monoguthealth
Optimal gut function in monogastric livestock

ICROFS
International Centre for Research in Organic Food Systems

Ministeriet for Fødevarer, Landbrug og Fiskeri
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AARHUS UNIVERSITY
DEPARTMENT OF FOOD SCIENCE
DEPARTMENT OF ANIMAL AND VETERINARY SCIENCE

Monoguthealth in Denmark:

Early interventions in pigs and broilers to improve their health and welfare

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

Monoguthealth in Denmark-
Two PhD projects

- Antibacterial plant combinations for better gastrointestinal health in piglets and broilers

Kevin Jerez-Bogota

- Poultry in Motion – pre-hatch interventions and the development of the locomotory system in broiler chickens

Tobias Kettrukat

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Antibacterial plant combinations for better gastrointestinal health in piglets and broilers

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Youngmonogastricsare vulnerable to gastrointestinal pathogens

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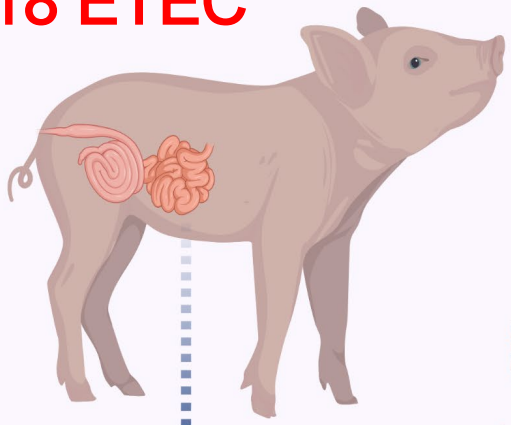
1-2 weeks post weaning

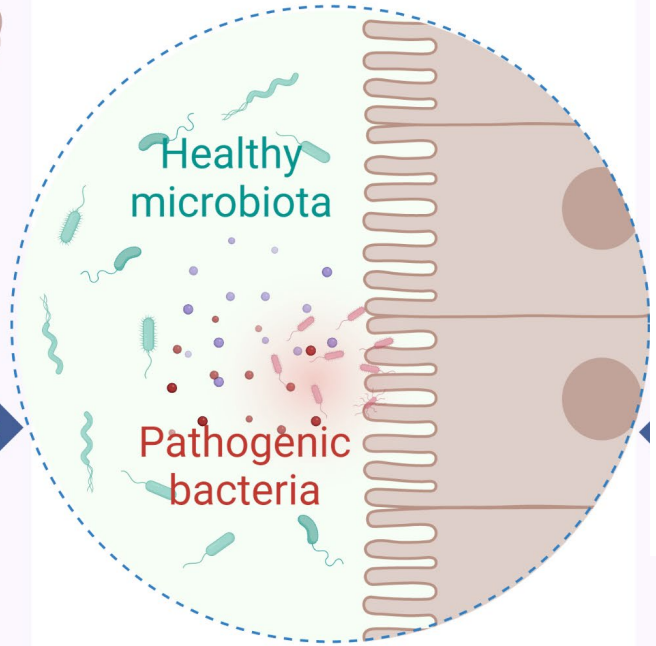
Postweaning diarrhea

F4 and F18 ETEC

PWD
- *Diarrhoea*
- *Growth retardation*
- *Mortality*



Antib NO GO oxide



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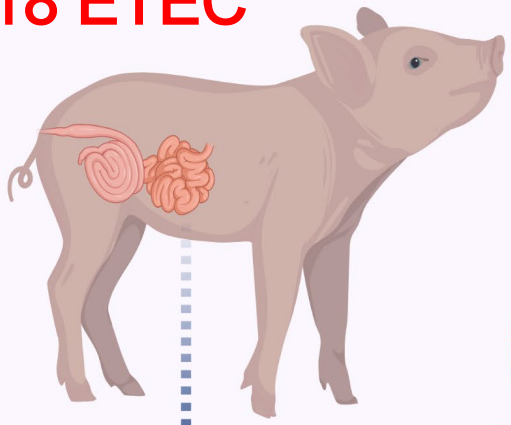
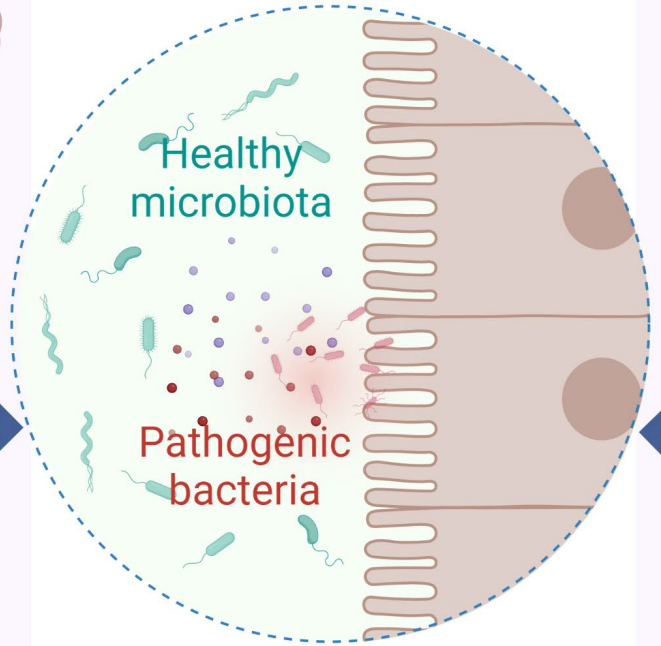


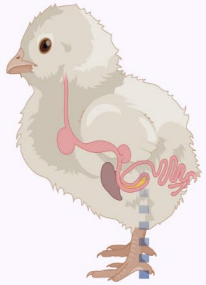


Antib NO GO oxide

2-6 weeks of age

Necrotic enteritis

***Cl. perfringens* (netB)**



Necrotic enteritis
- *Ruffled feathers*
- *Diarrhea*
- *Weight loss*
- *Mortality*

NO GO coccidiostats

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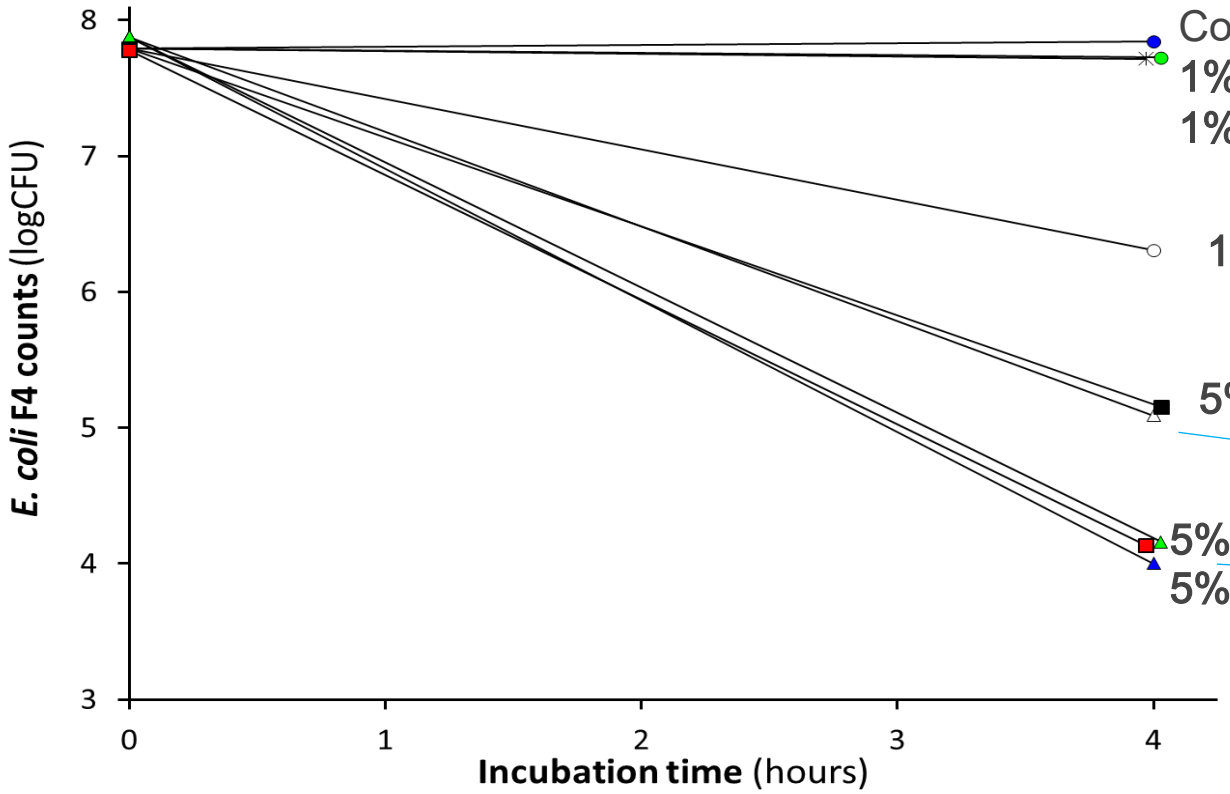
IN VITRO

Wild garlic high in allicin and acidic berries synergistic antibacterial action in stomach content



Synergistic effect …!

Canibe, et al. 2018. 14th International Symposium on Digestive Physiology of Pigs

Limitations

Wild Garlic (allicin) **+ Lingonberries** (acidity)

- Scalability: both scarce
- Does it work *in vivo*?

IN VITRO
E. coli vs. allicin content

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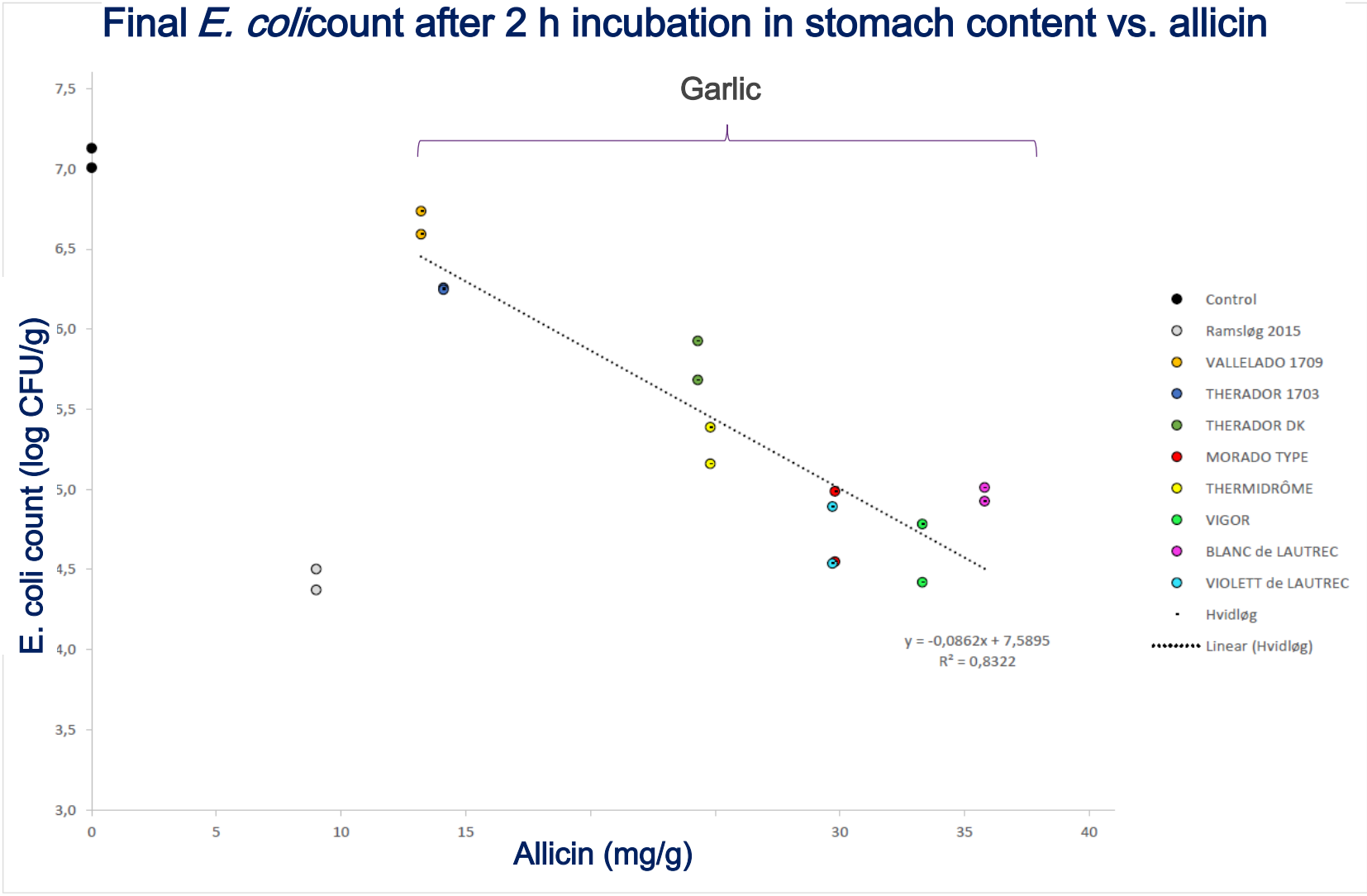
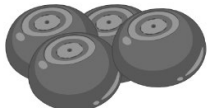


