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Optimal gut function in monogastric livestock

PROPHYBIOTICS, A NOVEL APPROACH FOR *IN-OVO* GUT MICROBIOME REPROGRAMMING OF BROILERS

Ramesha N. Wishna-Kadawarage (ESR3)

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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.

Abstract

PROPHYBIOTICS, A NOVEL APPROACH FOR *IN-OVO* GUT MICROBIOME REPROGRAMMING OF BROILERS

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Bioactives administered *in-ovo* may reprogram the gut microbiome of chickens before they are exposed to environmental pathogens. Although the antimicrobial and gut microbiome modulation potential of probiotics and phytobiotics alone have been studied intensively, their combined use in *in-ovo* model has yet to be investigated. We coined the term **Prophybiotics** (**pro**biotic + **phy**tobiotics) to describe such a combination. The current study aims to screen and validate the effects of prophybiotics in an *in-ovo* model to determine if their application can mitigate pathogenic stress in broilers. Six lactic acid bacteria, *Lactiplantibacillus plantarum, Lacticaseibacillus casei, Limosilactobacillus reuteri, Lacticaseibacillus rhamnosus, Leuconostoc mesenteroides* and *Pediococcus pentosaceus* and three plant extracts, turmeric, green tea and garlic were included for *in-vitro* screening. Growth curves with plant extract supplementation and antimicrobial assays against *Salmonella* and *Campylobacter* were performed to select the most effective anti-pathogenic and synergistic combination for *in-ovo* validation. *L. mesenteroides* (LM) with garlic (G) presented as the most promising prophybiotic *in-vitro*. Therefore, this prophybiotic and its probiotic component alone were injected to ROSS308 broiler hatching eggs on day 12 of incubation to validate the beneficial effects on the gut microbiome *in-vivo*. The hatchability of LM+G and LM alone treated groups were higher and lower, respectively than that of positive control (injected with physiological saline). The weight of chicks on day one was highest in LM group followed by LM+G group (p value<0.05). The chick length and Pasgar score were not significantly different between the groups although LM+G treatment resulted in the highest chick quality (Pasgar score 9). Moreover, the impact of LM+G and LM on the gut microbiome and physiology of the chickens will be presented. This project is funded by European Union's Horizon 2020 research and innovation program (Grant agreement N° 955374).

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Content

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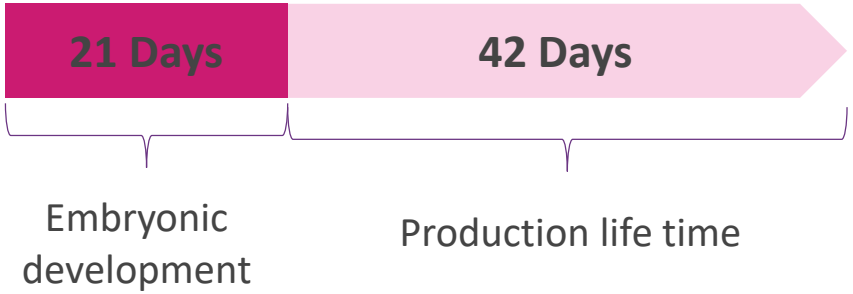
Background

Broiler chicken lifespan



Key events related to gut health happening during **embryonic development**

- Development of **immune system**
- Development of **gastrointestinal tract**
- Development of **gut microbiome**

ESR 3 project

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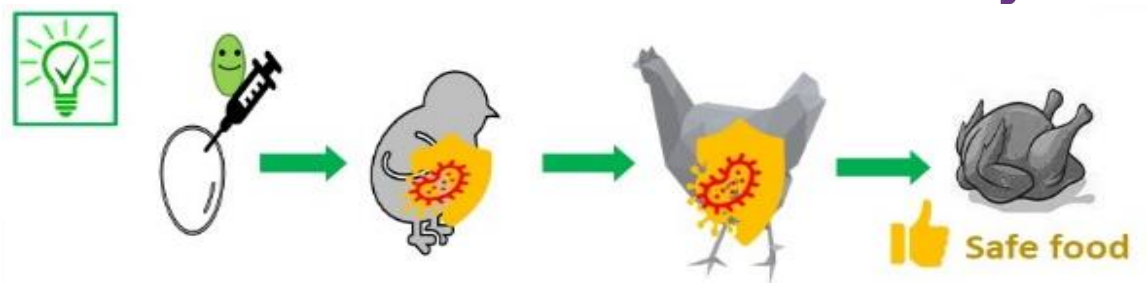

