monogut health

Optimal gut function in monogastric livestock

POLITECHNIKA BYDGOSKA

Effects of in-ovo stimulation on gut health and production of broiler chickens.

Maria Siwek

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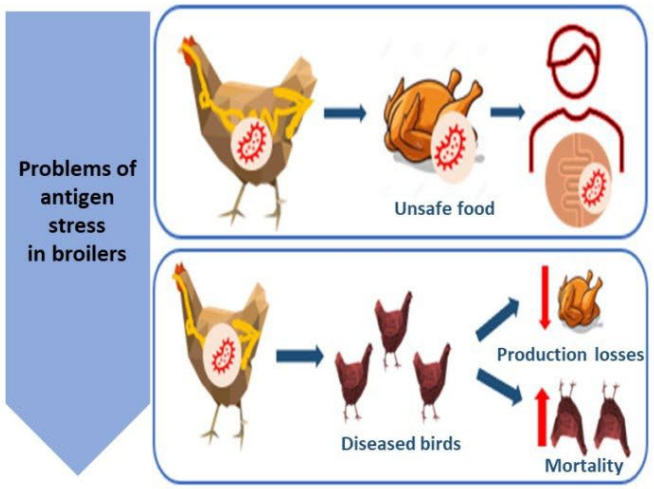
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

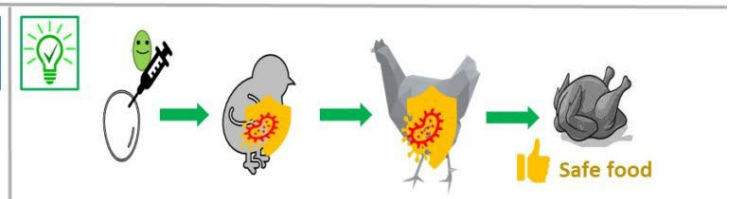
ESR3 & ESR4 projects

Ramesha N. Wishna-Kadawarage

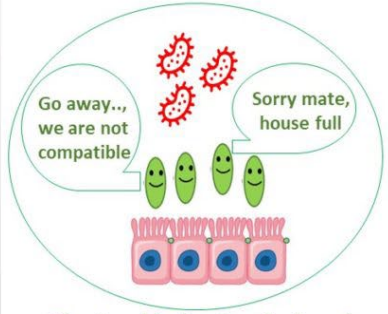
ESR 3

Mitigation of environmental antigen stress effects in poultry production using microbiome programming *in-ovo*

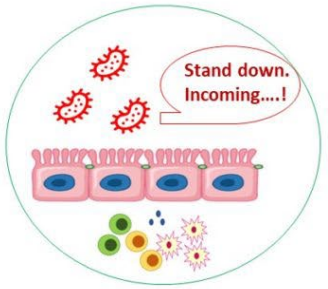




In-ovo injection of pre/probiotics will allow early programing of gut microbiota to gain a competitive advantage over environmental pathogens



Direct and indirect exclusion of pathogens via early colonization of gut with probiotics



Boosting immunity

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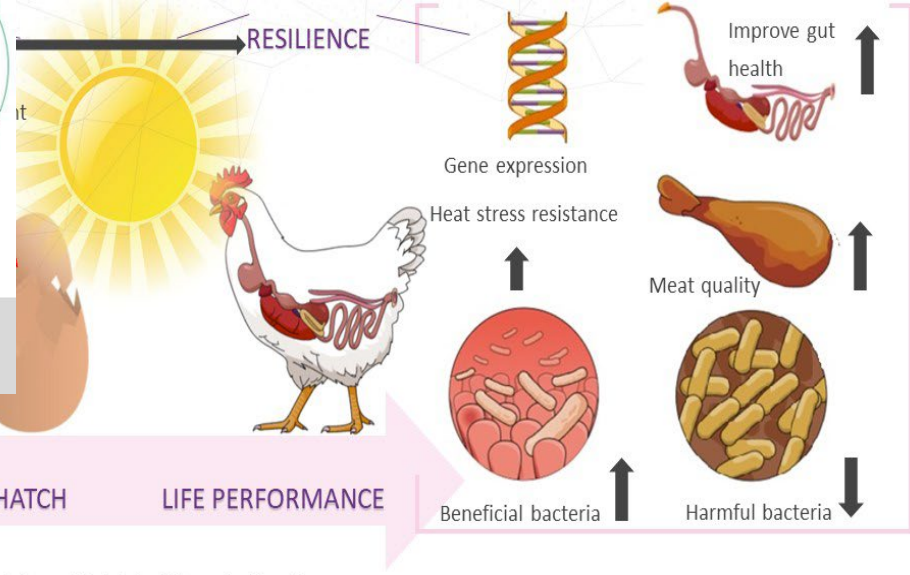
ffects in poultry production using microbiome

Modou Mangan (ESR4)



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Introduction & objective

Healthy gut → *"the absence of gastrointestinal symptoms and disease, as well as an absence of other unfavorable local conditions including increased intestinal permeability, mucosal inflammation, or deficiency (or even excess) of short-chain fatty acids"*

complex physiological status with utmost importance for an organism

Objective:

To determine effects of *in-ovo* stimulation with a selected prebiotic, probiotic and a prophybiotic combination on gut health and production of broiler chickens in **environmetal homeostasis** and **environmental challenge**.