Alternatives

Wild Garlic + Lingonberries

- Garlic

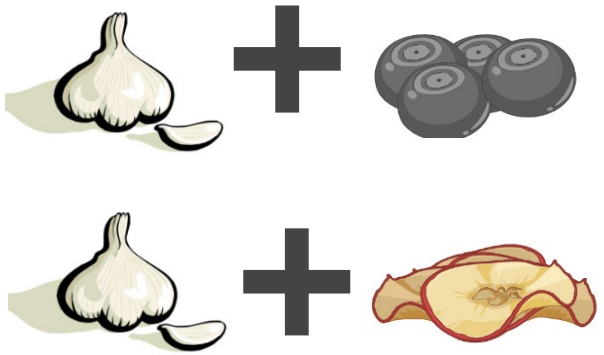
- Blackcurrant
- Apple pomace



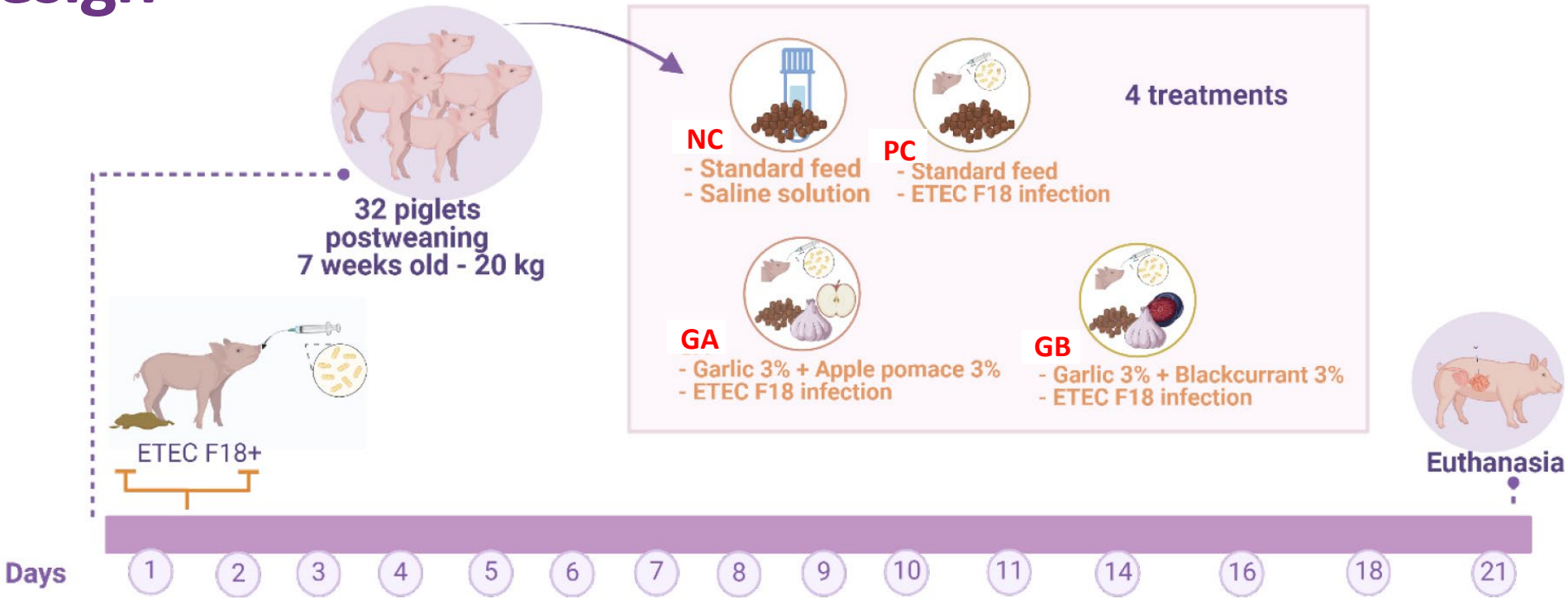
Hypotheses

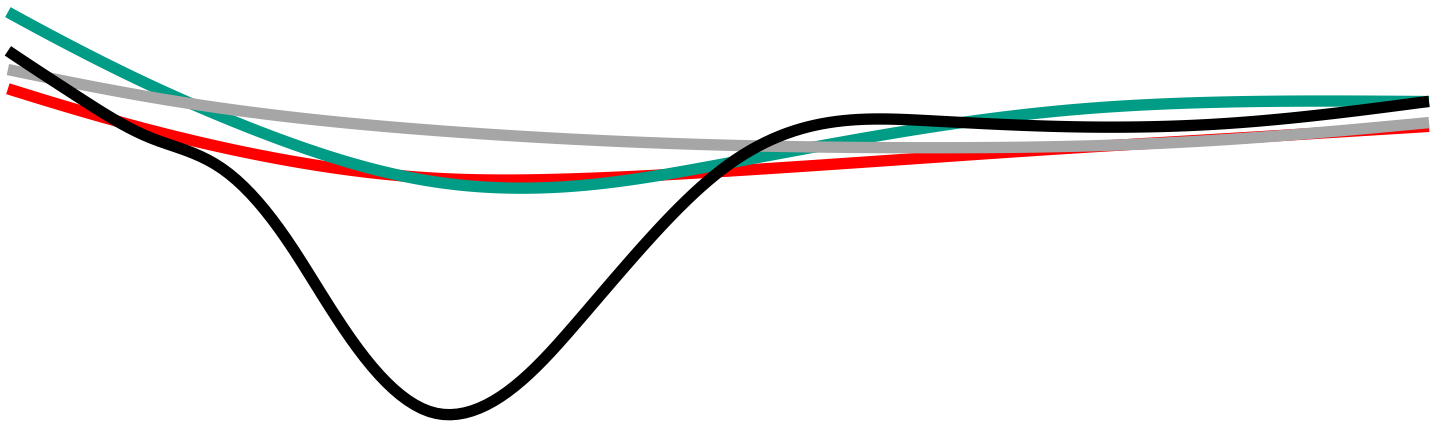
Dietary inclusion of garlic, and apple pomace or blackcurrants:

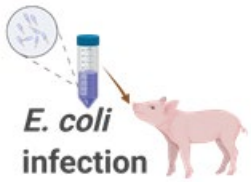
- Inhibit ETEC growth without harming the commensal microbiota.
- Prevent PWD under ETEC challenge.



