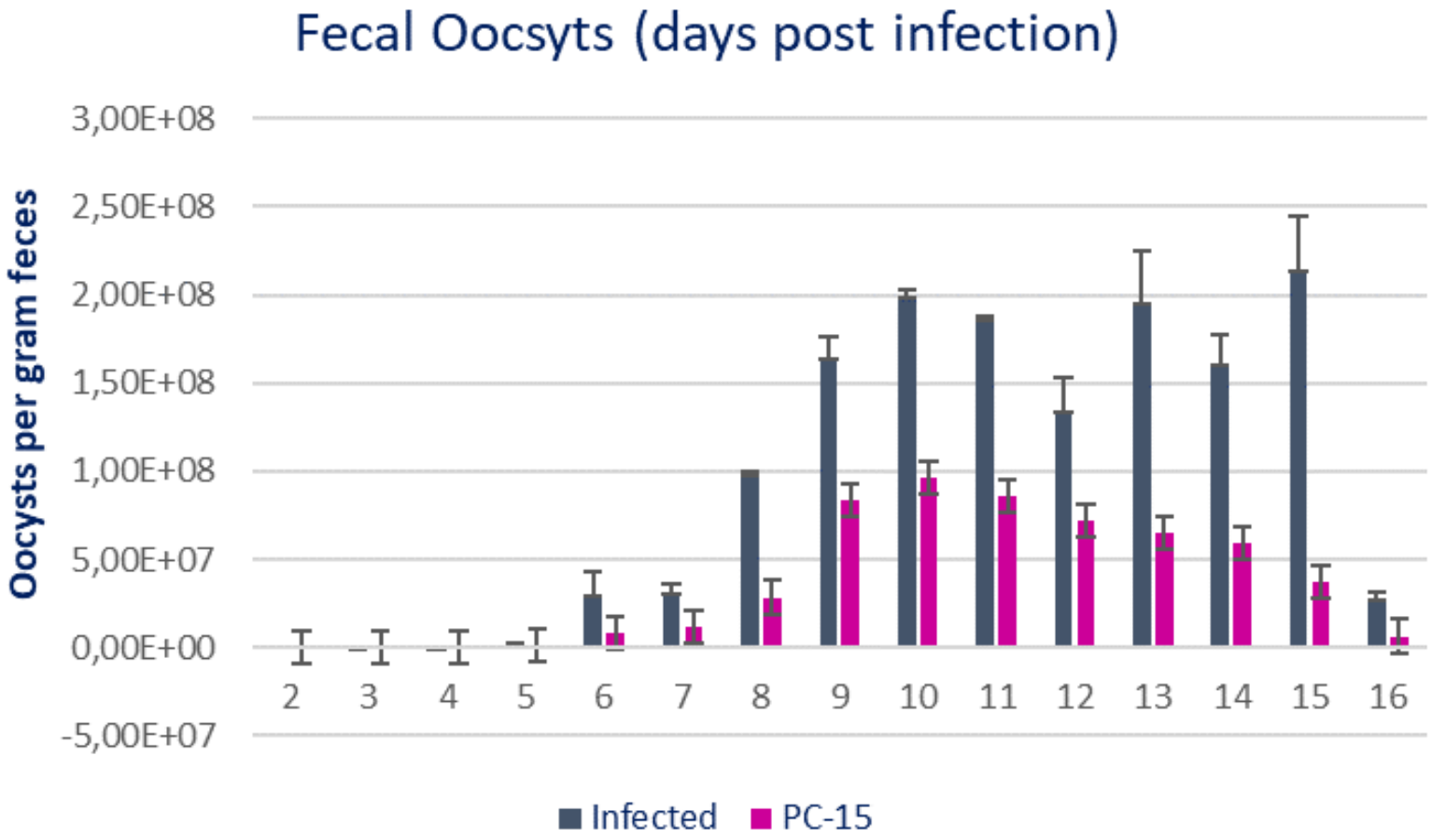
Proof of concept



Unpublished data, Nutreco T2022-14

PC15 reduced oocyst shedding

Proof of concept

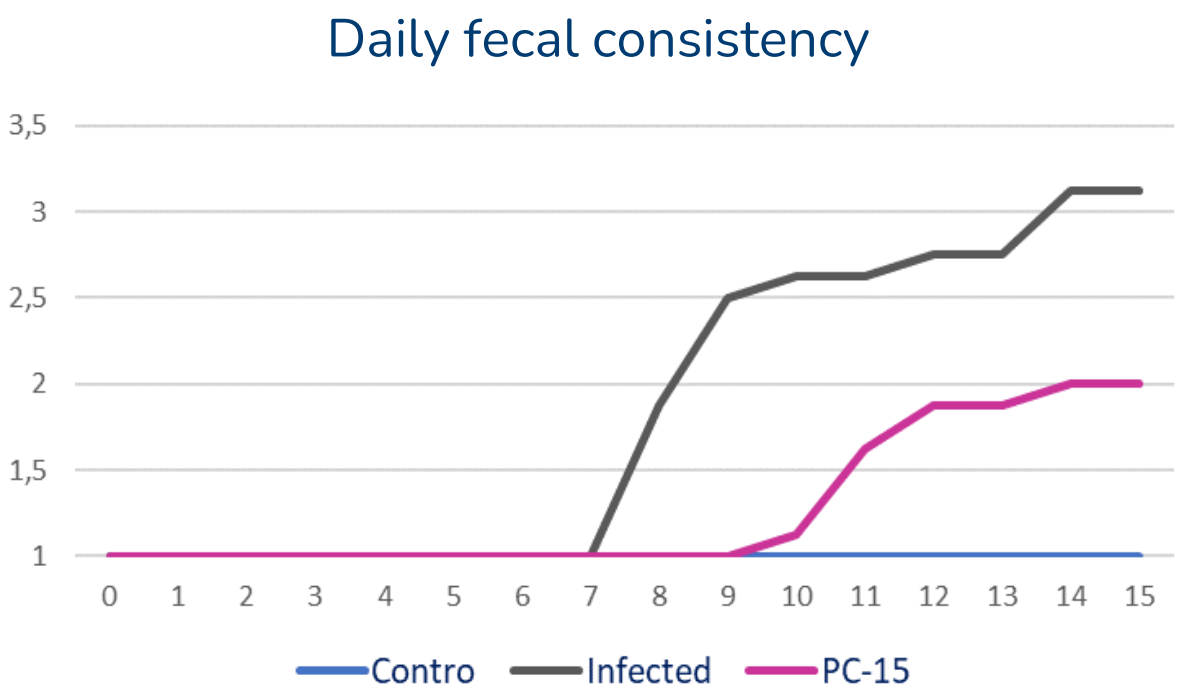


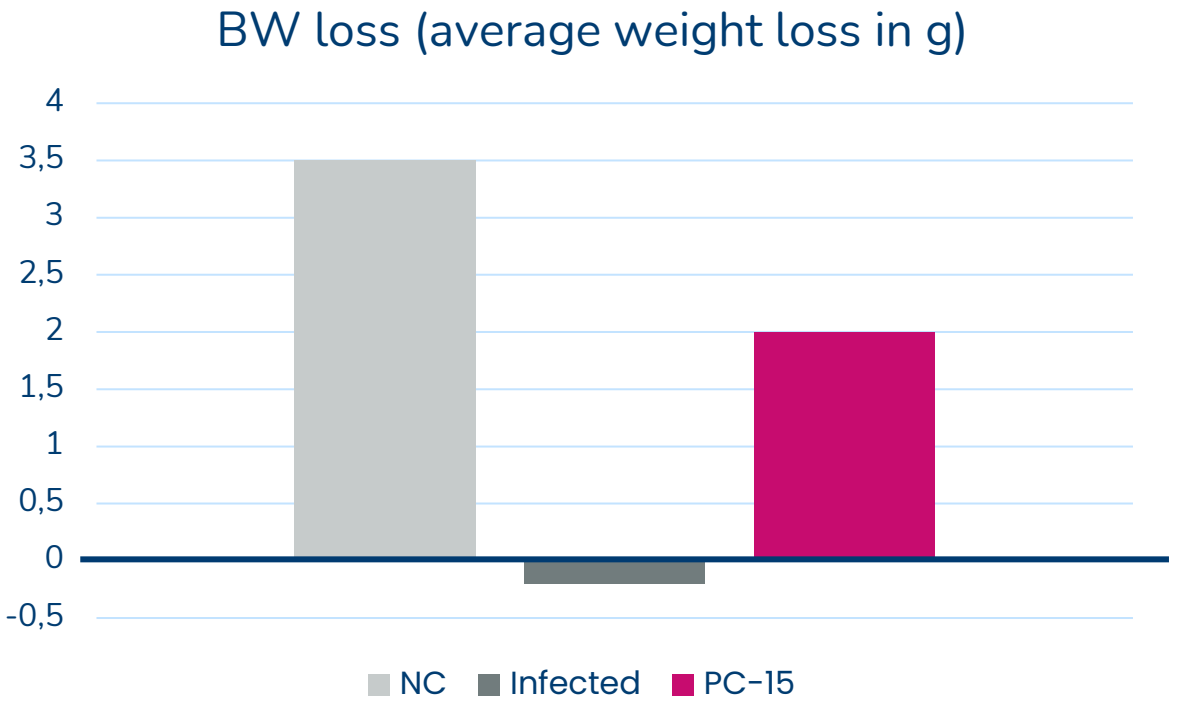
70% reduction of Oocyst shedding by 13 DPI