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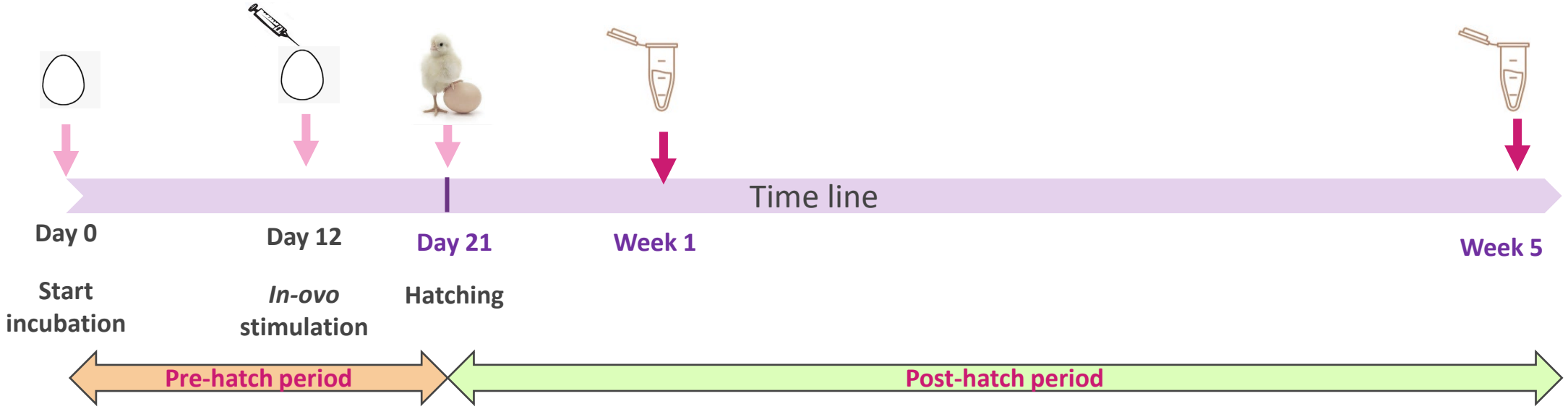
The in ovo concept



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im. Jana i Jędrzeja Śniadeckich

Environmental homeostasis

Effects of in-ovo stimulation on gut health and production of broiler chickens

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programme under the Marie Sklodowska-Curie grant agreement No 955374.

Methodology – experimental setup

Group	Abbreviation	In-ovo injection composition	Dose of bioactive /egg
Negative control	NC	Non injected	----------
Positive control	PC	Physiological saline	0.2ml of 0.90% w/v of NaCl
Probiotic 1	LP	**Lactiplantibacillus plantarum** bacterial suspension in physiological saline	10^6 CFU (in 0.2ml)
Probiotic 2	LM	**Leuconostoc mesenteroides** bacterial suspension in physiological saline	10^6 CFU (in 0.2ml)
Prebiotic	GOS	**Galactooligosaccharides** dissolved in physiological saline	3.5mg GOS (in 0.2ml)
Prophybiotic (Probiotic + Plant extract)	LMG	A mixture **of Leuconostoc mesenteroides** bacterial suspension in physiological saline and 0.5% **garlic** aqueous extract (in 2: 1 ratio, respectively)	10^6 CFU of bacterial suspension + Garlic aqueous extract 0.5% (w/v) (in 0.2ml)

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Methodology – environmental homeostasis

Experimental groups:

NC: **Negative control** (No injection)

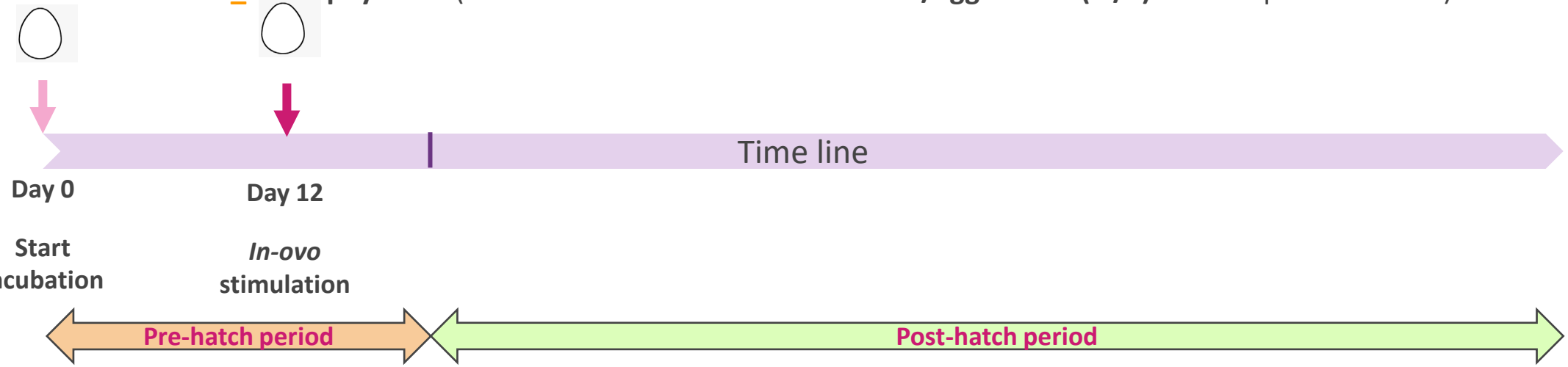
PC: **Positive control** (Physiological saline injection)

LP : **Probiotic** (*Lactiplantibacillus plantarum* **10^6 CFU/egg**)

LM: **Probiotic** (*Leuconostoc mesenteroides* **10^6 CFU/egg**)

GOS: **Prebiotic** (Galactooligosaccharide)

LM_G: **Prophybiotic** (*Leuconostoc mesenteroides* **10^6 CFU/egg** + **0.5% (w/v)** Garlic aqueous extract)