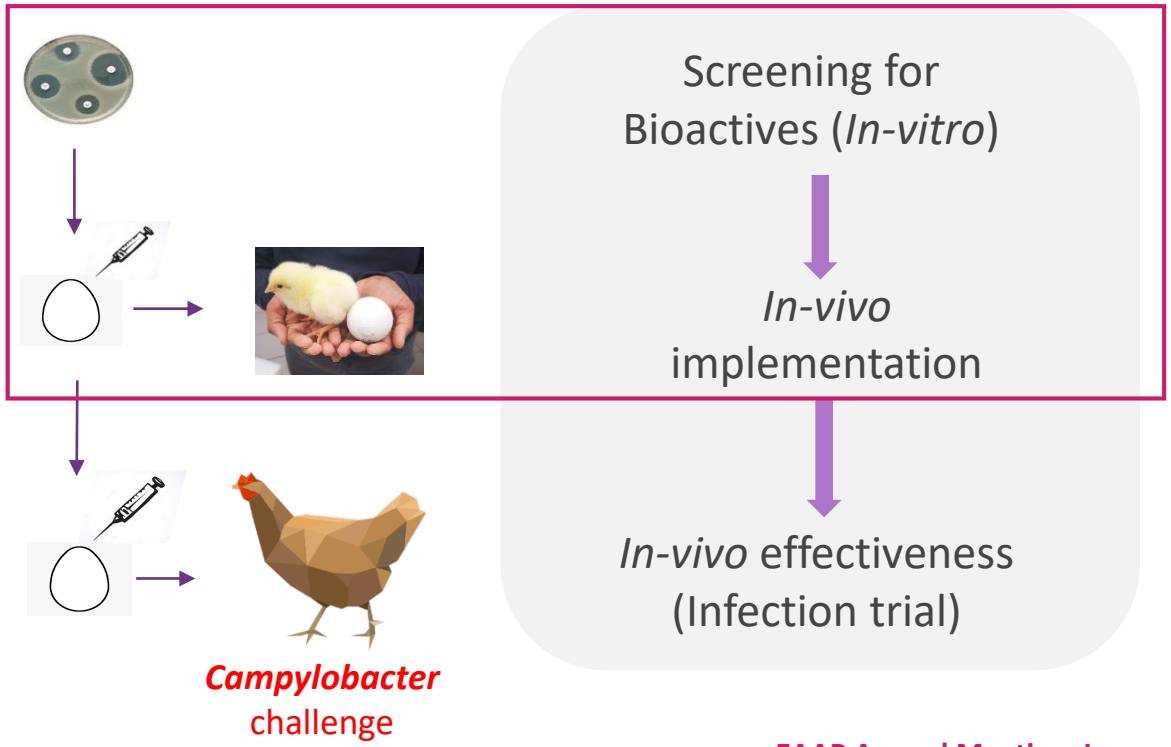

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Screening for bioactives (*In-vitro*)

Probiotics + **Phy**tobiotics = **Prophybiotics**

Research questions

- What is the **best plant extract** to be used in **combination** with the probiotics and at what dose?
- What is the **best probiotic** in terms of *in-vitro* **anti-pathogenic** activity?
- Does *in-ovo* application of **prophybiotics** adversely affect the **hatchability and quality of chicks**?

Objectives:

- To determine effects of supplementation of different plant extracts in variable doses on *in-vitro* growth of probiotics
- To determine anti-*Salmonella* and anti-*Campylobacter* effects of probiotics *in-vitro*
- To determine the effects of *in-ovo* application of the selected pro/prophybiotic on the hatchability and chick quality parameters

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Materials and Methods

Probiotic strains:

(JHJ Sp Zo.o, Poland)

1. *Lactiplantibacillus plantarum*
2. *Lacticaseibacillus casei*
3. *Limosilactobacillus reuteri*
4. *Lacticaseibacillus rhamnosus*
5. *Leuconostoc mesenteroides*
6. *Pediococcus pentosaceus*

Positive control: *Lactococcus lactis*

Plant supplements:

Aqueous extracts of

1. Turmeric
2. Green tea
3. Garlic

Pathogenic strains:

1. *Salmonella typhimurium* (DPC6463)
2. *Salmonella enterica* subsp. *Enterica* (ATCC 14028)
3. *Salmonella braenderup* (NRL-IE-22)
4. *Campylobacter jejuni* (DVI-SC181)

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Materials and Methods

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Selection of plant extracts

Methods:

- Growth curve analysis of probiotics supplemented with;
  - Turmeric (w/v: 0.06%, 0.1%, 0.6%)
  - Green tea (w/v: 0.06%, 0.1%, 0.6%)
  - Garlic (w/v: 0.25%, 0.5%, 1%)

Selection of best probiotic

Methods:

Anti- *Salmonella*

- Spot overlay assays
- Well diffusion assays
- Co-culture assays
- Co-aggregation assays

Anti- *Campylobacter*

- Well diffusion assays

Selection of best Prophybiotic

Methods:

Anti- *Salmonella*

- Co-culture assays with best probiotic + PEs

***In-vivo* implementation: Prophybiotics**

Methods:

In-ovo injection of the selected **prophybiotic and probiotic alone** to **ROSS 308** chicken eggs at **12th Embryonic day**. Recorded;