Unpublished data, Nutreco T2022-14

PC15 greatly reduced diarrhea and body weight loss

Proof of concept

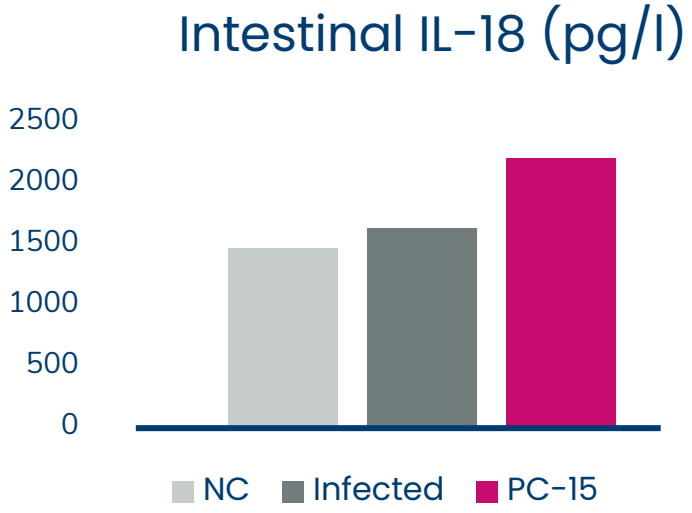


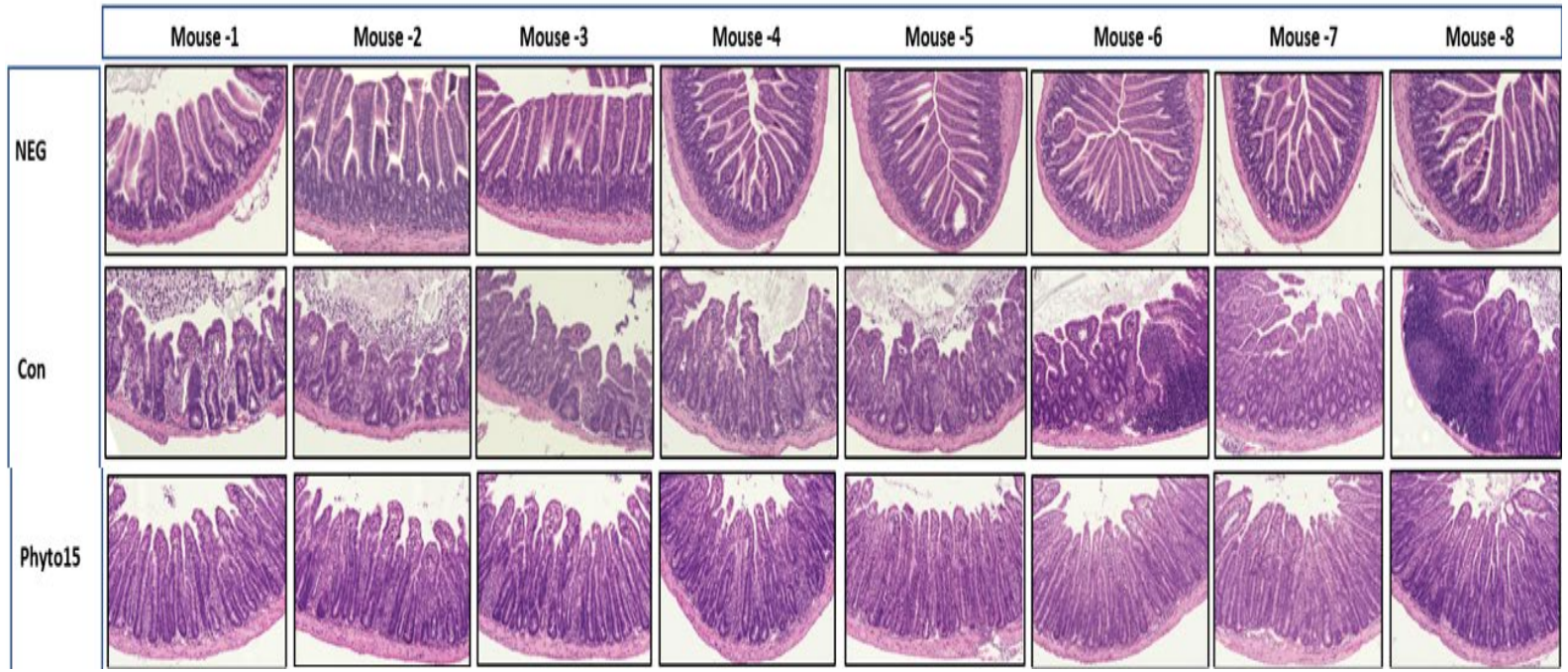


Unpublished data, Nutreco T2022-14

Proof of concept

PC15 increased intestinal IL-18 level and improved gut morphology





Increased intestinal IL-18 leads to
more AMP to inhibit intracellular parasites

Unpublished data, Nutreco T2022-14

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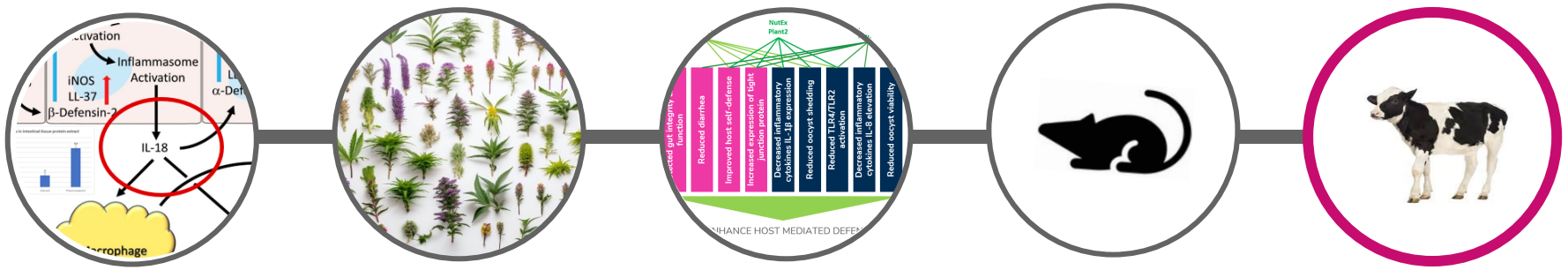
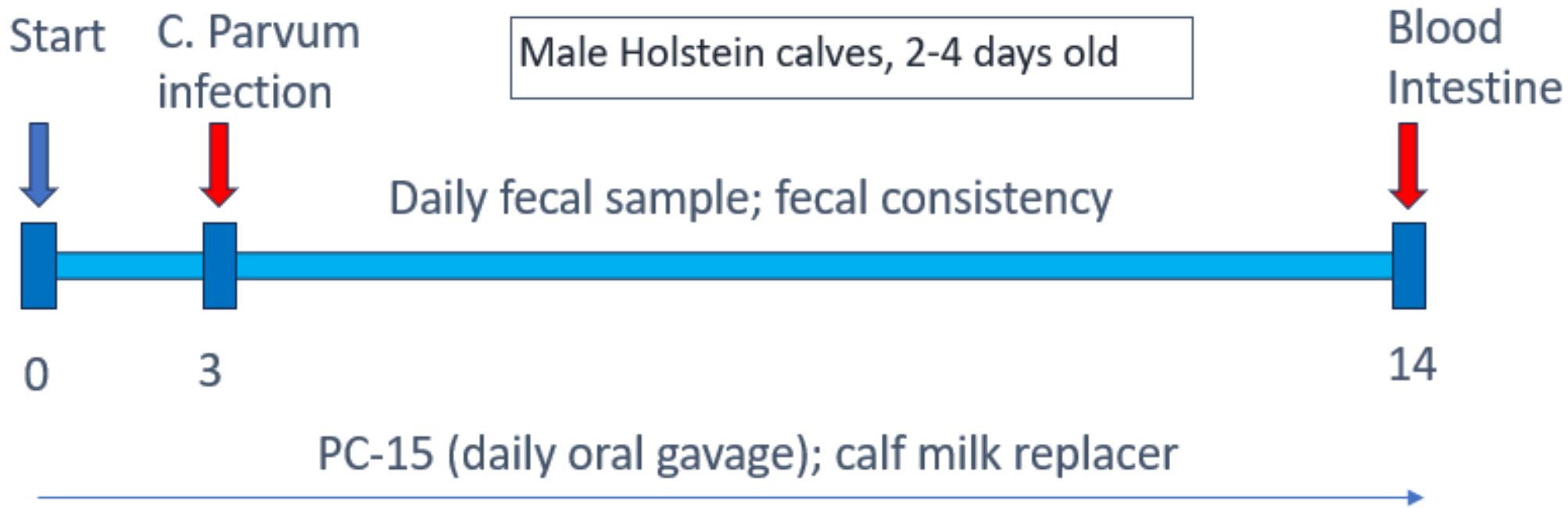