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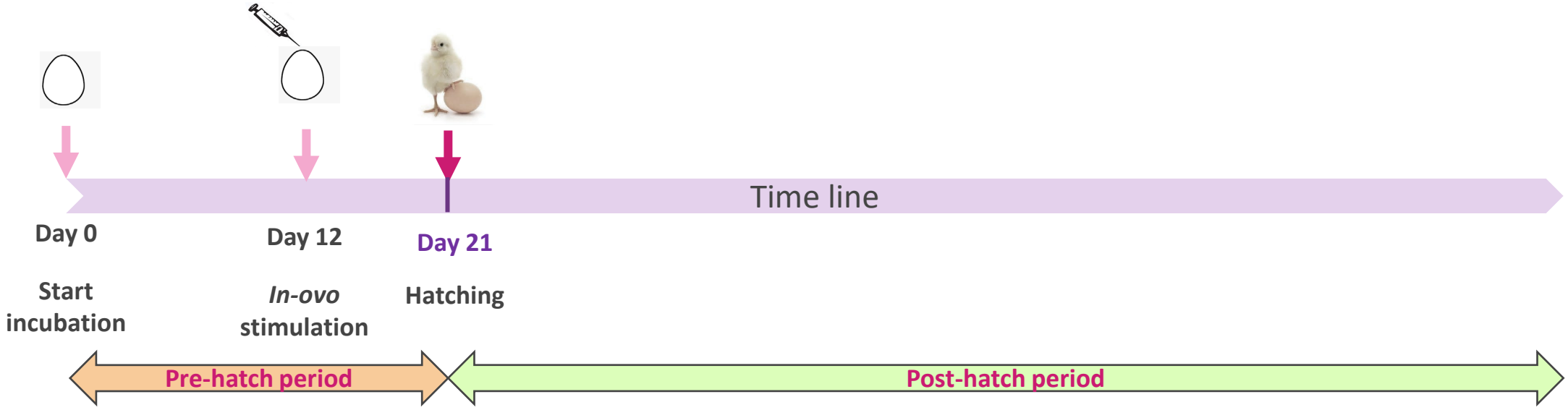
Methodology

Data collection:

- Chick weight
- Chick length
- Pasgar score



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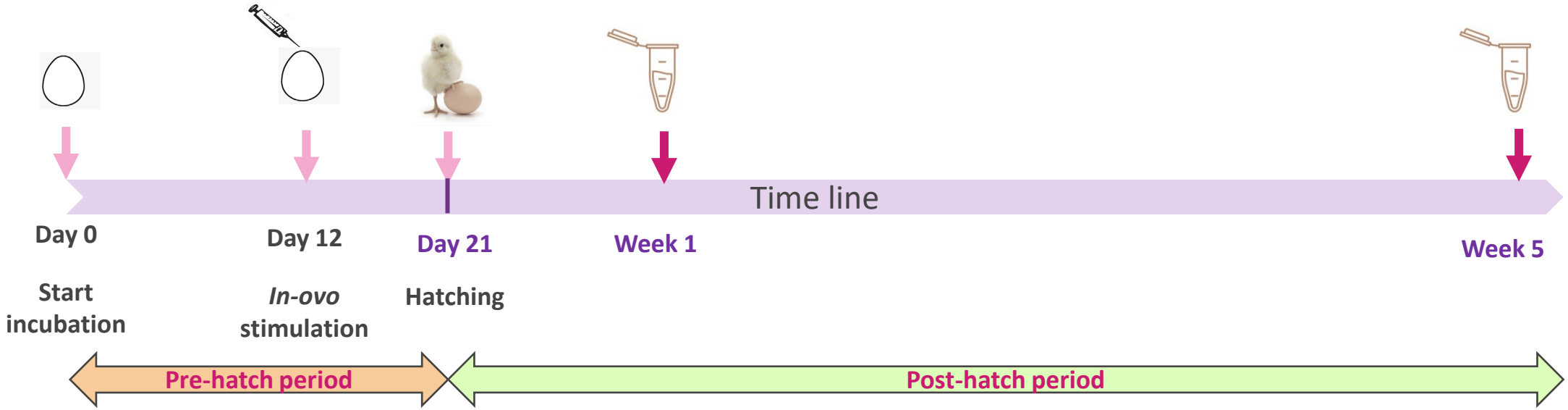
Methodology

Feces collection:

- Feces (8 birds/group)
- Week 1 and Week 5 (Bacterial abundance)



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Methodology

Slaughter (8 birds/group): Sample collection

- Cecal content (abundance of bacteria)
- Cecal tissue (Histo-morphometry)
- Cecal mucosa
- Cecal tonsils
- Spleen
- Liver

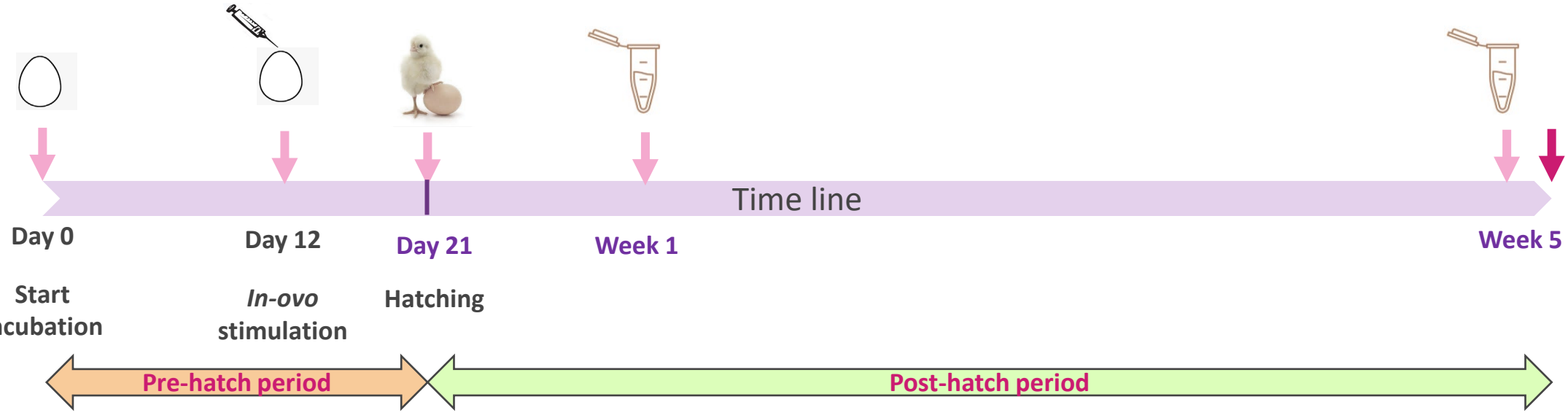
(Gene expression)