- Hatchability
- Chick quality
- Chick weight/length

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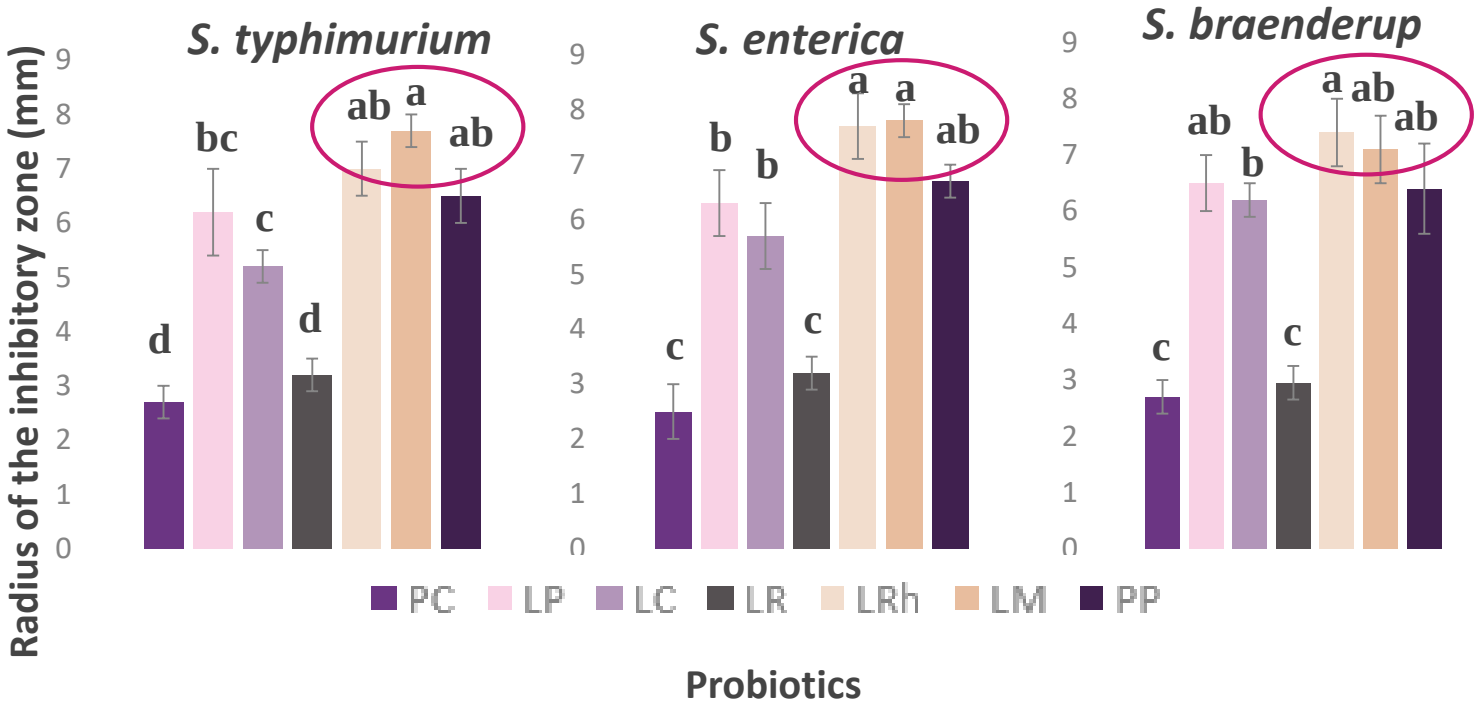
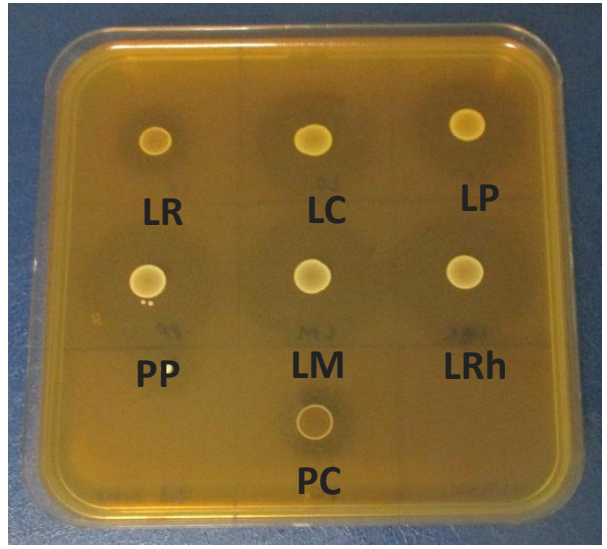
Results (What's the best probiotic?)

Anti-*Salmonella*

Spot overlay assays





Inhibition of *S. typhimurium* in probiotic spot overlay assay

PC: *Lactococcus lactis* **LP:** *Lactiplantibacillus plantarum* **LC:** *Lacticaseibacillus casei* **LR:** *Limosilactobacillus reuteri*

LRh: *Lacticaseibacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus*

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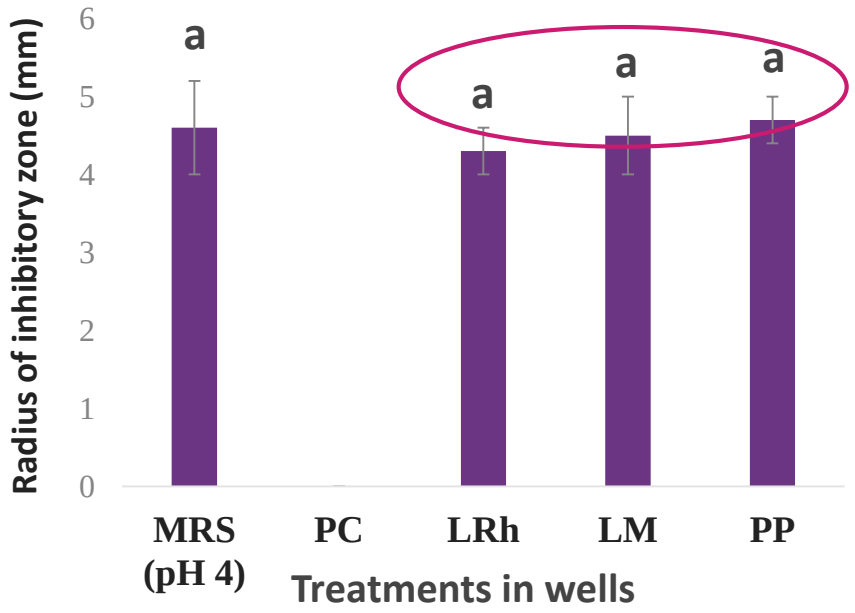
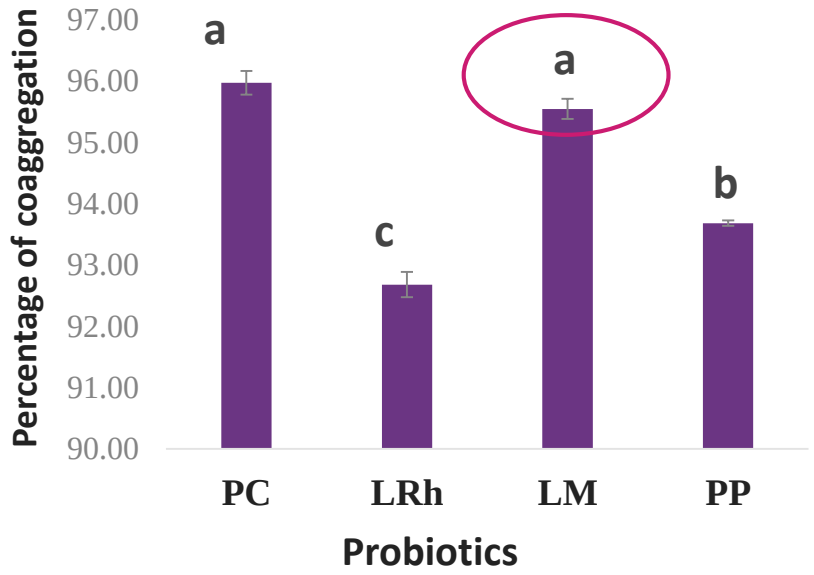
Results (What's the best probiotic?)