Efficacy of PC-15 in neonatal calves infected with *C. parvum*

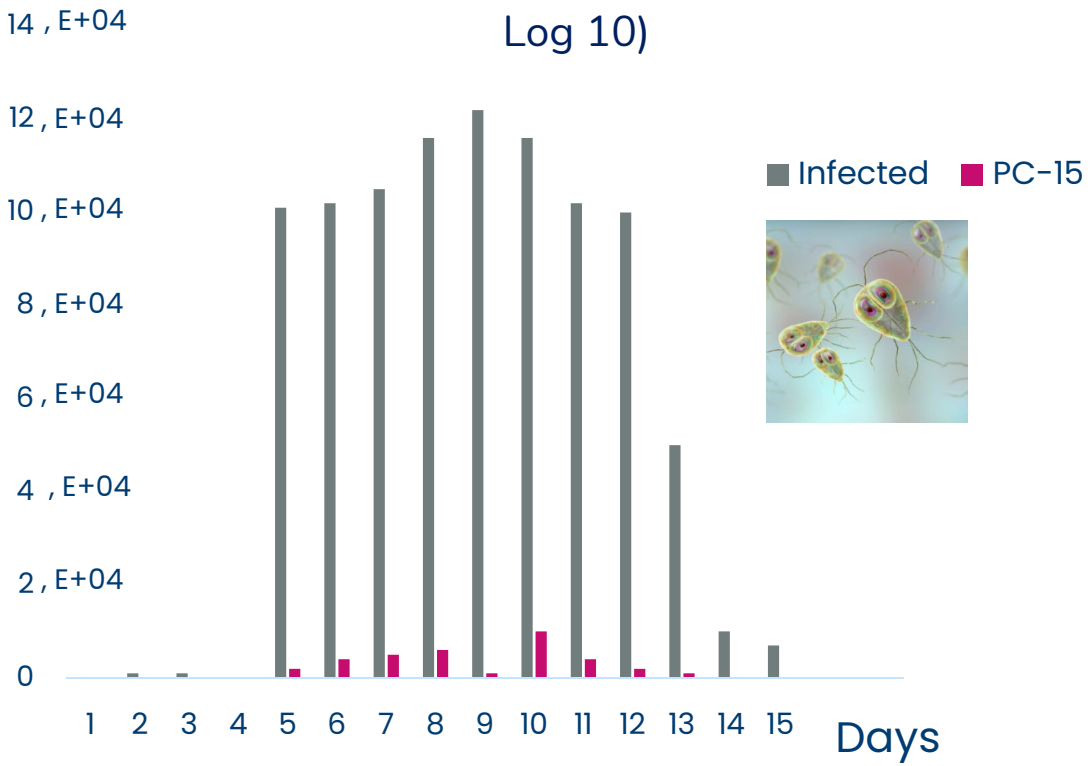


Unpublished data, Nutreco T2023-76

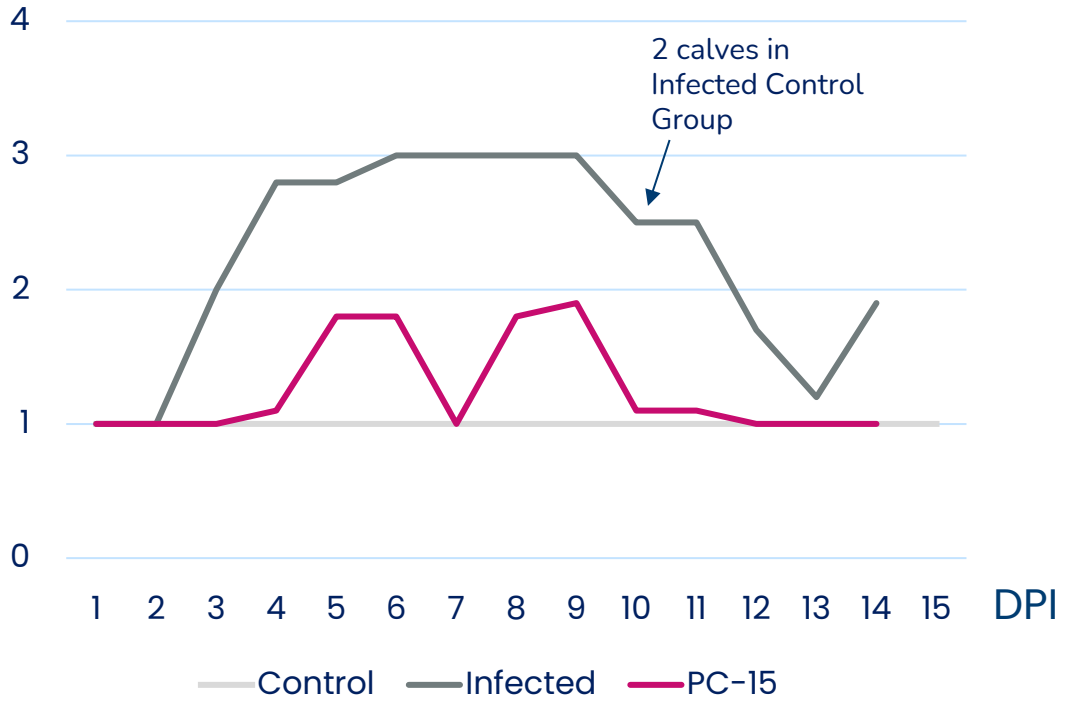
PC-15 reduced oocyst shedding & diarrhea