Slaughter analysis: (8 birds/group)

- Carcass dressing %
- Breast muscle %
- Leg muscle %
- Leg bones %
- Giblets %
- Abdominal fat %

Meat (Breast and thigh) quality analysis: (8 birds/group)

- pH
- Color
- Drip loss
- Thawing loss
- Cooking loss
- Shear force
- Texture



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Methodology

Statistical analysis

Data type	Method of statistical analysis
Hatch data	Linear mixed model Fixed effect: Treatment Random effect: Sex
Body weights	
Slaughter and meat quality parameters	
Histomorphometry parameters	
Relative abundances of bacteria	
Relative gene expression	Two sample t-test to compare between each treatment group and positive control group

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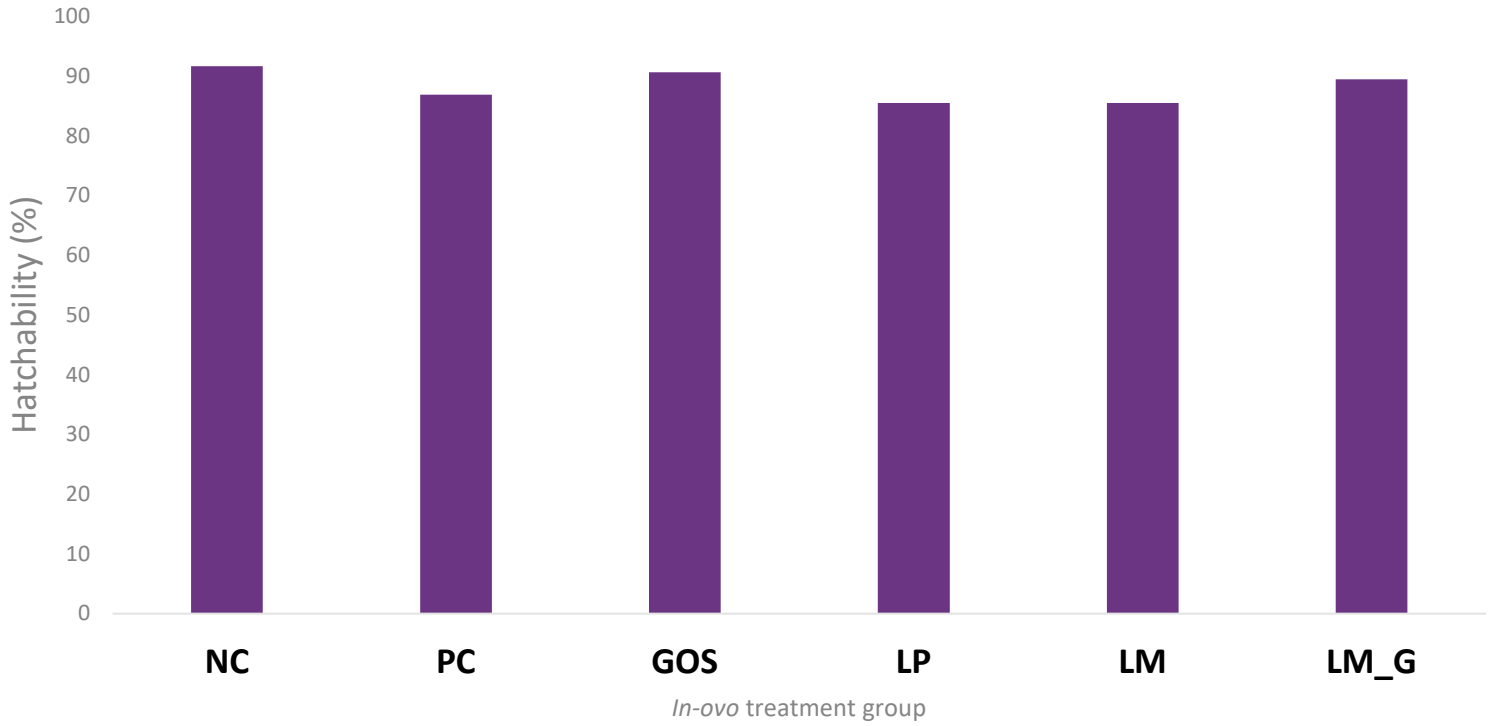
Results - hatchability



NC: Negative control, PC: Positive Control, GOS: Galactooligosaccharides, LP: *Lactiplantibacillus plantarum,* LM: *Leuconostoc mesenteroides* and LM_G: *Leuconostoc mesenteroides* + Garlic

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Results – bacteria abundance

Treatment group	Feces		Cecal content
	Early life	Adult stage	
GOS	Bifidobacterium sp. Lactobacillus sp. ↑	Bifidobacterium sp. ↑ Lactobacillus sp. ↑	Lactobacillus sp. ↑ Bifidobacterium sp. ↑
LP	Bifidobacterium sp. Lactobacillus sp. ↑	Bifidobacterium sp. Lactobacillus sp. ↑	Lactobacillus sp. ↑ Bifidobacterium sp. ↑
LM	Faecalibacteria ↑	No difference	Akkermansia sp. ↑
LMG	Faecalibacteria ↑ Bifidobacteria ↑	E. coli ↓	Akkermansia sp. ↑ Faecalibacteria ↓ E.coli ↓