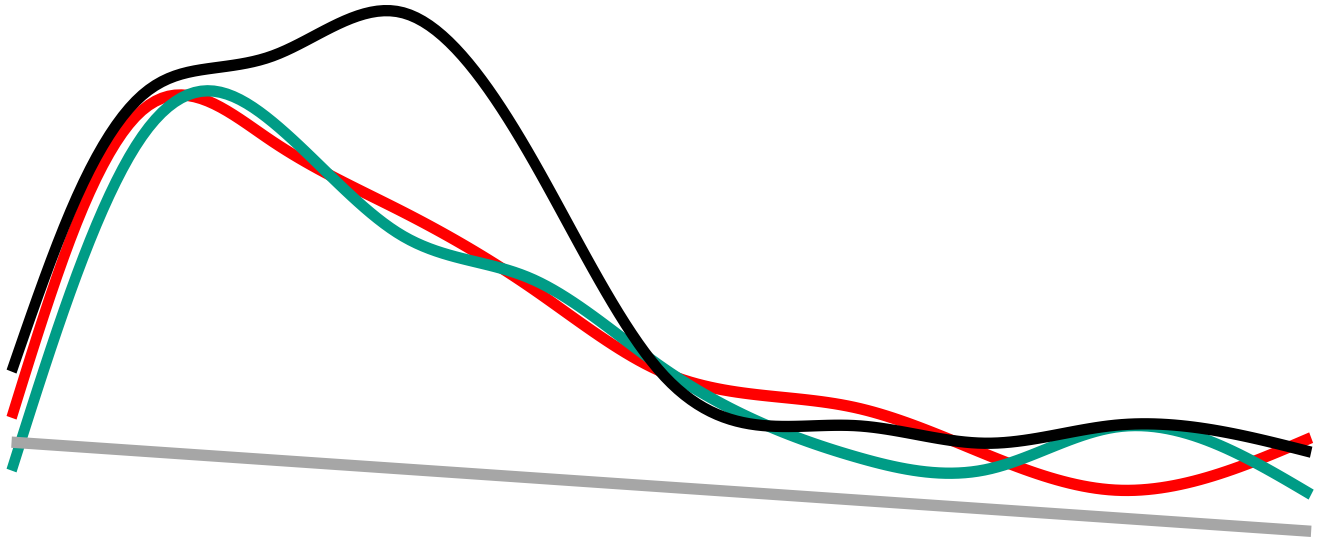
Study design

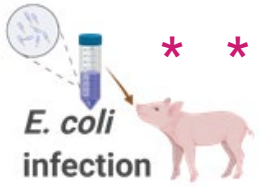




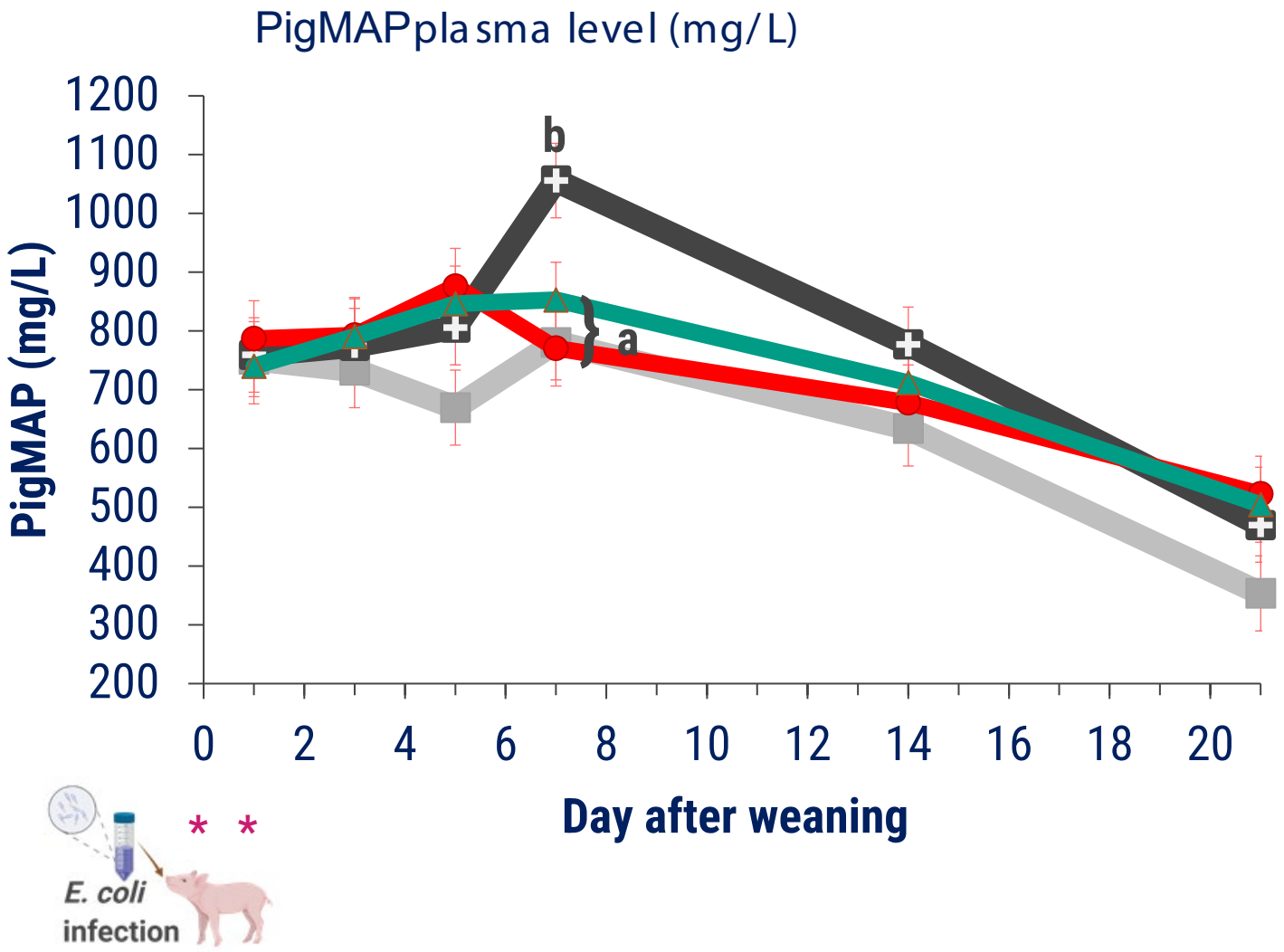

E. coli
infection

Plants reduce *E. coli* proliferation

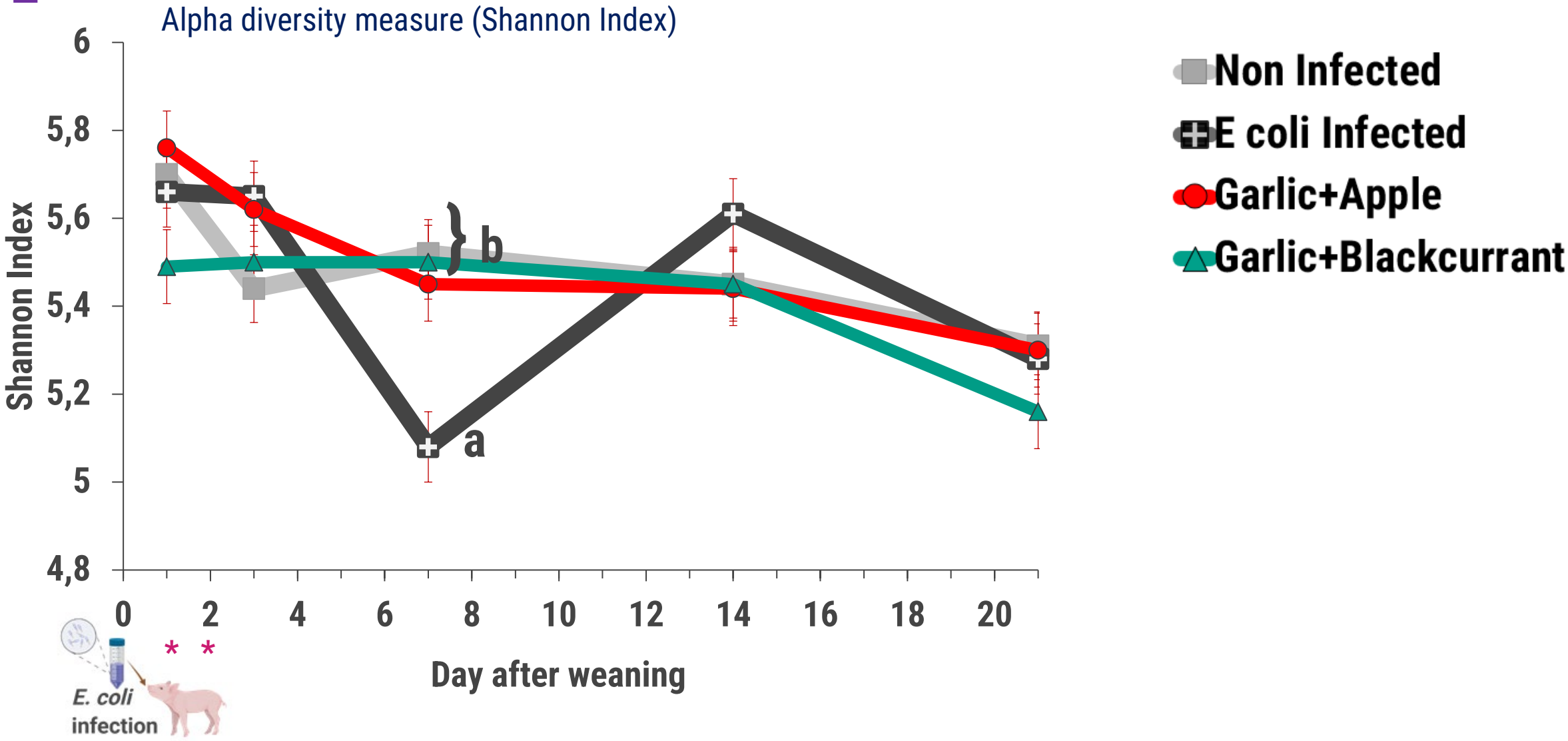




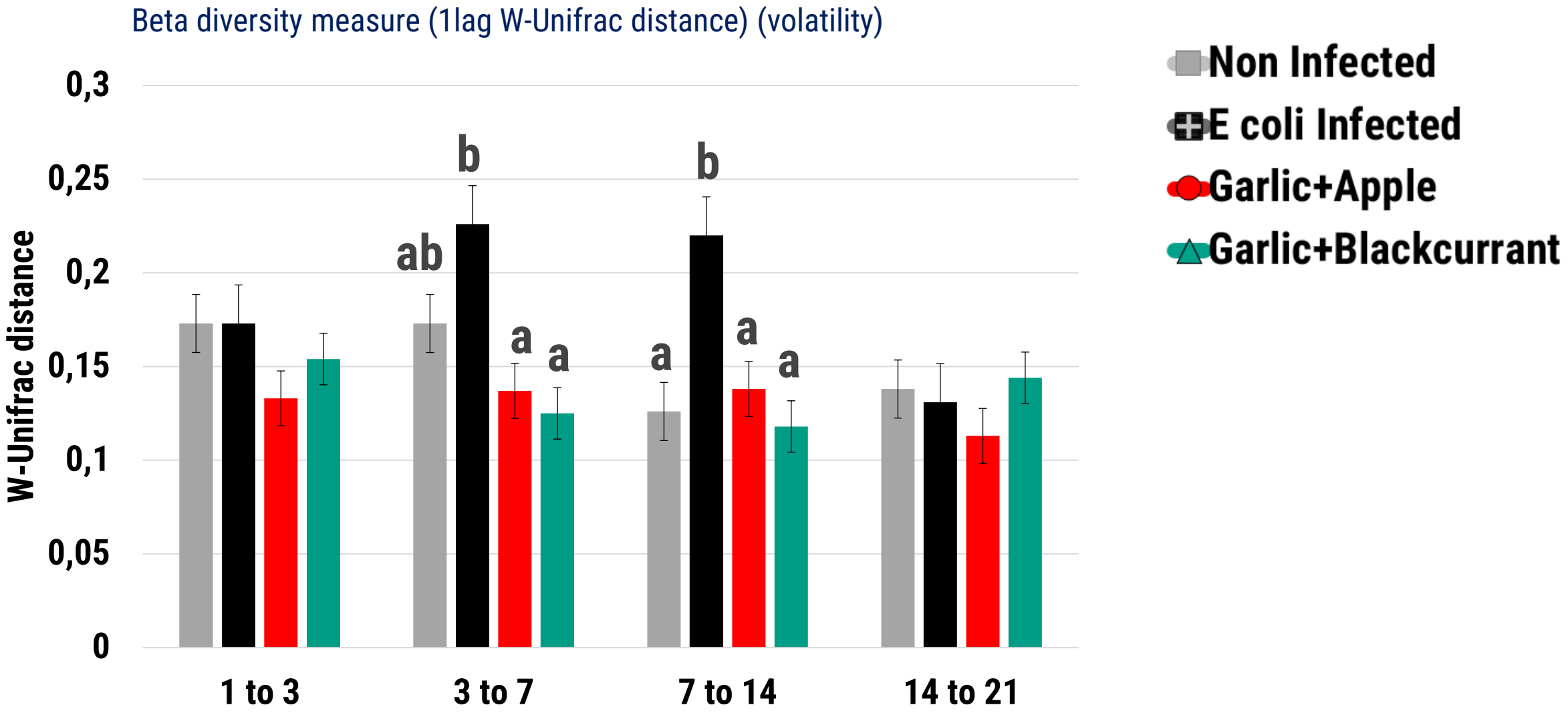
Plants reduce inflammation



Plants maintained the fecal microbiota richness and balance



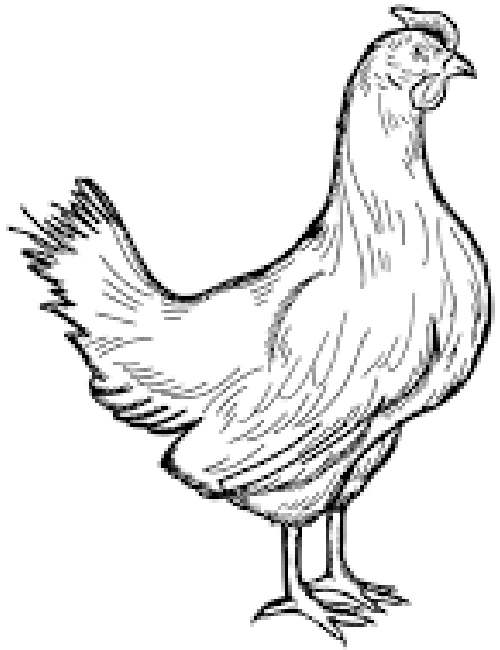
Plants promote a more stable microbiota



Conclusions

Garlic + Apple pomace or Garlic + Blackcurrant powder (3% and 3%)

- ✓ Prevented *E. coli* growth
- ✓ Prevented PWD
- ✓ Resulted in a more diverse microbiota
- ✓ Resulted in a more stable microbiota



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Optimal gut function in monogastric livestock

The influence of lauric acid and essential oils on the course of necrotic enteritis infection in broilers applying a subclinical disease model

Kevin Jerez-Bogota

Susceptibility of *C. perfringens* to lauric acid

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Inhibitory concentration of C_2–C_{18} fatty acids against *C. perfringens* strains CCM 4435 and CNCTC 5459 grown on glucose

Fatty acid	CCM 4435T	CNCTC 5459
C_2, C_3, C_4†, C_5†, C_6	>5	>5
C_8	1·92 ± 0·10	0·68 ± 0·06
C_{10}	0·28 ± 0·05	0·27 ± 0·06
C_{12}	0·04 ± 0·01	0·04 ± 0·01
C_{14}	0·08 ± 0·02	0·18 ± 0·01
C_{16}, C_{18}	>5	>5
$C_{18:1}$	0·48 ± 0·01	1·48 ± 0·05
$C_{18:2}$	>5	0·48 ± 0·30

Skřivanová, E. et al. Lett. Appl Microbiol., Volume 41, Issue 1, 1 July 2005, Pages 77–81, doi.org/10.1111/j.1472 - 765X.2005.01709.x