Anti-*Salmonella*

Well diffusion Assays



Co-aggregation Assay



LRh: *Lacticaseibacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus* **PC:** *Lactococcus lactis*

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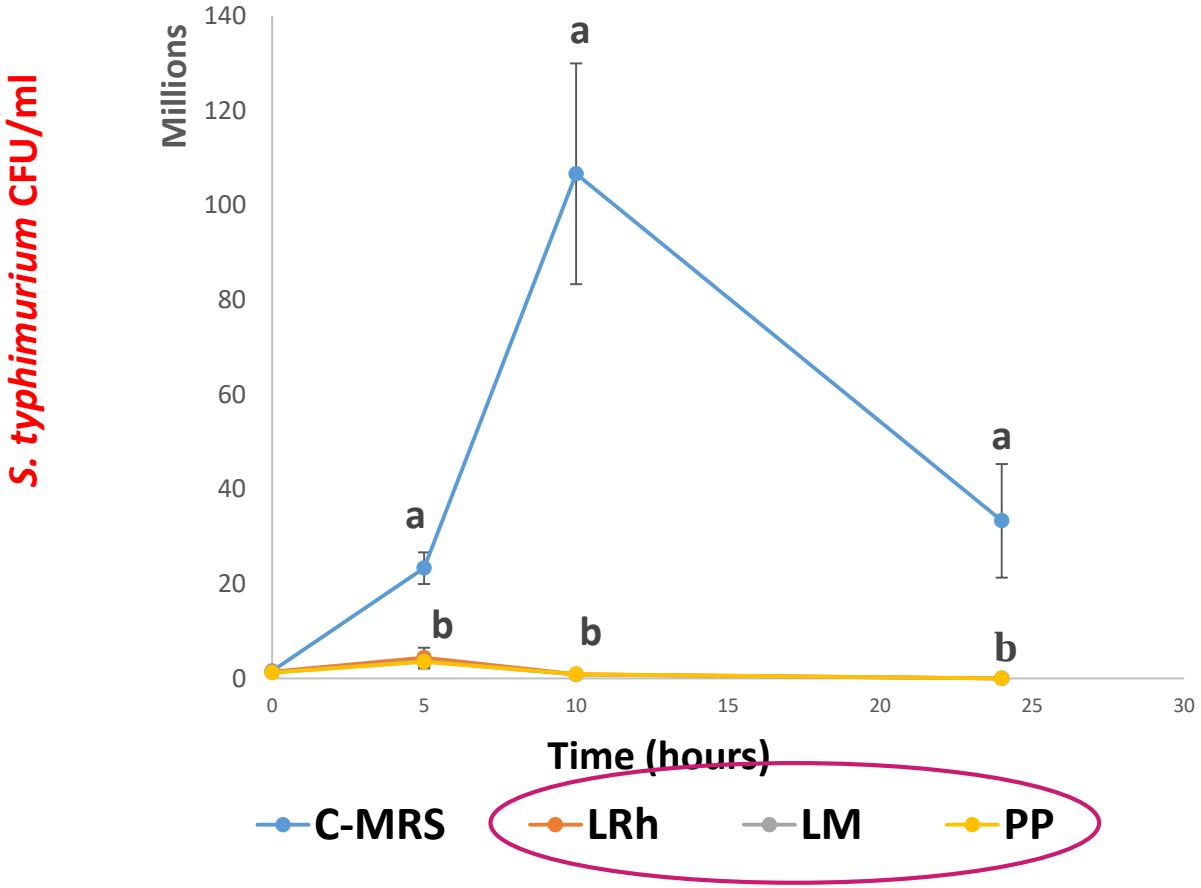
Results (What's the best probiotic?)