Animal studies

C. parvum oocysts in fecal sample (oocyst/g, Log 10)



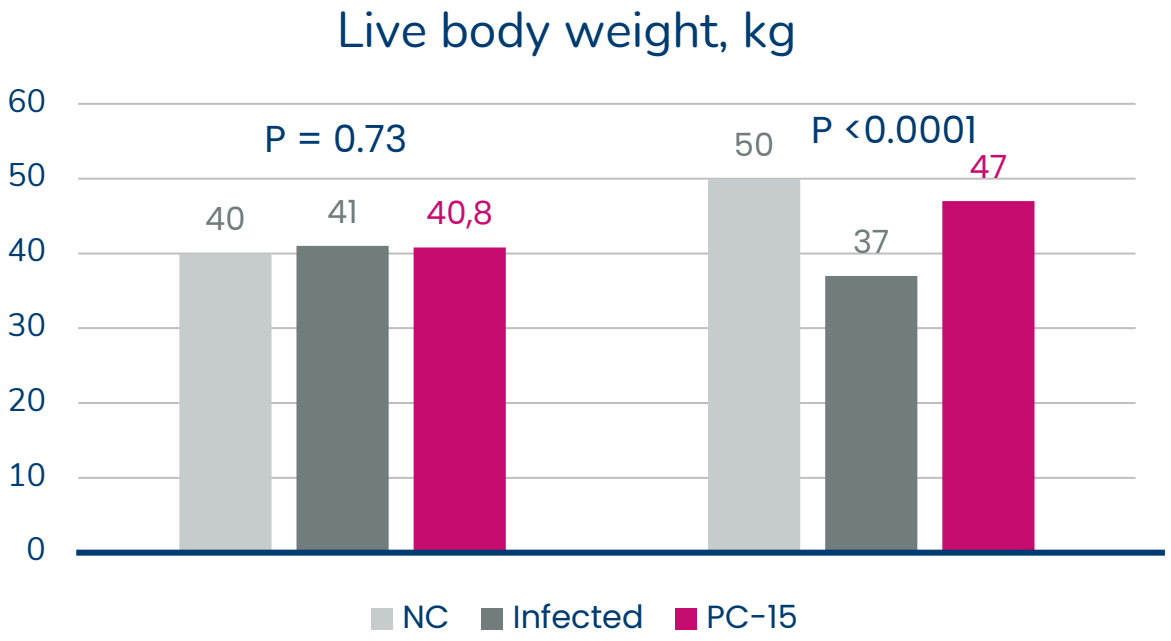
Diarrhea, fecal score

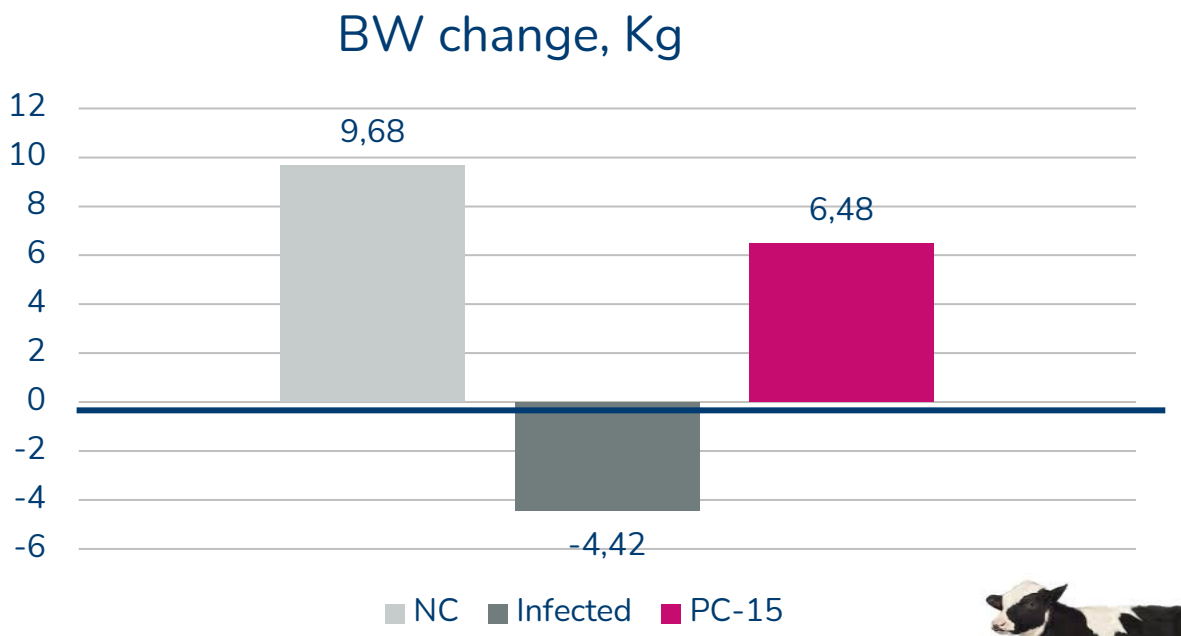


Unpublished data, Nutreco T2023-76

PC-15 reduced body weight loss

Animal studies

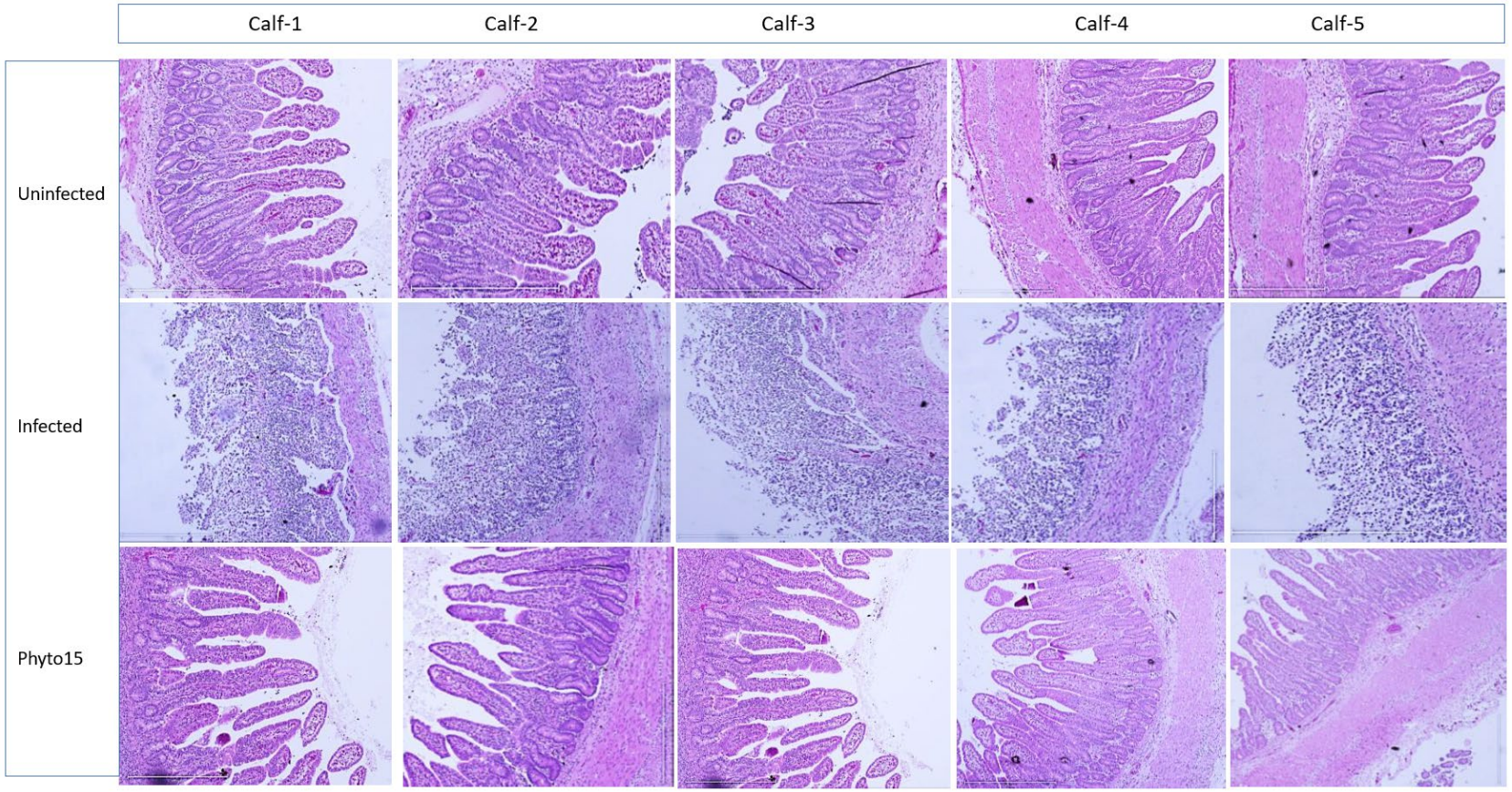




Unpublished data, Nutreco T2023-76

PC-15 maintained healthy gut morphology

Animal studies



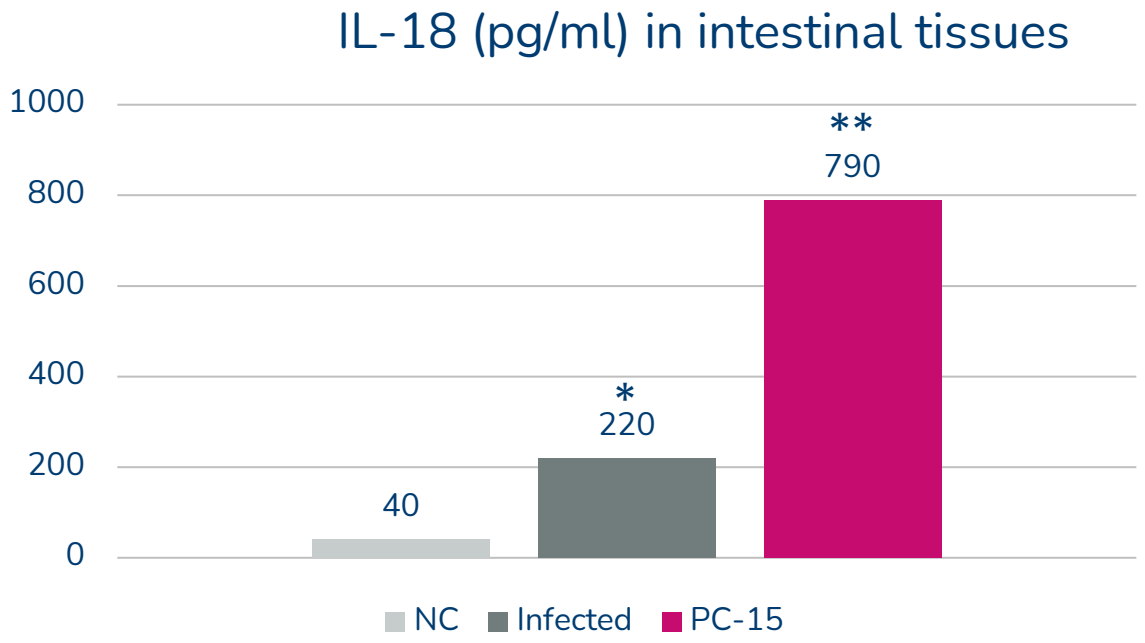
Uninfected: Healthy mucosa and prominent villi