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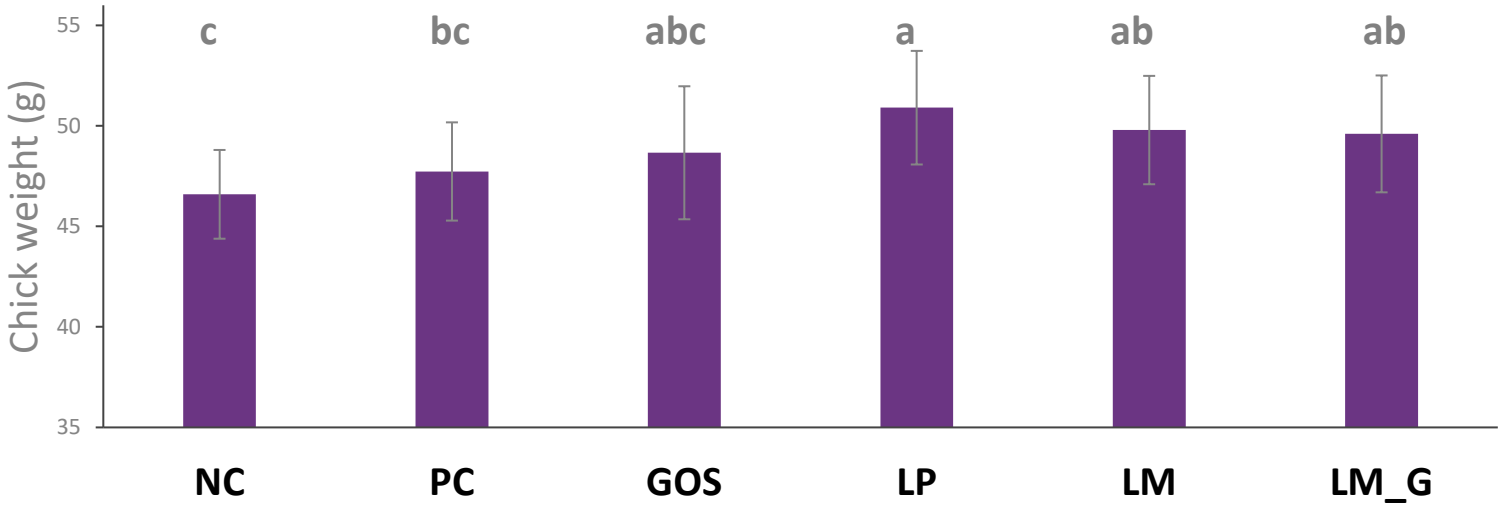
Results – chick weight



Error bars: ± SD. Homogenous means have been indicated by similar letters (in descending order) as identified by Tuckey HSD test (p value <0.05). **NC**: Negative control, **PC**: Positive Control, **GOS**: Galactooligosaccharides, **LP**: Lactiplantibacillus plantarum, **LM**: Leuconostoc mesenteroides and **LM_G**: Leuconostoc mesenteroides + Garlic

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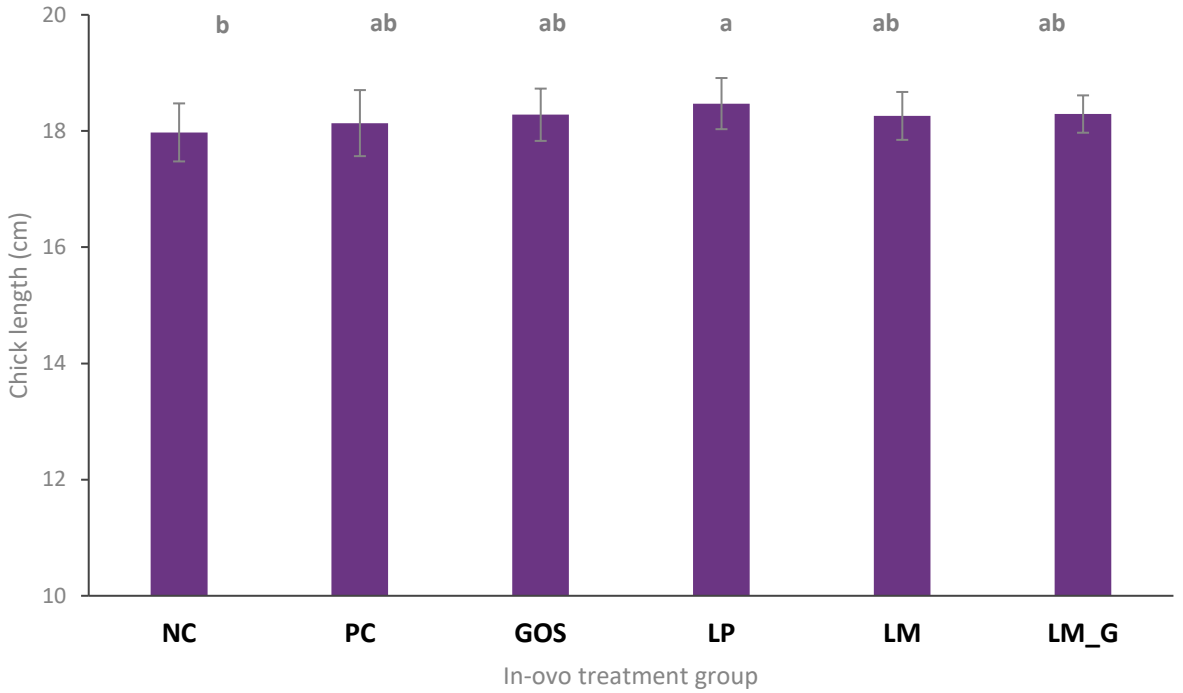
Results – chick length