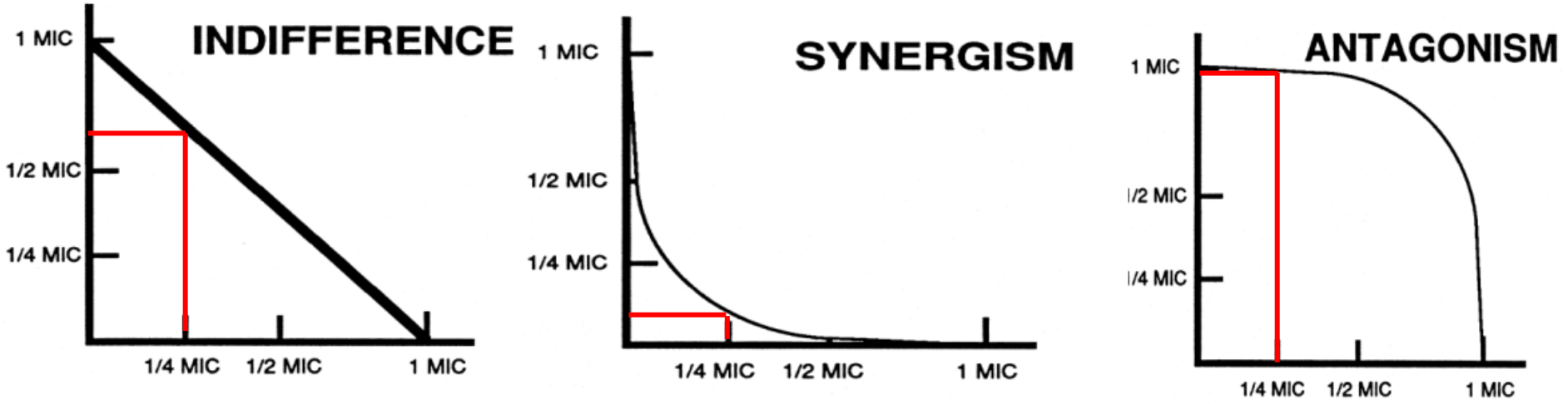
Lauric acid oils and EO reduced NE in broilers

Number of birds with macroscopic necrotic enteritis lesions and mean lesion scores on three sampling days

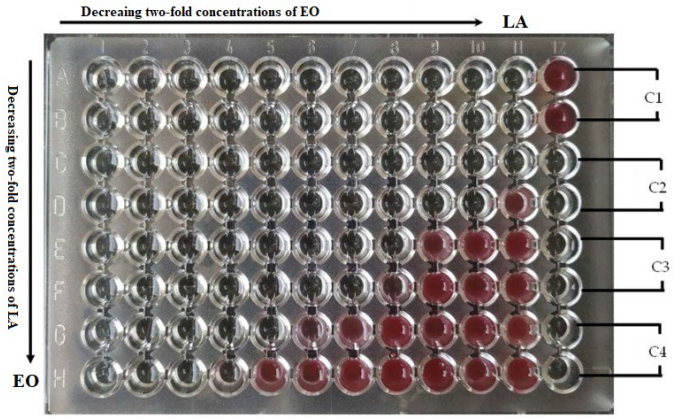
Group	Total	Total (%)	Mean score
Negative control	0/22	0	0
Only coccidia	0/21	0	0.43
Positive control	13/26	50	1.73
Butyric acid	6/26	23[B]	1.19
Lauric acid oil	6/24	25[B]	1
EO	6/25	24[B]	1.16
But/LA	2/25	8[B]	0.4
But/LA/EO	11/26	42	1.77

Timbermont, L. et al Avian Pathol., Volume 39, Issue 2, 2010, Pages 111–121, doi.org/10.1080/03079451003610586

IN VITRO
Synergism Testing: Microdilution Checkerboard

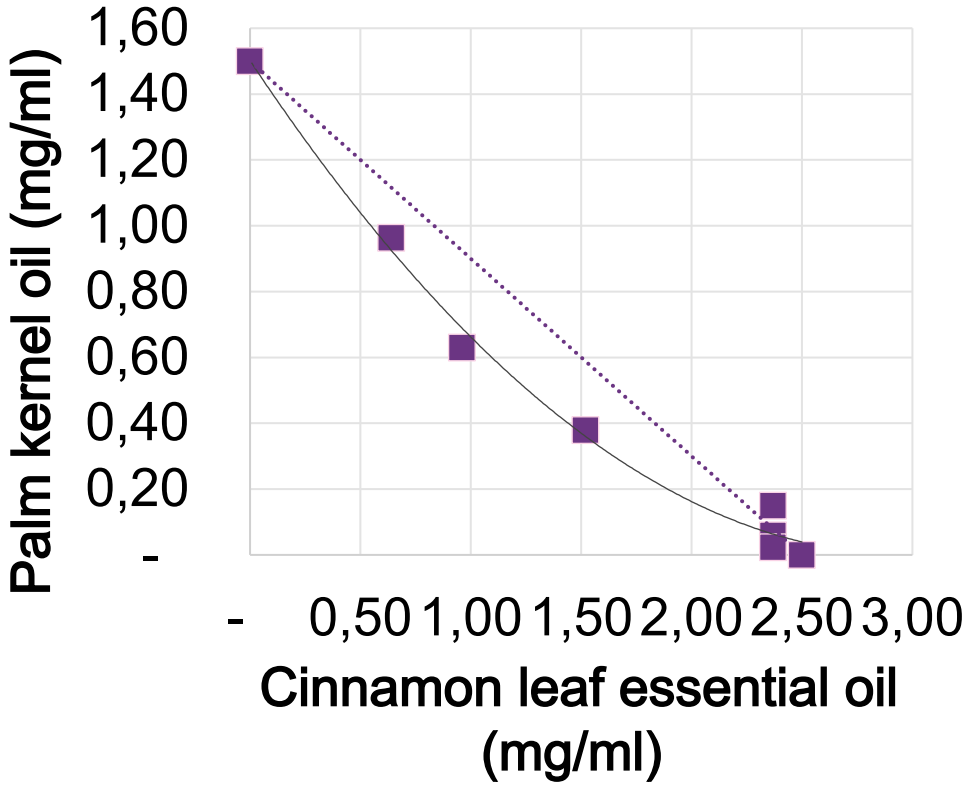


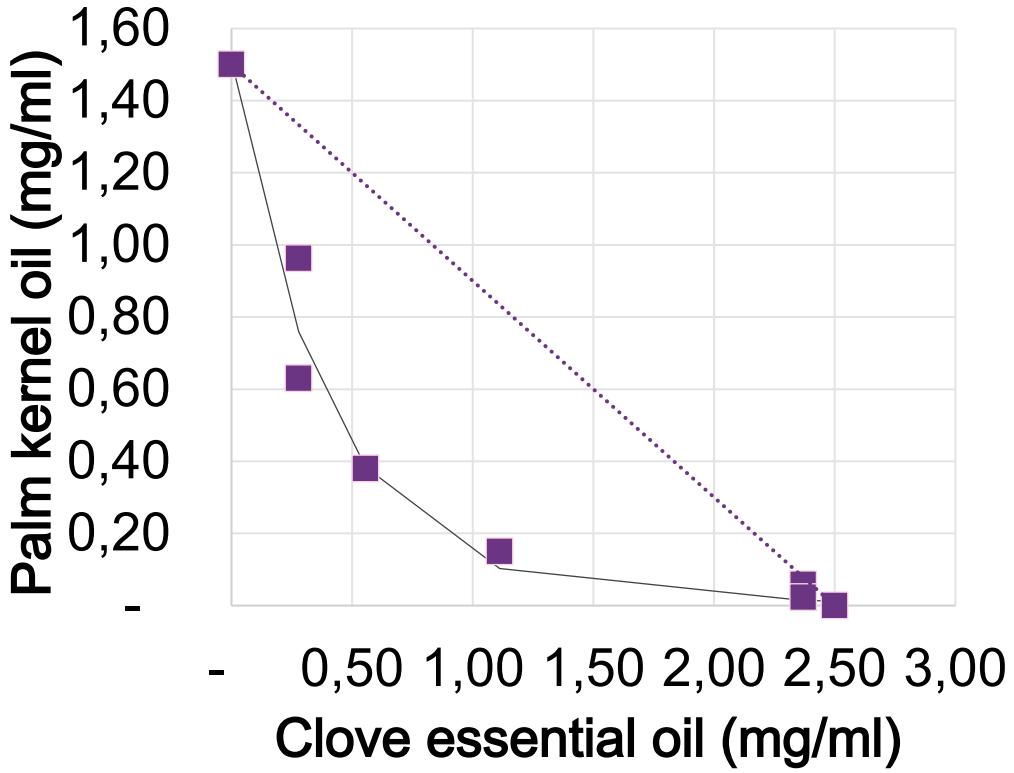
Name	Product information
Grolux™ Lauri	Split palm kernel fatty acids (94%) Typically, 47% C12:0 (lauric acid)
Oregano essential oil	Min. 70 % carvacrol, max. 20 % thymol
Thyme essential oil	Min. 35 % thymol and carvacrol
Anise essential oil	Min. 95 % trans-anethole
Clove essential oil	85 % eugenol
Cinnamon leaf essential oil	80 % eugenol, 2.5 % cinnamol



IN VITRO

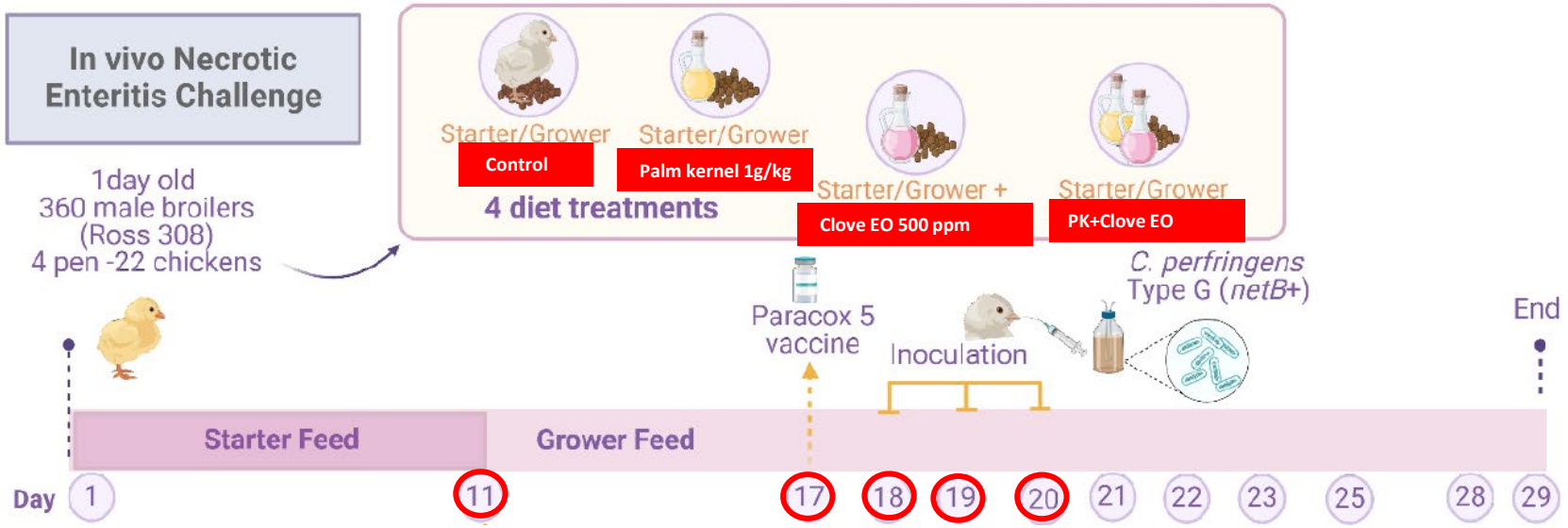
Synergism Testing: Microdilution Checkerboard





Intersection in the Y and X axis represents the MIC for palm kernel oil (Grolux Lauri; 1.5 mg/ml) and either ***cinnamon leaf*** *(2.5 mg/ml) or* ***clove*** *(2.5 mg/ml) essential oils.*