Infected/untreated: Intestinal lesions characterized by villus atrophy

Infected+PhytoComplex-15: Mostly intact mucosa with prominent villi

Unpublished data, Nutreco T2023-76

PC-15 increased intestinal IL-18 level

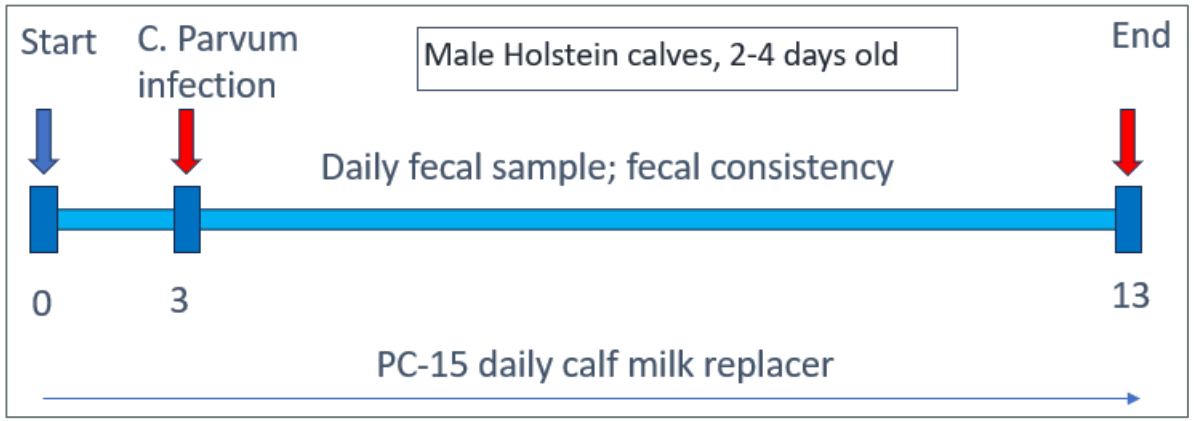


Increased intestinal IL-18 leads to more AMP to inhibit intracellular parasites

Unpublished data, Nutreco T2023-76

Animal studies

Optimizing the dose of PC-15 in neonatal calves under C. parvum challenge conditions