Chick length of *in-ovo* treatment groups. Error bars: ± SD. Homogenous means have been indicated by similar letters (in descending order) as identified by Tuckey HSD test (p value <0.05). NC: Negative control, PC: Positive Control, GOS: Galactooligosaccharides, LP: *Lactiplantibacillus plantarum,* LM: *Leuconostoc mesenteroides* and LM_G: *Leuconostoc mesenteroides* + Garlic

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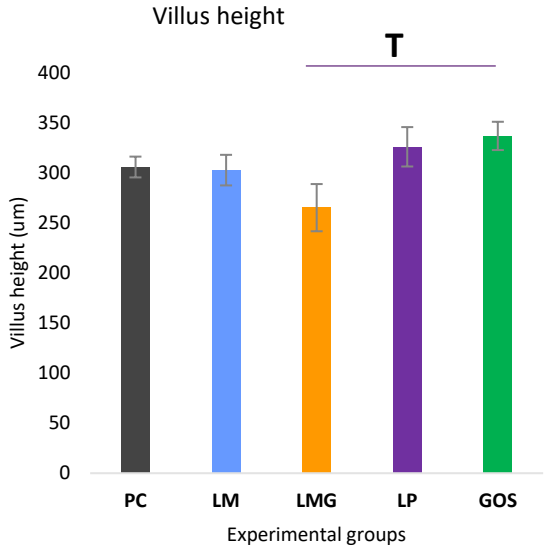
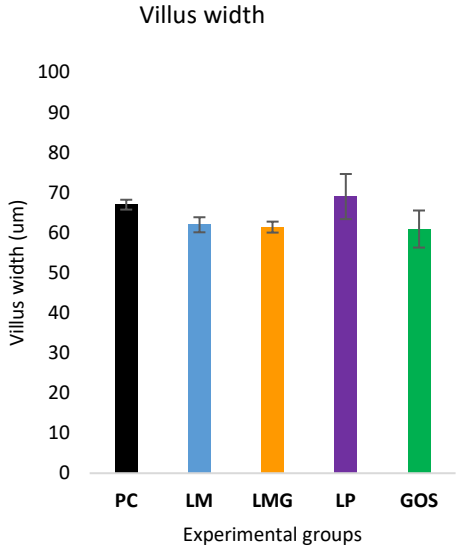
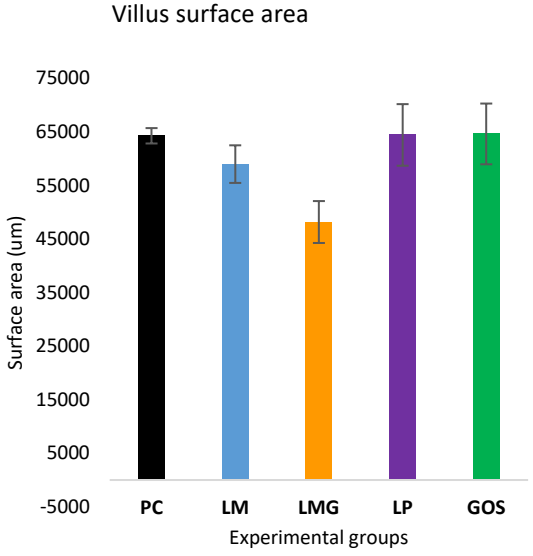
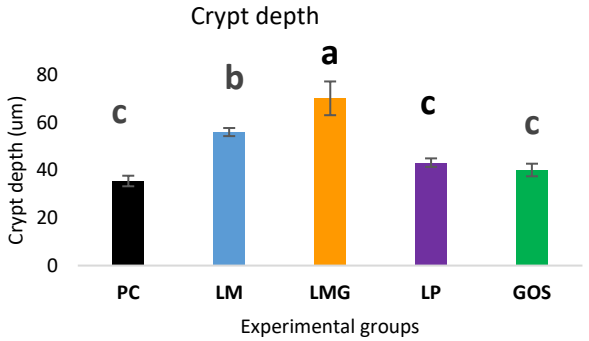
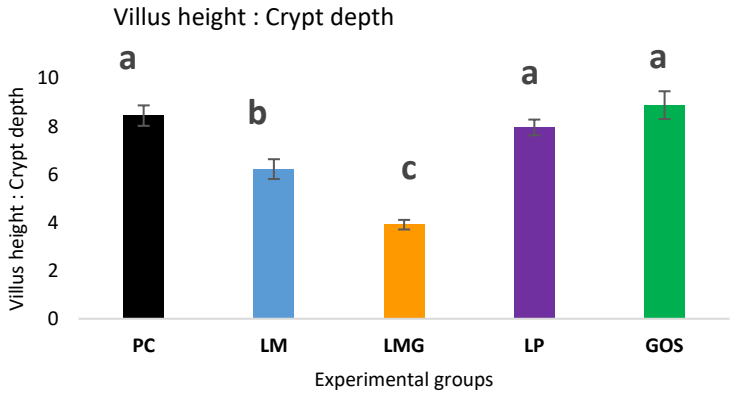
Results – cecal histomorphometry











Error bars: ± SE. Homogenous means have been indicated by similar letters (in descending order). “T” indicates a statistical tendency (P value <0.1) PC: Positive control, LM: Probiotic Leuconostoc mesenteroides, LMG: Prophybiotic (Leuconostoc mesenteroides + garlic aqueous extract), LP: Probiotic Lactiplantibacillus plantarum, GOS: Prebiotic galactooligosachcharides