Anti-*Salmonella*

Co-culture Assay



C-MRS: Control medium **LRh:** *Lacticaseibacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus*

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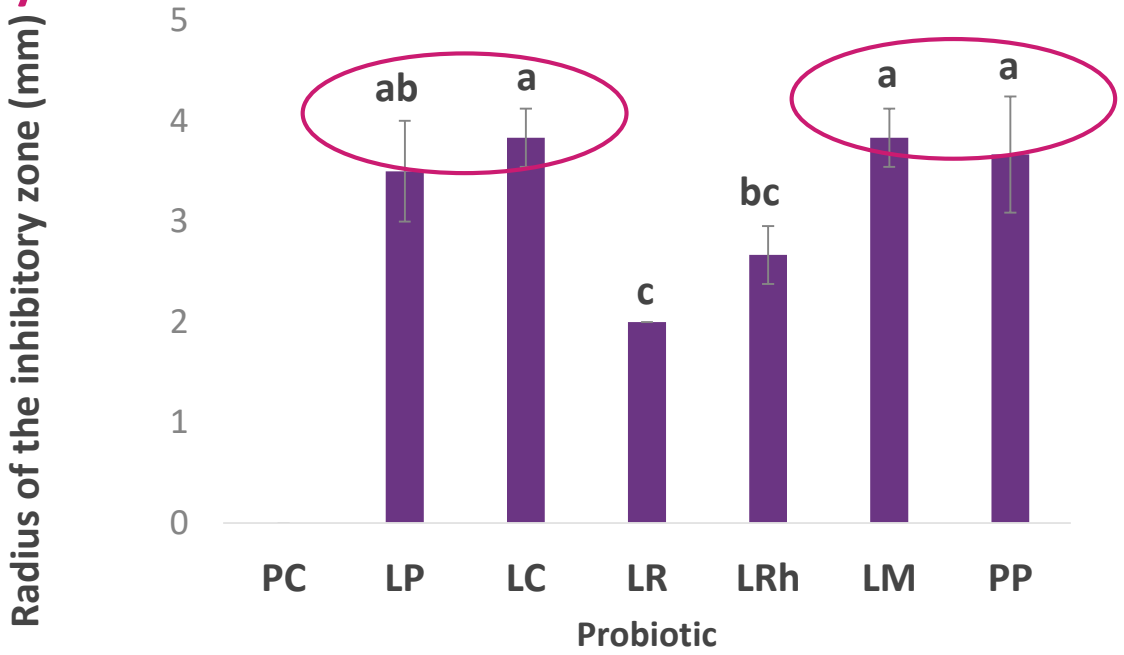
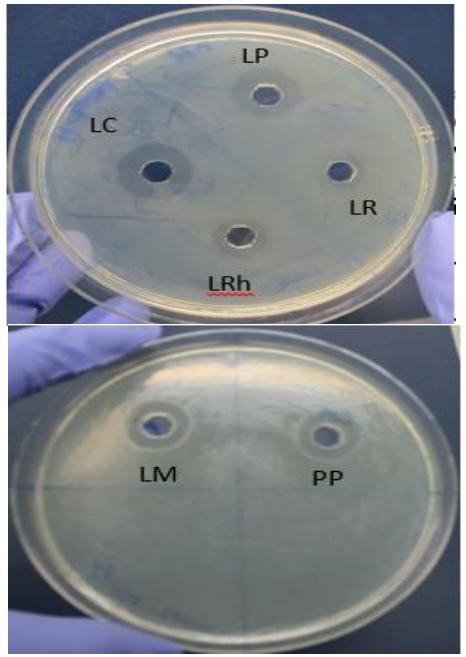
Results (What's the best probiotic?)

Anti-*Campylobacter*

Well diffusion assays





Anti-*Campylobacter* well diffusion assay

PC: *Lactococcus lactis* **LP:** *Lactiplantibacillus plantarum* **LC:** *Lacticaseibacillus casei* **LR:** *Limosilactobacillus reuteri* **LRh:** *Lacticaseibacillus rhamnosus* **LM:** *Leuconostoc mesenteroides* **PP:** *Pediococcus pentosaceus*

* Best probiotic selected: ***Leuconostoc mesenteroides***

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Results (What's the best plant extract?)