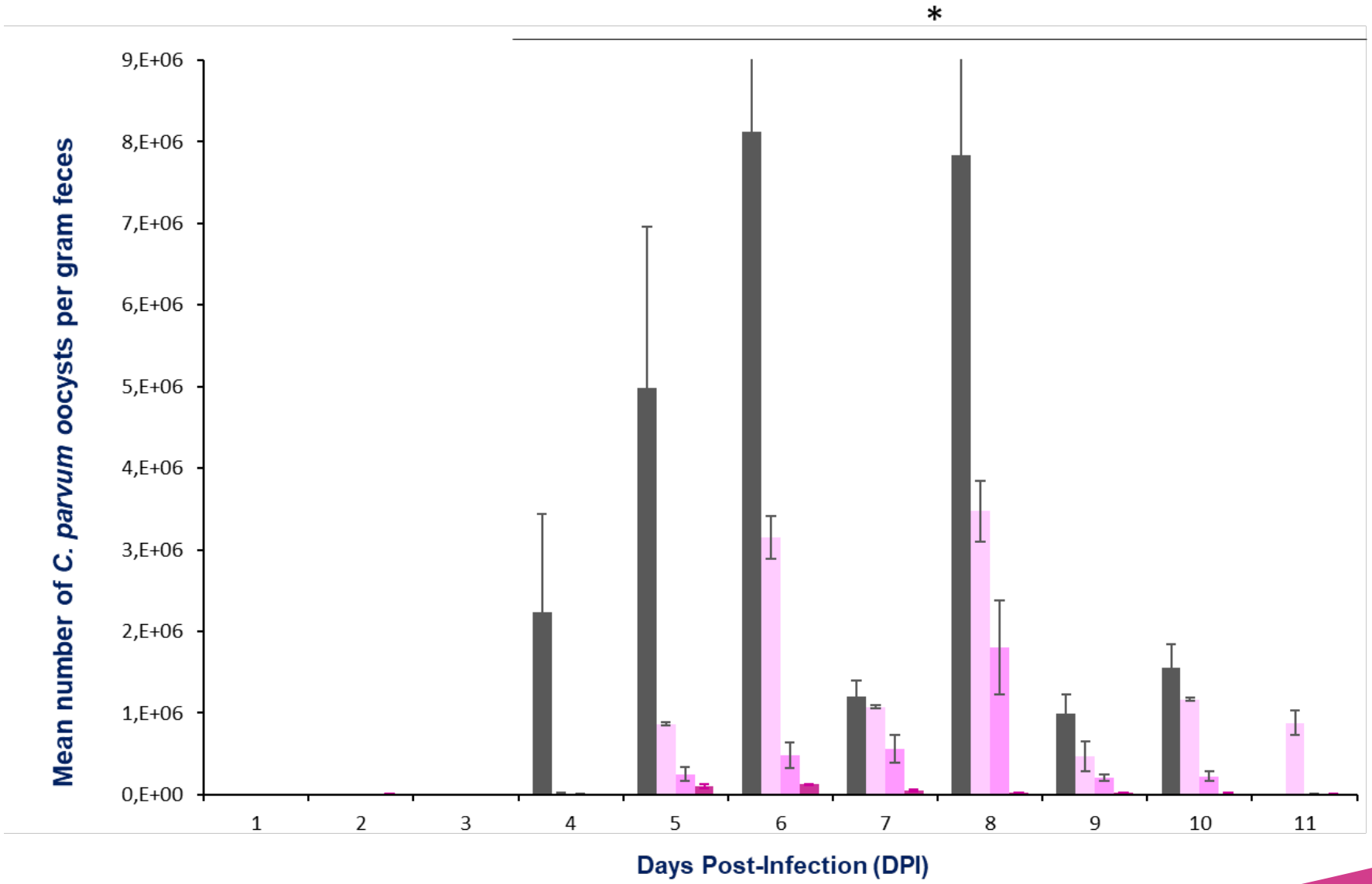


- 25 neonatal Holstein male calves, aged between 2-4 days old
- Pathogenic challenge: Cryptosporidium parvum
- 5 treatments, 5 calves per treatment
  - No infection & no supplement (Negative control)
  - Crypto challenge & no supplement (Infected)
  - Crypto challenge + PC-15 at **50 ppm** (PC50)
  - Crypto challenge + PC-15 at **100 ppm** (PC100)
  - Crypto challenge + PC-15 at **150 ppm** (PC150)
- The supplement was provided on days 0 to 13 in milk replacer
- On day 3 all groups but NEG were subjected to infection by oral administration of 5x10^5 C. parvum isolate oocysts