Study design



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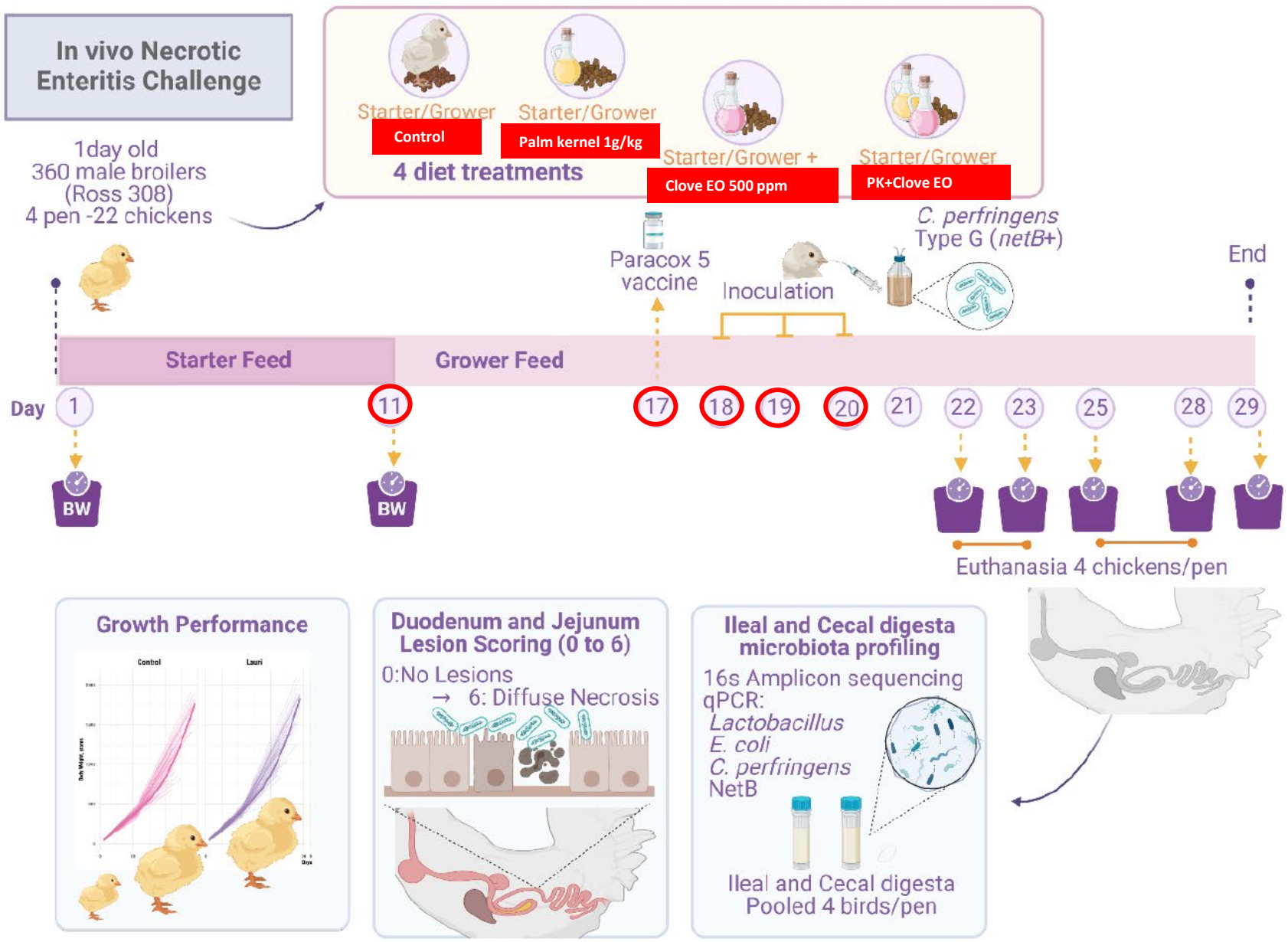
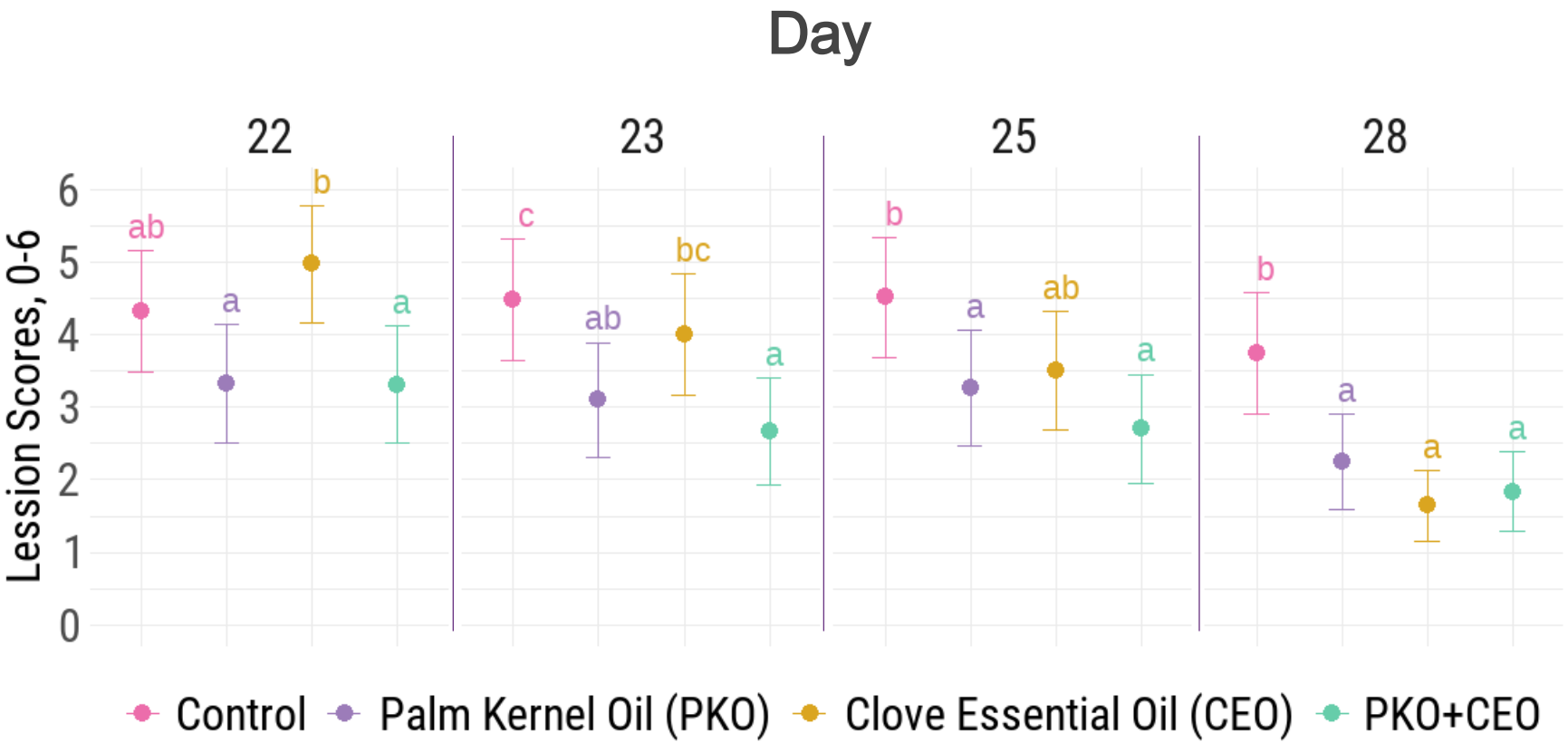
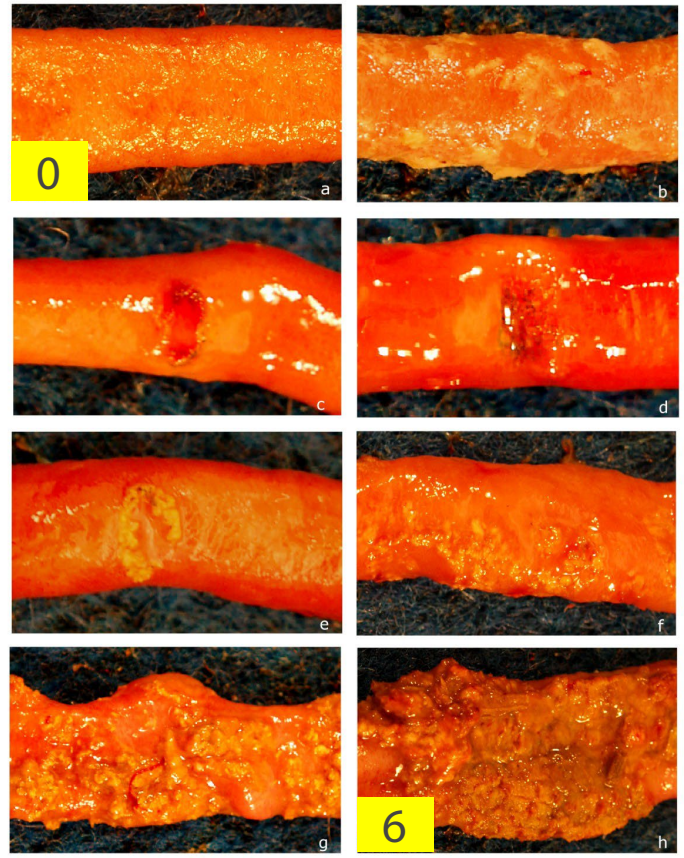


Growth performance

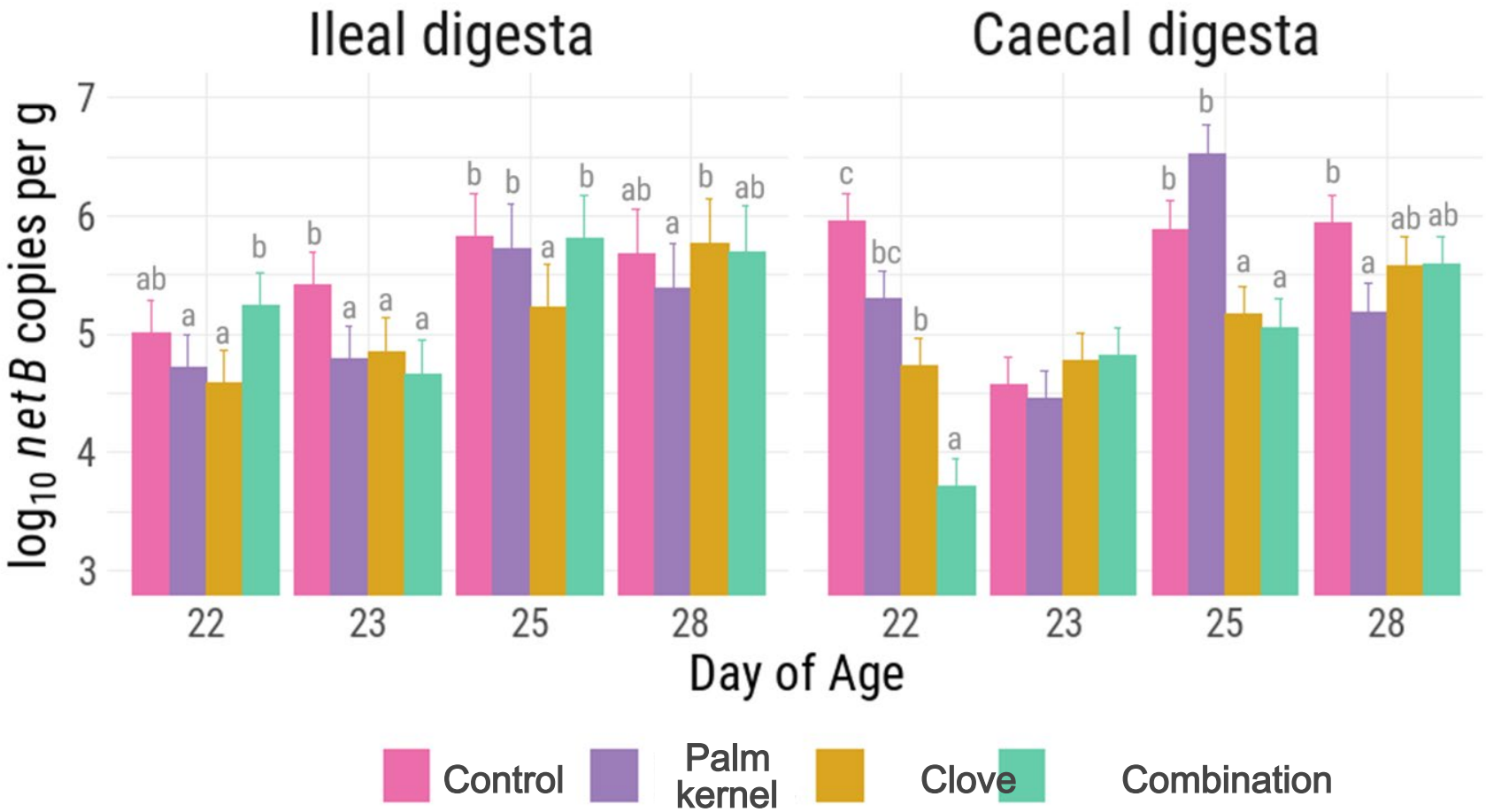
Item[1]	Control	Palm kernel	Clove	Combination	SEM	*P* - value
Day 1 to 11						
ADG, g	31.88	32.19	31.7	32.35	0.327	0.495
ADFI, g	33.17	33.11	32.91	33.29	0.348	0.891
G:F	0.96	0.97	0.96	0.97	0.007	0.474
Day 11 to 29						
ADG, g	64.09^{a}	66.79^{ab}	68.47^{ab}	72.27^{b}	2.572	0.045
ADFI, g	73.77^{a}	78.73^{ab}	78.69^{ab}	83.83^{b}	3.258	0.04
G:F	0.87	0.85	0.87	0.86	0.032	0.301