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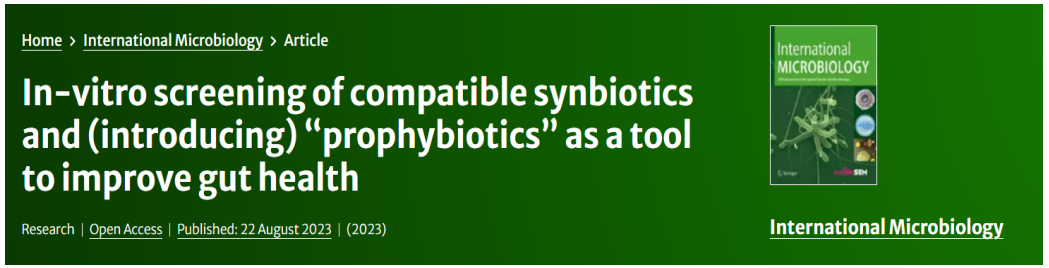
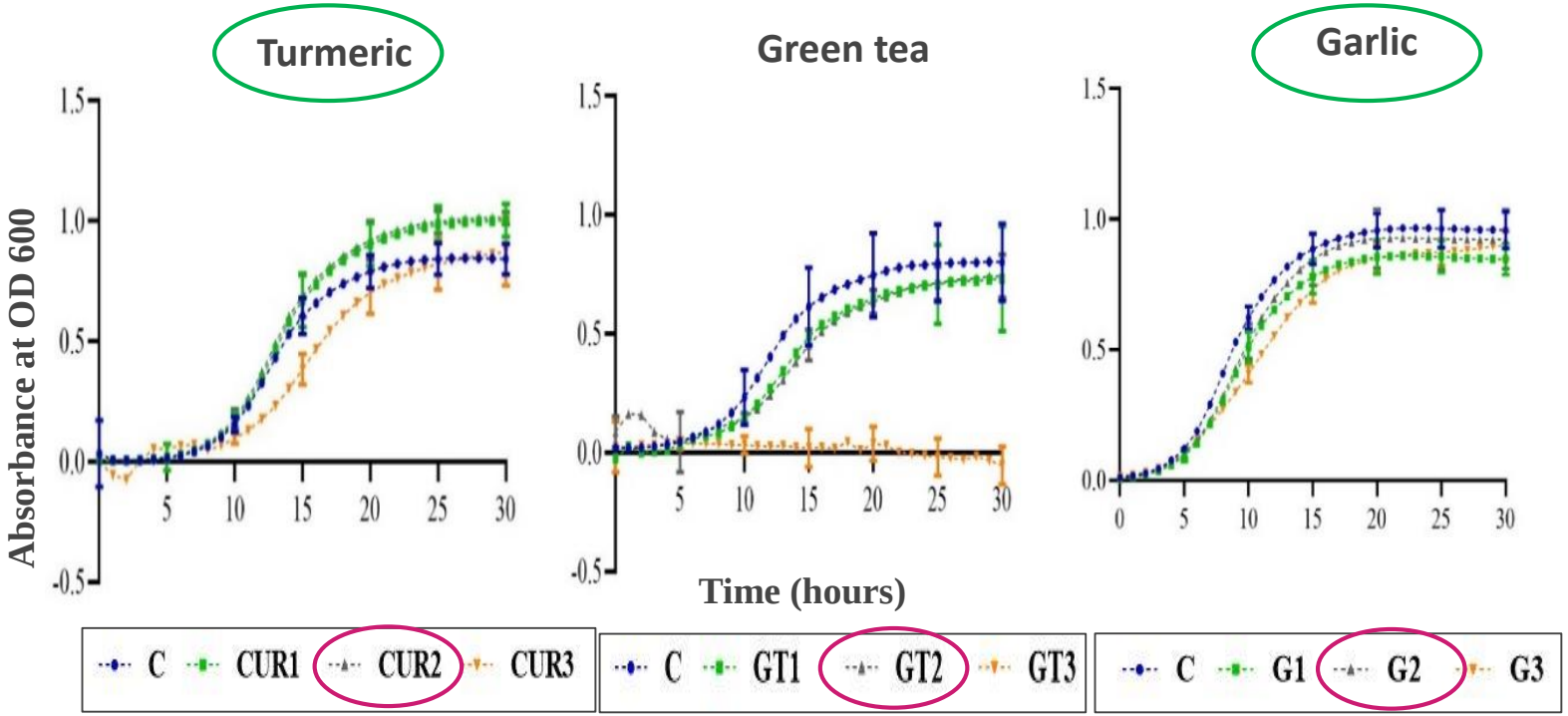
https://doi.org/10.1007/s10123-023-



Growth curve assays: *Leuconostoc mesenteroides*



* Best plant extracts for *L. mesenteroides*:

- **Turmeric (0.1% and 0.06%)**
- **Garlic (0.5%)**

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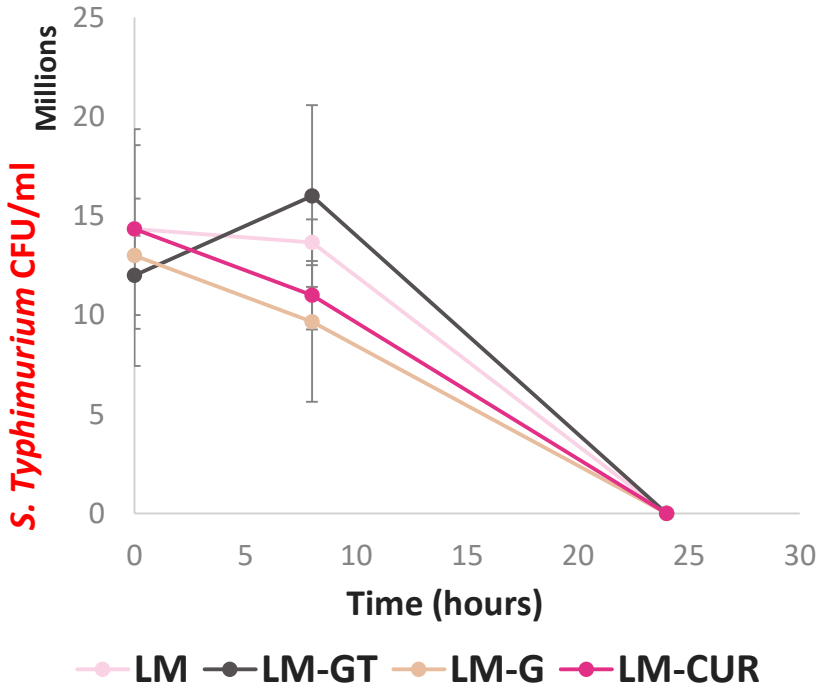
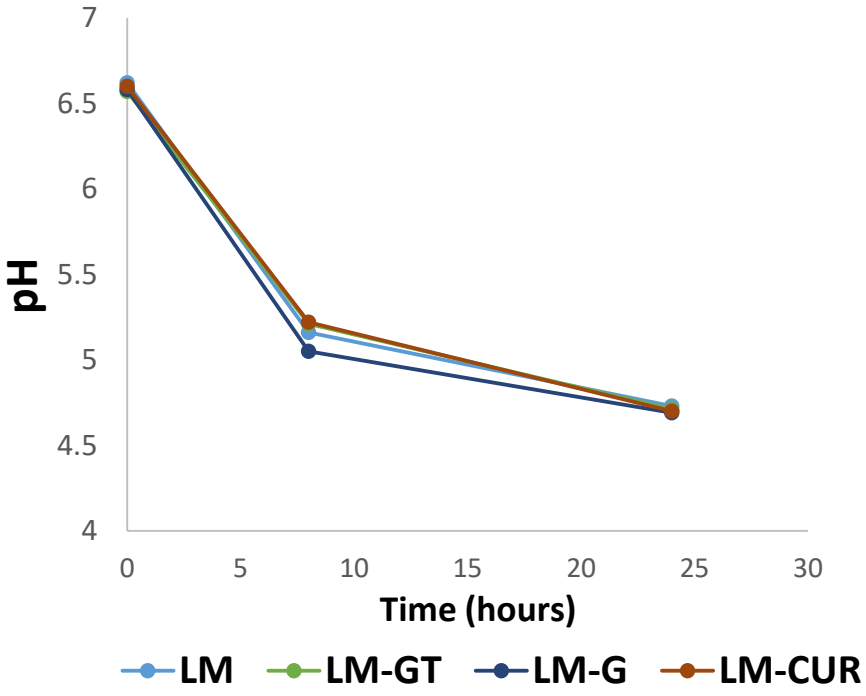
Results (What's the best Prophybiotic?)