Unpublished data, Nutreco T2024-143

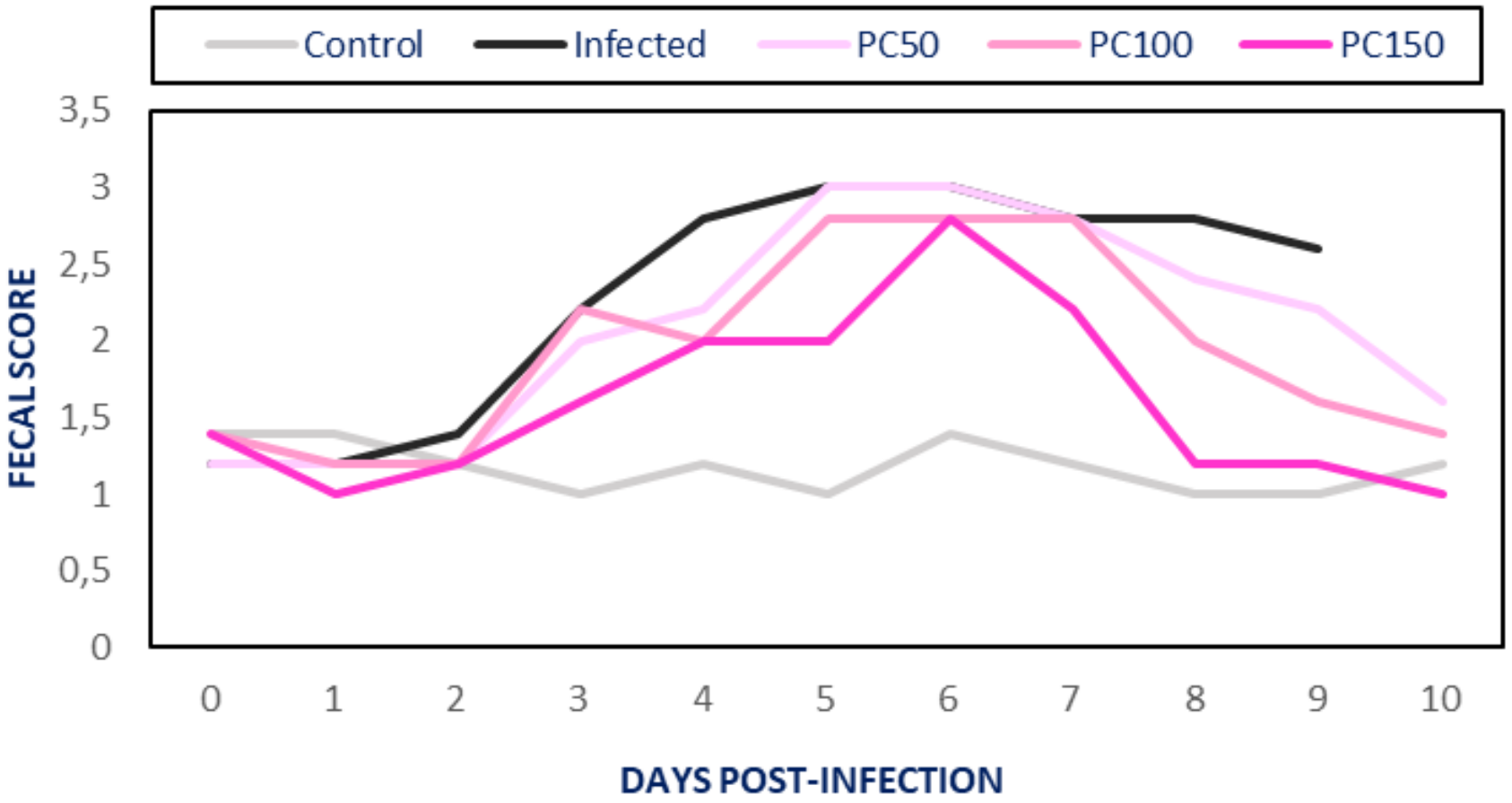
PC-15 reduced oocyst shedding

Animal studies



Unpublished data, Nutreco T2024-143

PC-15 reduced diarrhea



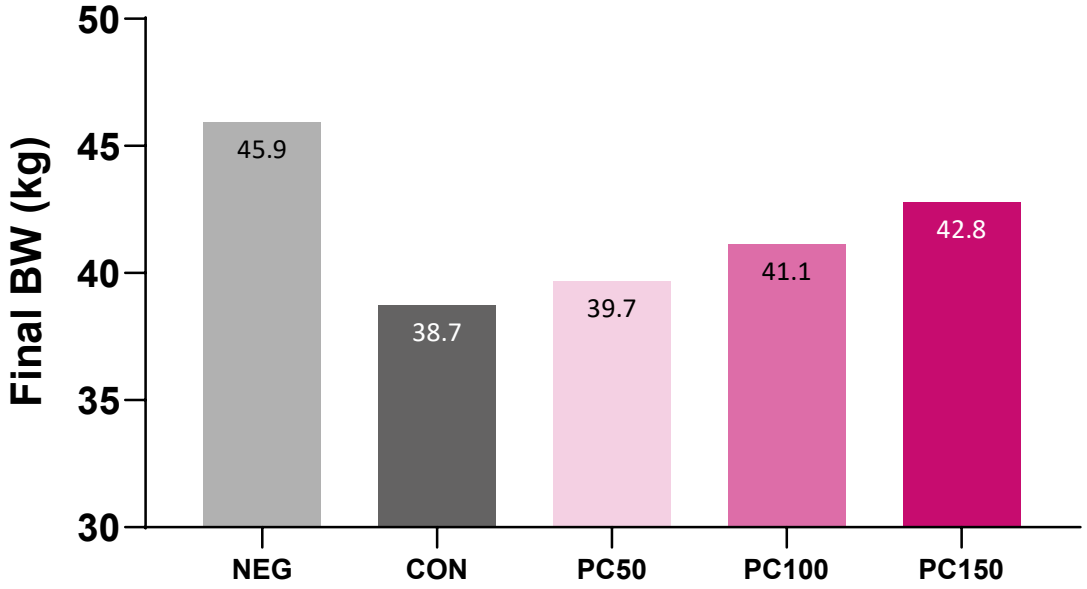
All infected control animals were euthanized on d10 due to illness

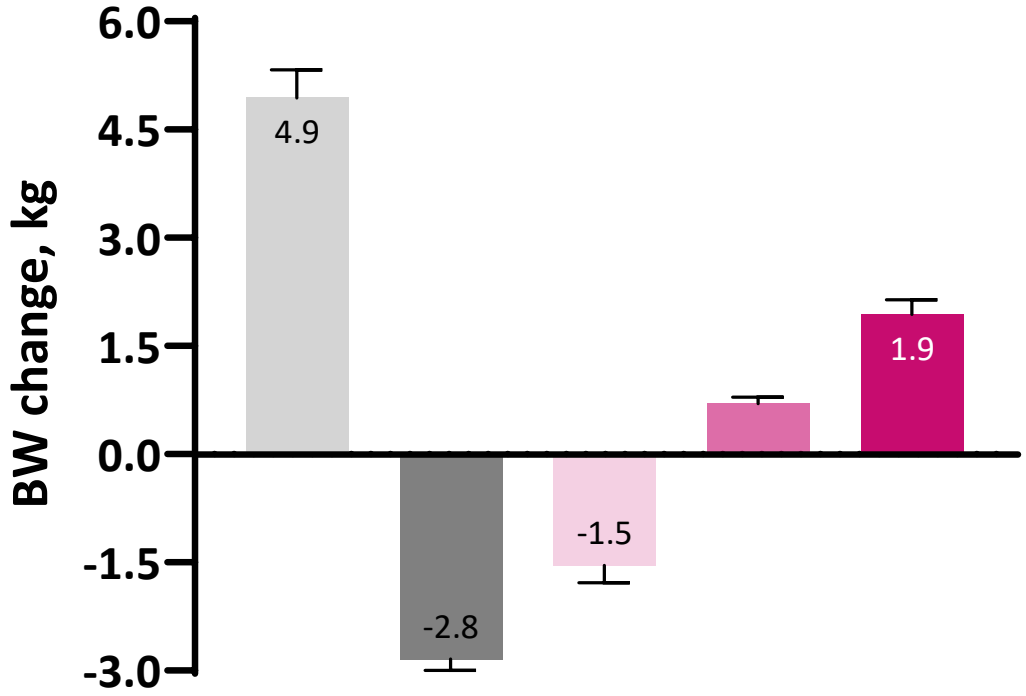
>> PC-15 reduced diarrhea dose-dependently

Unpublished data, Nutreco T2024-143

PC-15 improved performance of calves

Animal studies





Unpublished data, Nutreco T2024-143

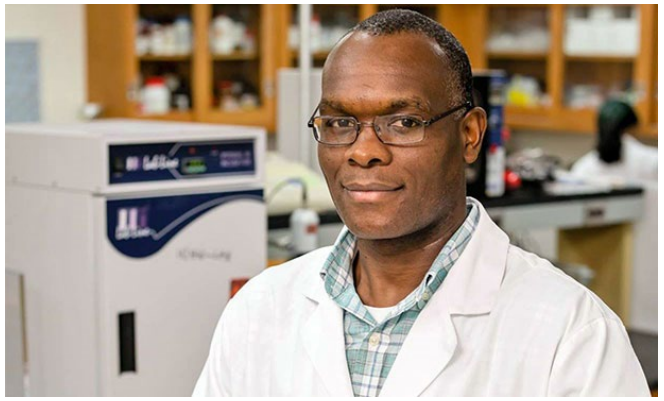
Overall summary

- Novel and unique approach at Nutreco enables us to discover and develop PhytoComplex with sound mechanisms and consistent efficacy
- PC-15 is the outcome of our work and shows the impact on the animal
  - Consistent effectiveness in reducing diarrhea in neonatal calves
  - Proven efficacy in 2 biological models (coccidiosis, cryptosporidiosis)
  - Proven efficacy in 3 animal species (chicken, mice, calves)
  - Optimized doses determined
  - Elucidated mode of actions – host mediated!
- Potentially positive impact on diarrhea caused by virus, bacteria, etc

Acknowledgment

Prof. Dr. Hyun Lillehoj

Prof. Dr. William Witola



Eile van der Gaast