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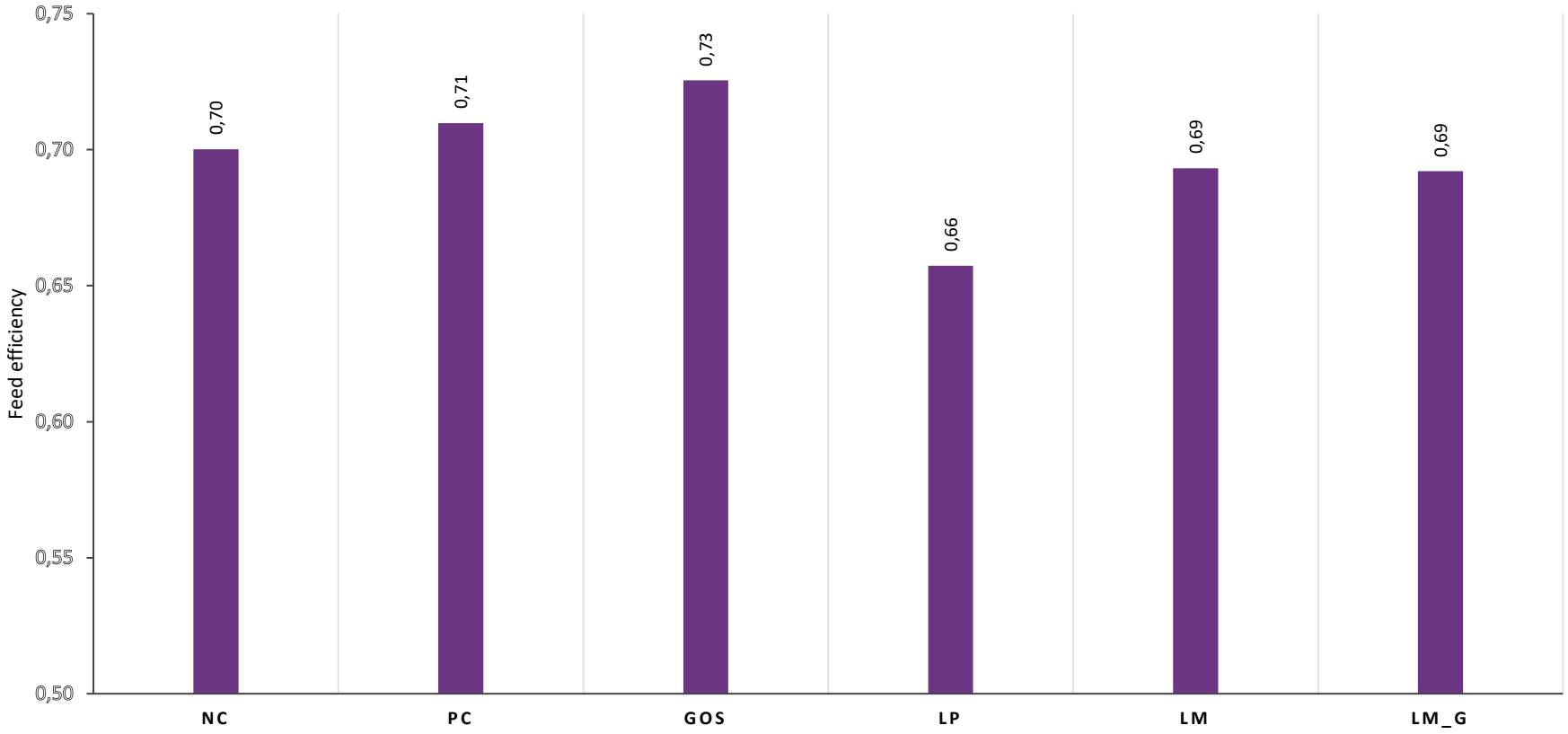
Results – feed efficiency



Overall feed efficiency of the chickens from the six *in-ovo* treatment groups. NC: Negative control, PC: Positive Control, GOS: Galactooligosaccharides, LP: *Lactiplantibacillus plantarum,* LM: *Leuconostoc mesenteroides* and LM_G: *Leuconostoc mesenteroides* + Garlic

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Results – body weight

Day of life	Treatments (T)						T effect
	NC	PC	GOS	LP	LM	LM_G	
7	**180.5 ± 25.8^{c}**	**177.3 ± 23.0^{c}**	**179.6 ± 26.2^{c}**	**195.2 ± 24.0^{ab}**	**206.1 ± 25.6^{a}**	**190.2 ± 30.7^{b}**	***
14	**480.2 ± 71.5^{b}**	**500.0 ± 47.2^{b}**	**485.9 ± 63.3^{b}**	**518.8 ± 66.1^{ab}**	**536.9 ± 79.9^{a}**	**521.2 ± 62.1^{a}**	**
21	1014.4 ± 143.1	1011.3 ± 113.5	1017.7 ± 113.9	1044.3 ± 112.0	1042.8 ± 141.6	1052.7 ± 129.4	NS
28	1681.5 ± 197.9	1663.8 ± 191.5	1655.4 ± 168.3	1716 ± 147.5	1718.3 ± 230.7	1711.9 ± 200.6	NS
35	2437.5 ± 254.9	2433.6 ± 301.7	2526.9 ± 276.0	2499.7 ± 225.4	2502.3 ± 255.7	2455.6 ± 266.3	NS

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Results – slaughter analysis