Prophybiotics = **Leuconostoc mesenteroides + Plant extracts**

Salmonella counts in co-culture with prophybiotics



pH of the co-culture with prophybiotics



* Selected prophybiotic: ***Leuconostoc mesenteroides*** **+ Garlic (0.5%)**

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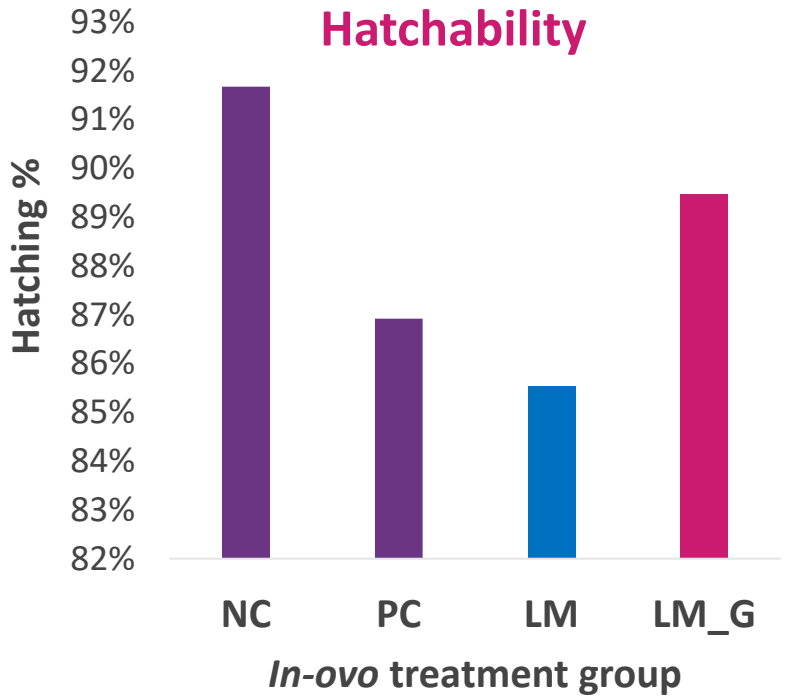
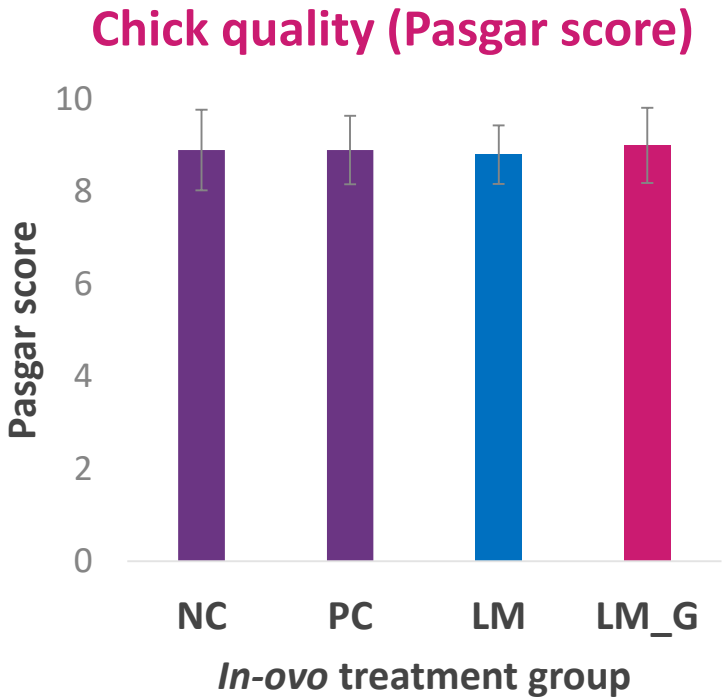
Results (*In-vivo* implementation)





NC: Negative control (No *in-ovo* injection)
PC: Positive control (*In-ovo* injection with physiological saline - 0.2µl/egg)
LM: *In-ovo* injection with 10^6 CFU of *L. mesenterodies* (0.2µl/egg)
LM_G: *In-ovo* injection with 10^6 CFU of *L. mesenterodies* + 0.5% garlic (0.2µl/egg)

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Results (*In-vivo* implementation)