Lesion Scoring





NetB toxin in digesta



Conclusions

- **In vitro**: Palm kernel oil (lauric oil) and clove EO acted synergistically against the pathogenic *C. perfringens*
- **Performance**: the combination increased weight and feed intake after the first challenge element (diet).
- **Lesions** reduced after 23, but no synergy
- **NetB toxin**: reductions but not consistent

monogut health

Optimal gut function in monogastric livestock

The impact of early incubation temperature on development of the locomotory system of broilers and thereby on walking ability and meat quality

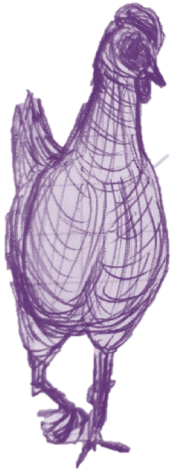
Tobias Kettrukat

Broiler locomotion

broiler

vs.

layer



- Shifted centre of motion due to high breast muscle weight; no similar selection on leg muscles
- More time spent on two legs (double support)
- Smaller strides, larger step width (instability)

Higher energy cost for standing/walking + rapid growth = high stress on the locomotory system

Welfare issue!

TIMING OF INTERVENTIONS

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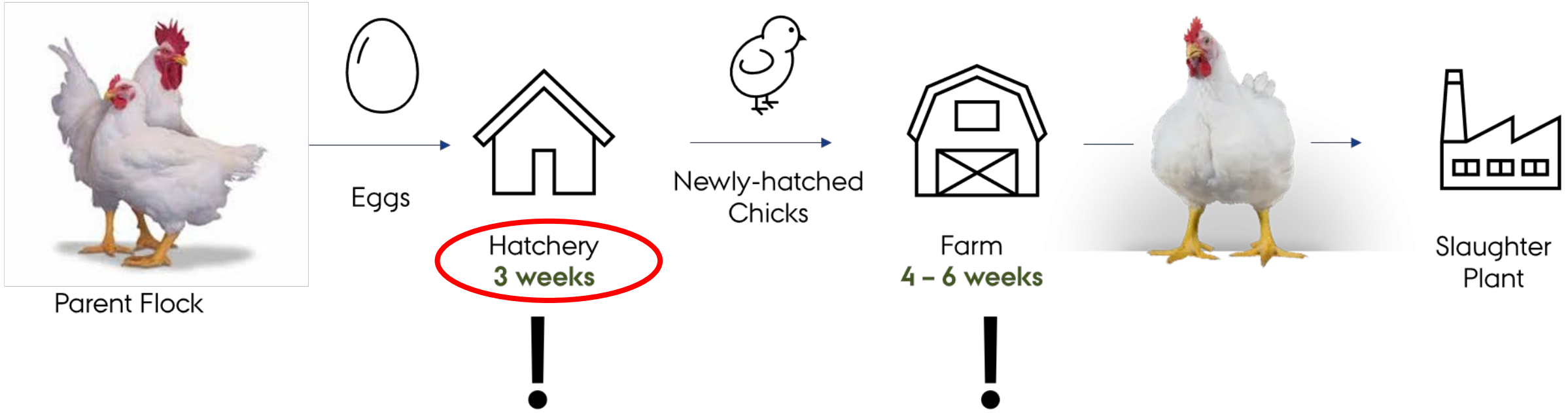
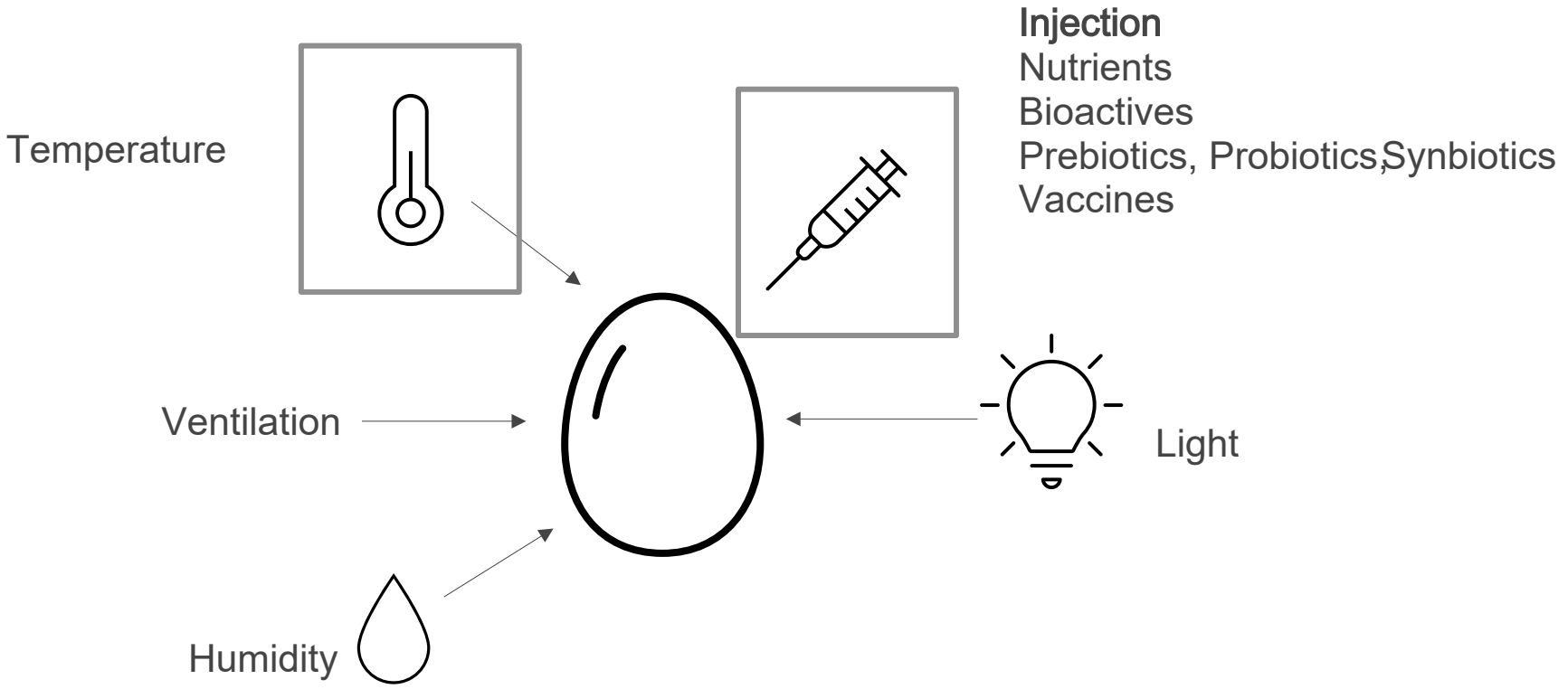


Influences in the Hatchery



Incubation Temperature and the Locomotory System

Livestock Science 276 (2023) 105326

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/livsci

The effect of incubation temperature on the development of the locomotory system and welfare in broiler chickens: A review

Tobias Kettrukat [a,*], Ewa Grochowska [b], Margrethe Therkildsen [a]

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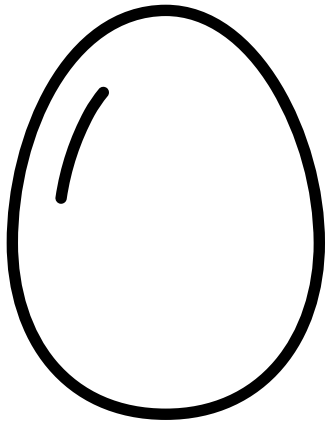
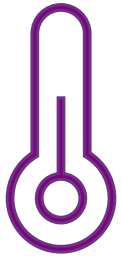
HIGHLIGHTS

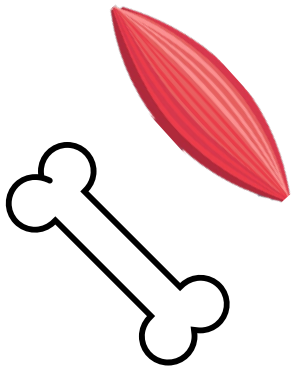
- Embryonic muscle responds to higher temperatures by proliferation.
- The timing of temperature manipulation is crucial for the resulting changes.
- Incubation temperature influences leg bone growth, mineralisation and breaking strength.
- The reflection of changes in the muscles and bones on walking ability needs more study.
- Future studies of temperature manipulation should include welfare assessments.

Incubation temperature and embryonic development



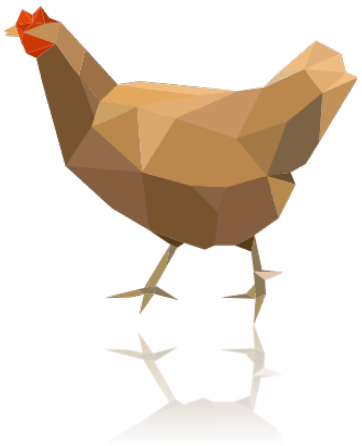


Incubation temperature



Muscle and Bone Development

Final walking ability?



AIM:
to investigate incubation temperature as a tool to improve the walking ability of broiler chickens by modifying muscle and bone development

The Experiment

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Day 0 — Day 4 — Day 8 — Day 10 — Day 18

400 eggs
(Ross-308)

8 incubators

4 Treatment groups:

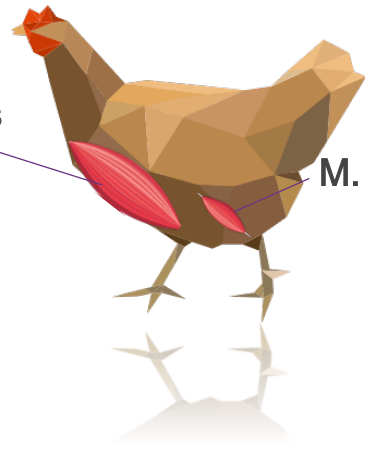
- 37.5°C (55% RH) control
- 38.5°C (65% RH)
- 39.0°C (65% RH)
- 36.5°C (55% RH)

Candling

Transfer to Hatcher

Hatch

Rearing for 35 days



- Walking ability
- Microstructure, gene expression, energy metabolism of M. gastrocnemius and M. pectoralis
- Performance
- Meat quality
- Tibia characteristics

Gait score

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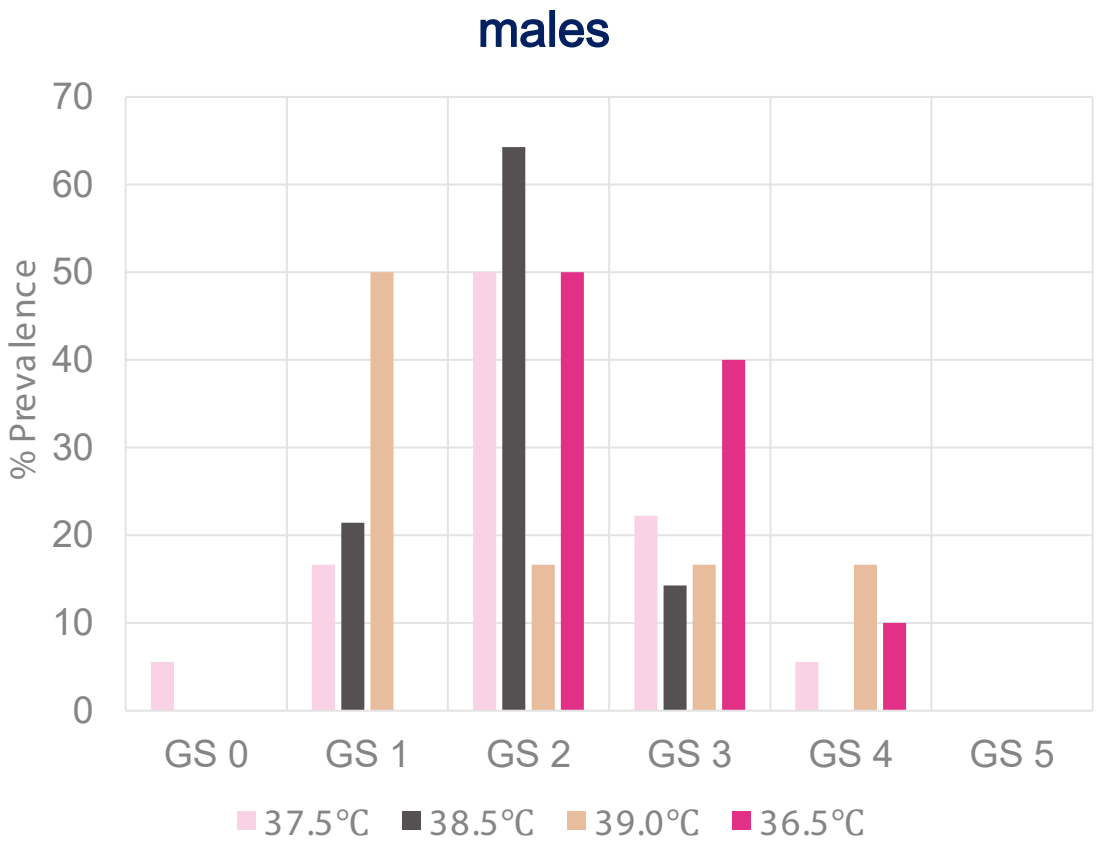
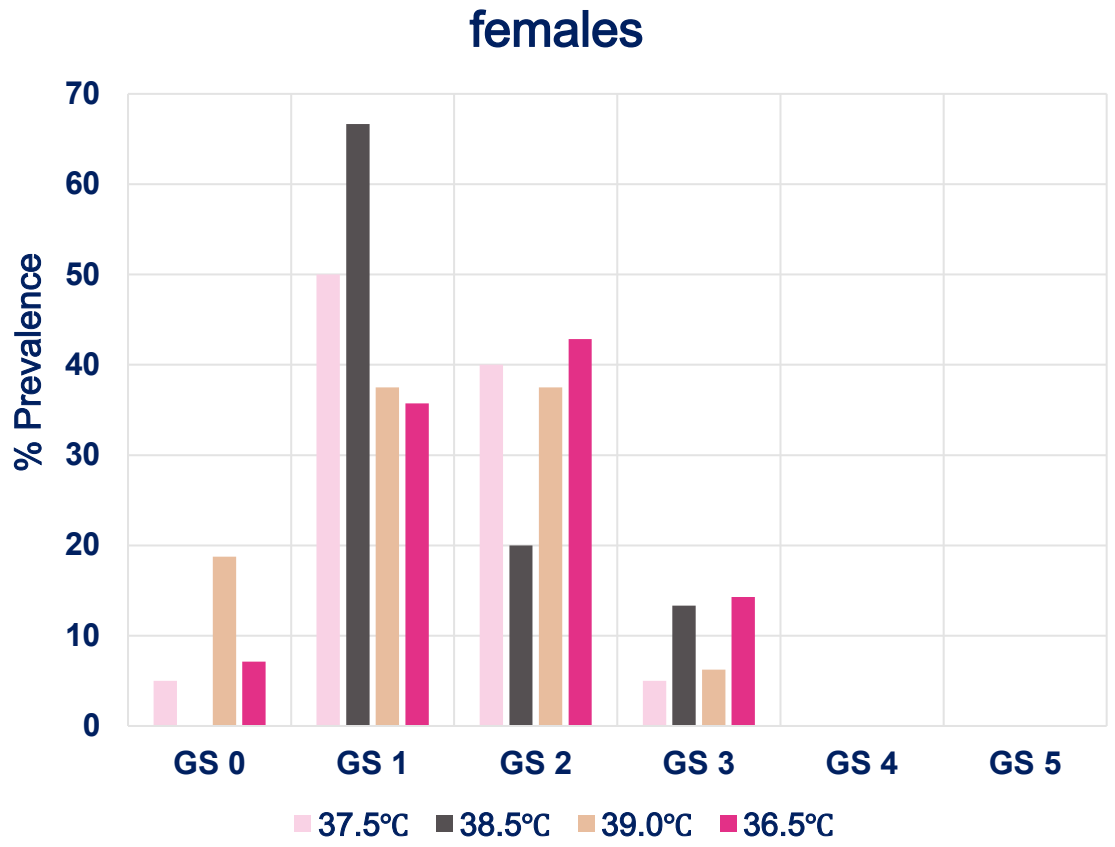
TABLE 1: The Bristol scale for scoring of walking ability (after Kestin *et al*[15])

Gait score	Criteria
GS0	No detectable abnormality, fluid motion.
GS1	Slight defect. Difficult to define.
GS2	Definite and identifiable defect, but it does not hinder movement.
GS3	An obvious gait defect that affects the broiler's ability to manoeuvre and accelerate.
GS4	A severe gait defect. The broiler will only walk a couple of steps if driven before sitting down.
GS5	Complete lameness. Either cannot walk or cannot support weight on the legs.

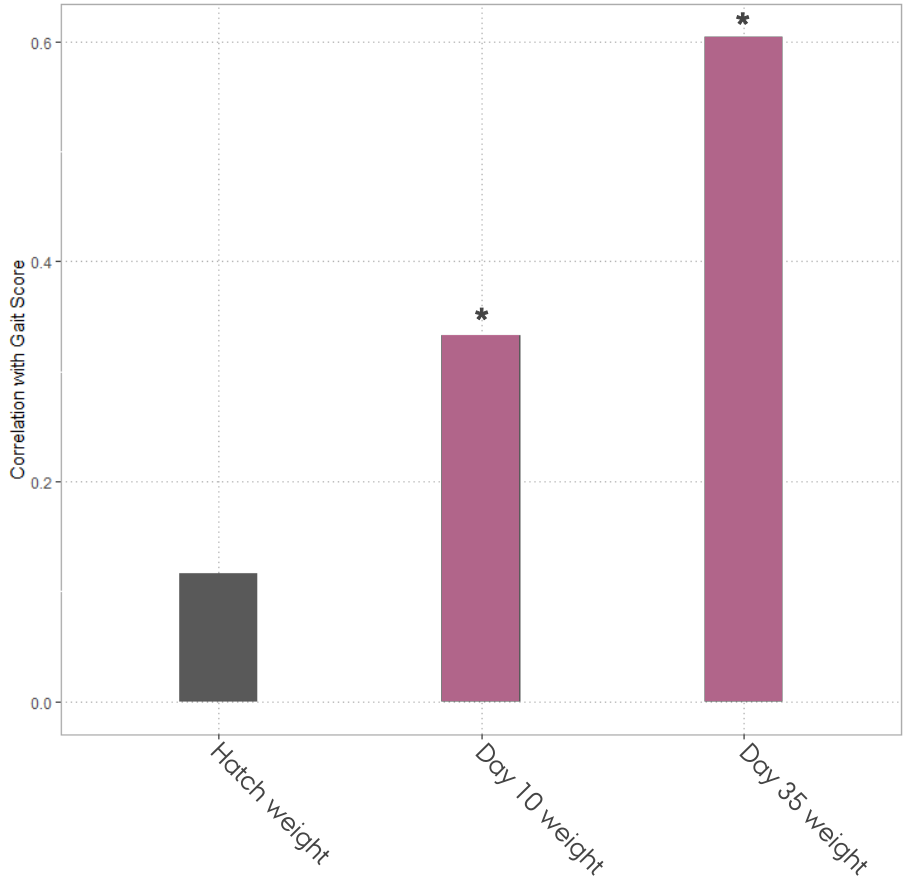
Incubation temperature and gait score

- Higher average gait score in group subjected to 36.5℃ than other groups
- Higher gait score in males than in females
- Likely related to higher body weight