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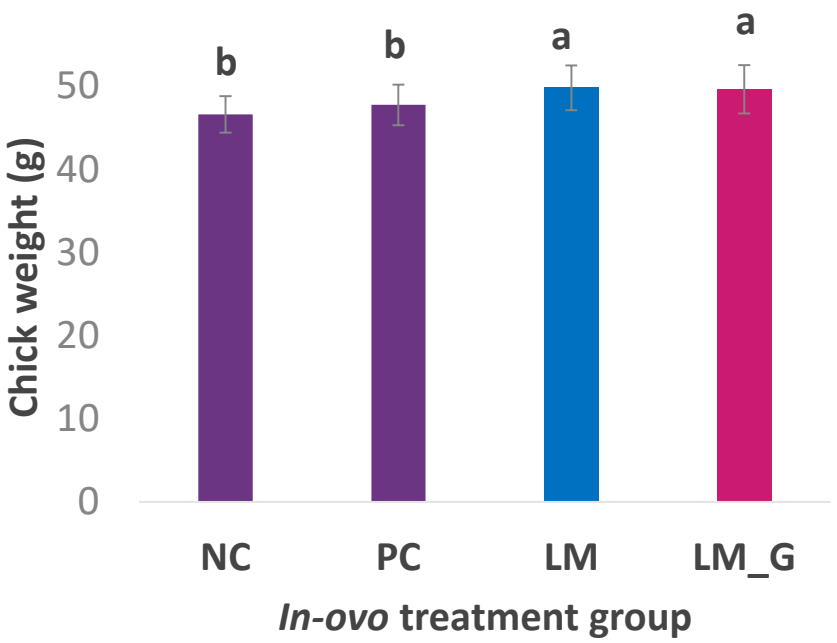
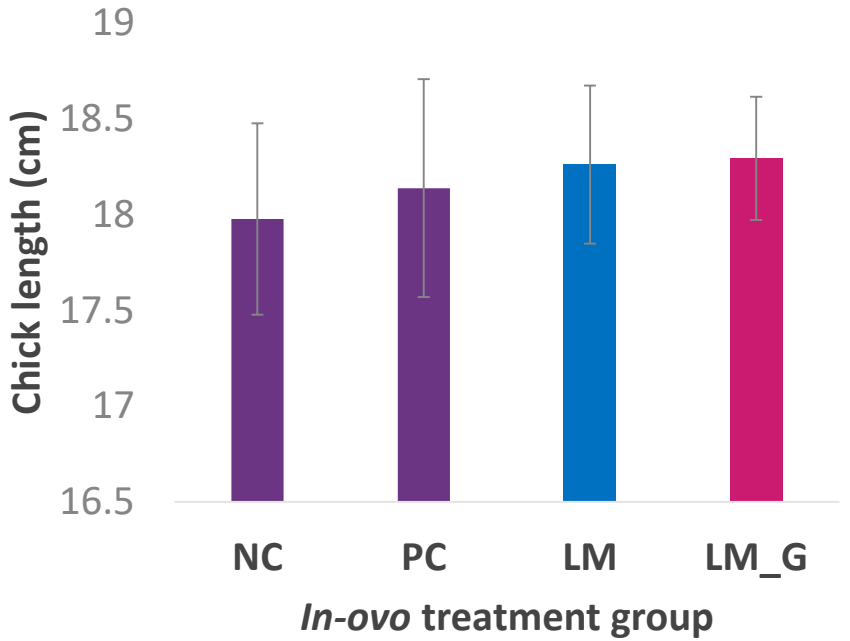
Chick weight



Chick length



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Conclusion and Future prospects

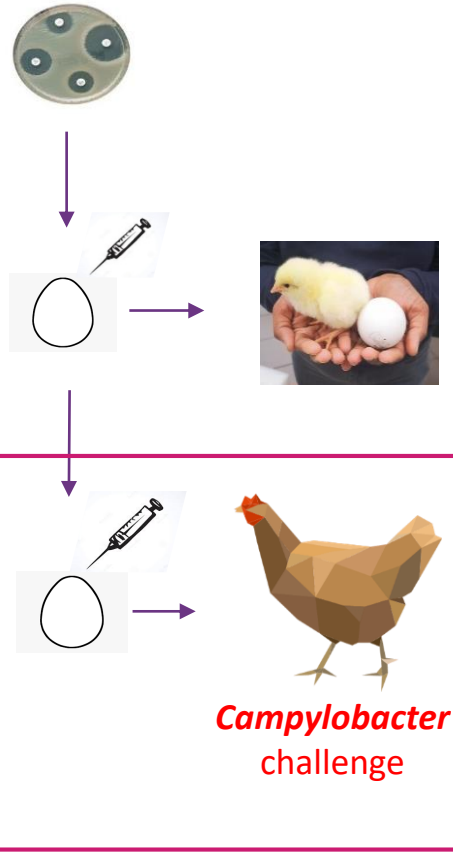
Conclusions

The selected **prophybiotic** (*L. mesenteroides* + Garlic) displays;

a promising **antimicrobial potential** and

the **potential to be used in an *in-ovo* application without compromising the hatchability and affecting the chick quality parameters**

Further research is necessary **to validate the *in-vivo* effectiveness** of the protocol



Screening for Bioactives (*In-vitro*)

In-vivo implementation

In-vivo effectiveness (Infection trial)

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THANK YOU

Ramesha N. Wishna-Kadawarage (ESR3)

Supervisors:

Maria Siwek (PBS)

Rita M. Hickey (Teagasc)

POLITECHNIKA BYDGOSKA

teagasc

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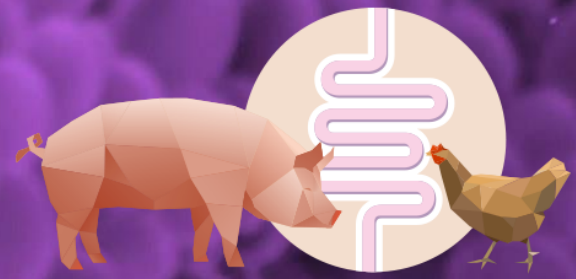

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