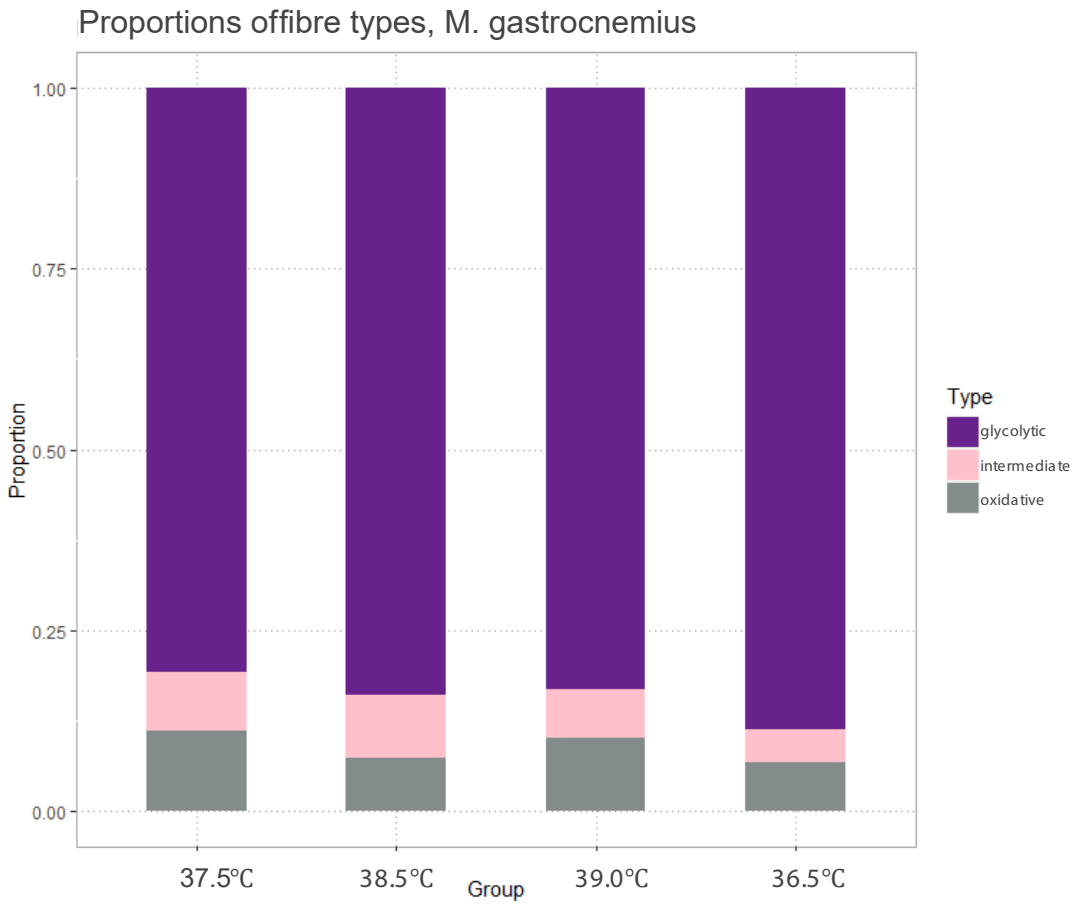


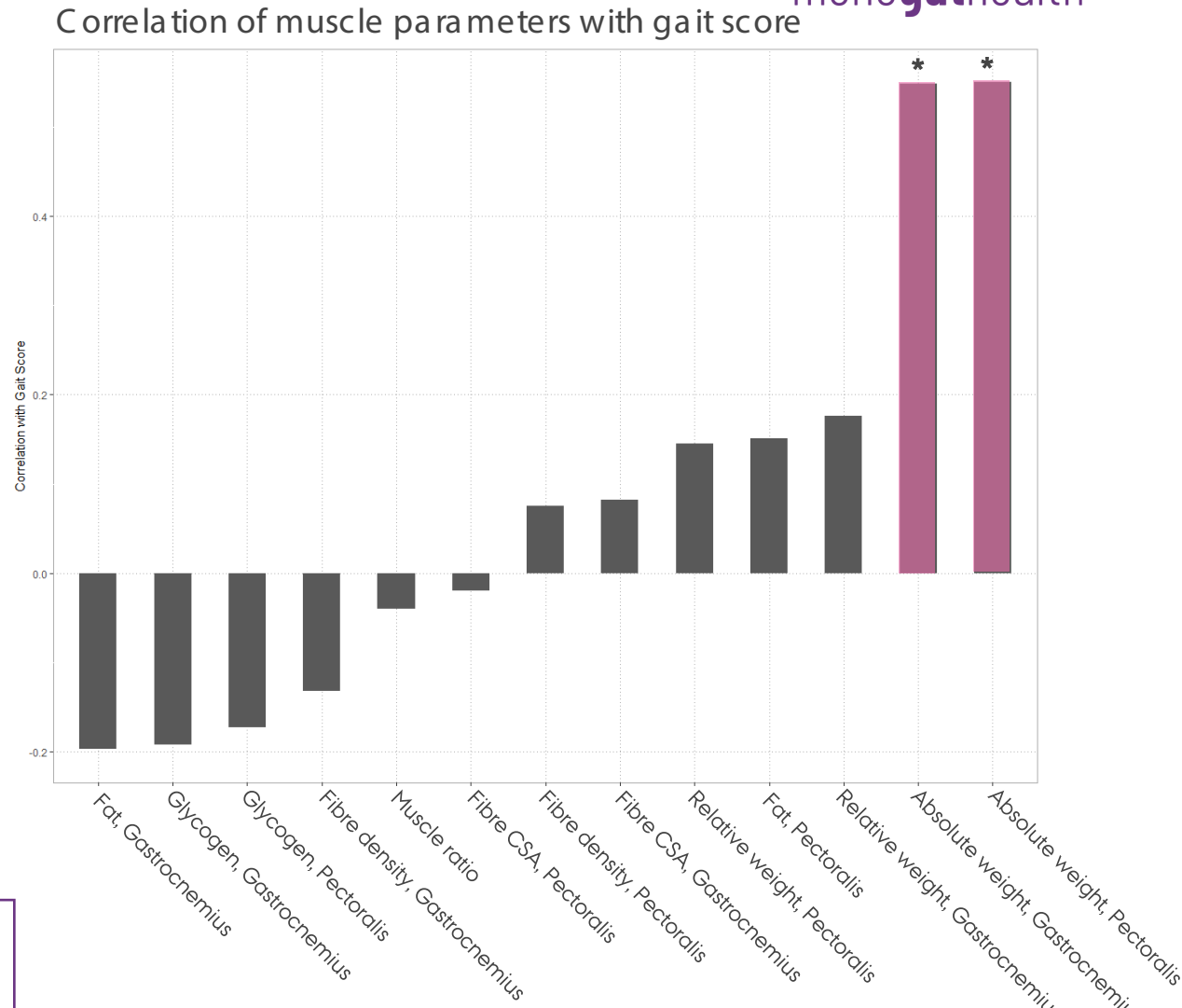


Performance vs. gait score



Muscle parameters



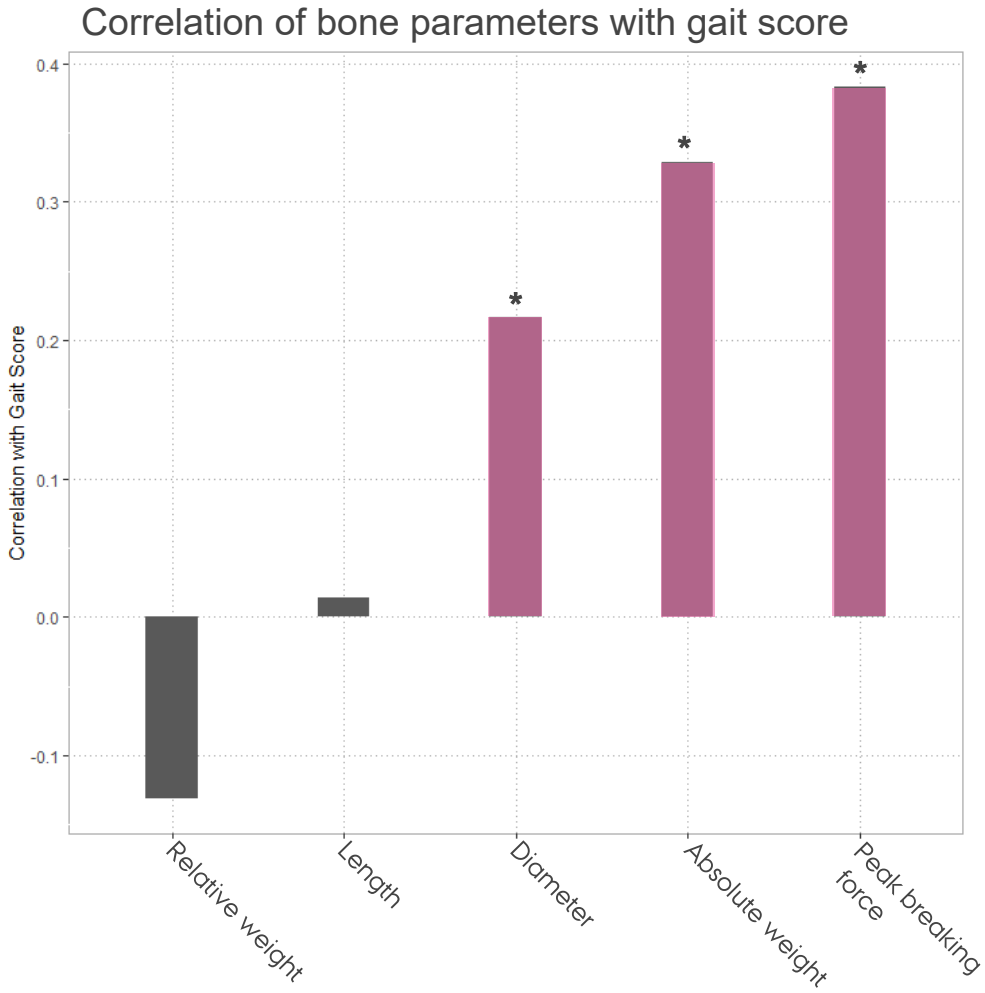


- Incubation temperature did not influence muscle microstructure
- Most muscle parameters were not correlated with walking ability

Bone parameters

	37.5°C		38.5		39.0		36.5		T	S	T x S
	m	f	m	f	m	f	m	f			
Length (mm)	95.2	94.5	96.4	94.8	95.6	94.1	95.8	94.2	0.77	0.02	0.92
Weight (g)	13.4	12.3	14.0	11.8	14.0	11.1	14.4	11.4	0.91	<.001	0.07
Relative Weight (%)	0.41	0.44	0.39	0.45	0.47	0.42	0.45	0.41	0.84	0.08	0.18
Diameter (mm)	7.9	7.6	7.7	7.3	7.9	6.7	7.8	6.9	0.22	<.001	0.26
Peak Force (N)	372	320	361	318	344	305	390	329	0.29	<.001	0.91

- Incubation temperature did not alter bone traits
- Relation of bone parameters with walking ability could be traced back to overall size/weight of the chickens



Meat quality

	37.5°C		38.5		36.5		T	S	T x S
	m	f	m	f	m	f			
pH_{24h}	5.87	5.84	5.85	5.83	5.87	5.84	0.80	0.09	0.96
Drip $loss_{48h}$ (%)	3.5	2.8	3.2	3.1	2.6	3.2	0.79	0.80	0.23
Cooking loss	15.7	18.0	17.7	17.7	17.2	18.0	0.43	0.06	0.29
Lightness	58.28	56.95	58.34	56.70	57.94	57.52	0.92	0.01	0.50
Redness	1.30	1.57	1.45	1.10	1.24	1.45	0.64	0.74	0.12
Yellowness	6.60	6.75	6.29	6.50	6.49	6.79	0.41	0.24	0.94
Force (N) (texture)	20.29	20.28	20.24	20.92	20.06	19.76	0.76	0.87	0.86

No detrimental effect of incubation temperature on meat quality

Conclusions

- Early incubation temperature manipulation did not result in changes in the muscles or bones that affected the final walking ability
- Gait score differences between the groups were a result of different body weights
- Leg muscle traits apart from the weight do not seem to be related to walking ability
- The sex of the chickens influenced almost all parameters measured, including gait score
- Gait scoring is a subjective method – future experiments should try to include other methods for quantifying chicken activity and mobility

Perspectives

- Plant materials
  - Influence on stress
  - Influence on feed intake of offspring via the dam
  - Chemical synthesis of phytochemicals

- Walking ability and animal welfare in broilers
  - Genetic selection for
    - better walking ability
    - slower growth
    - more animal welfare-friendly anatomy
    - better immune system

monogut health

THANK YOU!

ICROFS
International Centre for Research in Organic Food Systems

Ministeriet for Fødevarer, Landbrug og Fiskeri
gudp

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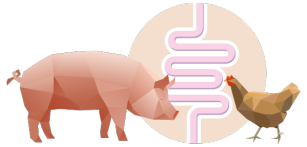
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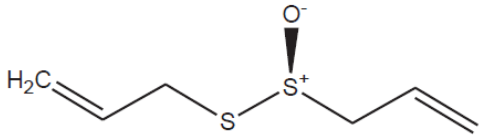
Allicin mechanism….!?!



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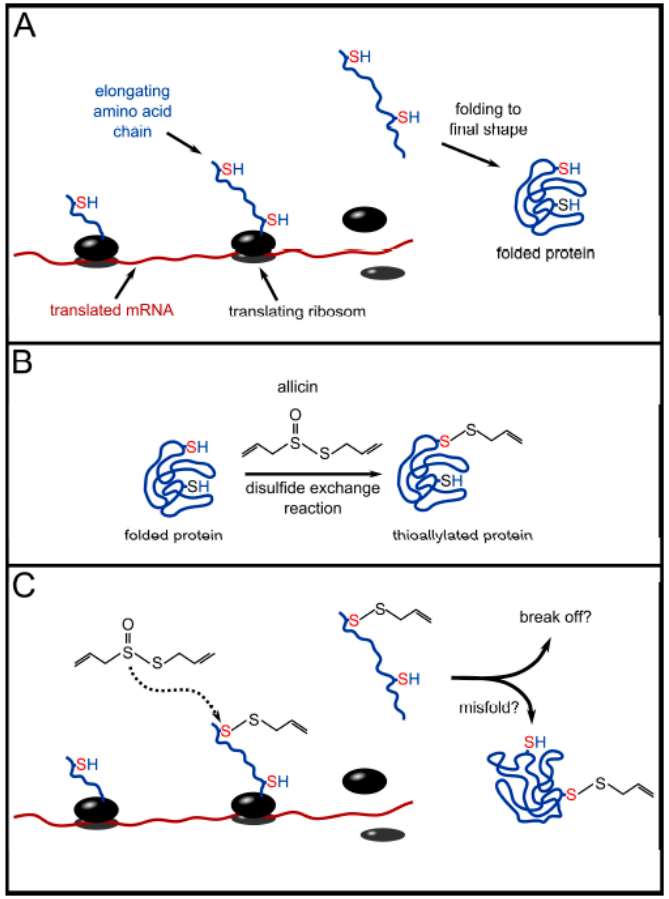
- Potential mechanism of allicin…

- Normal protein synthesis (A)
- The cysteine/methionine residue (accessible for attack) reacts with allicin via a disulfide exchange reaction => compromised protein/enzyme activity (B)
- Allicin may attack cysteine residues during protein synthesis => protein is misfolded (C)



Cavalitto 1946; Borlinghaus et al 2014