Parameter	Treatments (T)					T effect
	PC	GOS	LP	LM	LM_G	
Cooling losses	**1.79 ± 0.21^{a}**	**1.43 ± 0.16^{b}**	**1.31 ± 0.37^{b}**	**1.35 ± 0.29^{b}**	**1.55 ± 0.09^{ab}**	***
Dressing percentage with giblets (%)	79.81 ± 1.14	80.19 ± 1.09	80.32 ± 1.09	79.51 ± 1.25	79.82 ± 1.24	NS
Dressing percentage without giblets (%)	76.83 ± 1.19	77.19 ± 1.15	77.35 ± 1.16	76.49 ± 1.26	76.7 ± 1.30	NS
Breast muscle (%)	31.35 ± 2.05	30.64 ± 0.84	31.34 ± 1.53	29.39 ± 1.53	30.77 ± 2.37	NS
Pectoral muscles (%)	19.19 ± 1.47	18.47 ± 1.14	18.7 ± 1.70	19.39 ± 1.27	18.89 ± 2.07	NS
Giblets (%)	3.75 ± 0.42	3.73 ± 0.34	3.63 ± 0.15	3.93 ± 0.24	3.91 ± 0.30	NS
Liver (%)	2.23 ± 0.30	2.19 ± 0.21	2.14 ± 0.07	2.42 ± 0.29	2.34 ± 0.19	NS
Gizzard (%)	0.96 ± 0.20	0.94 ± 0.13	0.89 ± 0.16	0.92 ± 0.19	0.97 ± 0.12	NS
Heart (%)	0.53 ± 0.06	0.53 ± 0.03	0.57 ± 0.07	0.55 ± 0.07	0.53 ± 0.05	NS
Pectoral bones (%)	3.98 ± 0.48	4.03 ± 0.38	4.16 ± 0.65	4.44 ± 0.49	4.18 ± 0.40	NS
Abdominal fat (%)	1.83 ± 0.30	1.85 ± 0.24	1.9 ± 0.32	1.94 ± 0.46	1.7 ± 0.34	NS

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Conclusions

- ***In-ovo* stimulation** with the **selected prebiotic** / **probiotic/prophybiotic** confer **long term benefits** on ***in-vivo***;
  - **gut microbiome**
  - **Cecal histo-morphology**

- The treatments **did not adversely affect** the **hatch, production or meat quality parameters**

- The **prophybiotic** application can be **potentially more beneficial** than using probiotic alone for ***in-ovo* stimulation**

- **Future research** is necessary to **test different prophybiotic combinations** to **maximize the benefits** on gut health of broiler chickens.

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Environmental challenge

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Campylobacter jejuni – environmental antigen

- *Campylobacter* is the most common foodborne pathogen reported within Europe transmitted to humans mainly from chicken sources.
- Poultry ceca can carry a large number of *Campylobacter* without showing symptoms.
- *Campylobacter* infection impacts the intestinal integrity, intestinal permeability, tight junction proteins causing inflammation and leaky gut in chickens.
- controlling *Campylobacter* in broiler chickens is imperative for food safety as well as in addressing animal welfare concerns

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Campylobacter challenge experiment

Incubation:

ROSS308
N = 200 eggs

***In-ovo* stimulation:**

NC: 38 eggs
PC: 40 eggs
LM: 41 eggs
LMG: 40 eggs

Hatching and rearing:

Collection of;
Hatchability/group
Chick weight (20/group)
Chick length (20/group)
Pasgar score (20/group)

Rearing of; 32 chicks/group

***Campylobacter* infection:**

Infection of; 16 birds/group
6 × 10^8 CFU of *C. jejuni*/bird

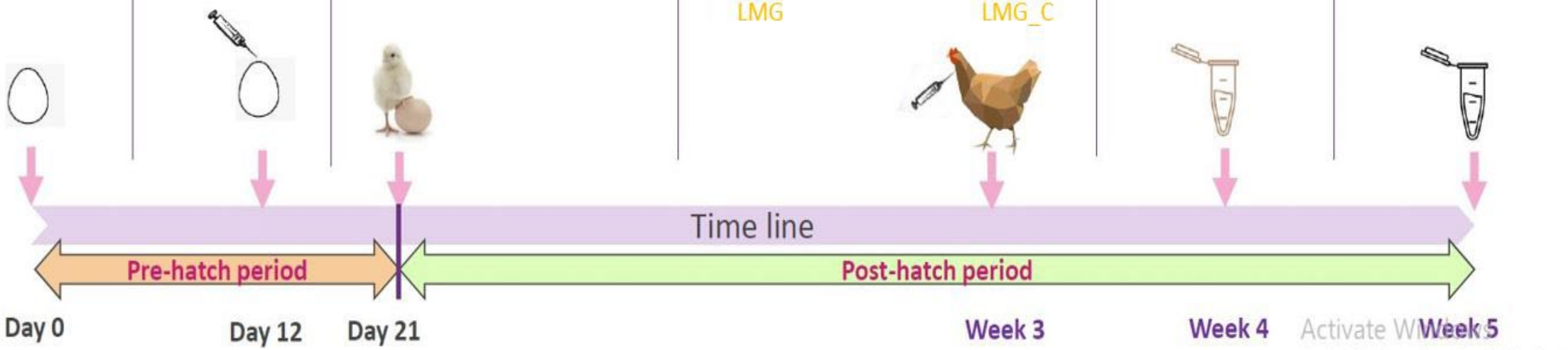
Non-Infected	Infected
NC	NC_C
PC	PC_C
LM	LM_C
LMG	LMG_C

Feces collection:

***C. Jejuni* quantification of;**
8 birds/group

Cecal content collection:

***C. Jejuni* quantification of;**
8 birds/group



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Methodology – experimental setup

Group	Abbreviation	In-ovo injection composition	Dose of bioactive /egg
Negative control	NC	Non injected	----------
Positive control	PC	Physiological saline	0.2ml of 0.90% w/v of NaCl
Probiotic	LM	**Leuconostoc mesenteroides** bacterial suspension in physiological saline	10^6 CFU (in 0.2ml)
Prophybiotic (Probiotic + Plant extract)	LMG	A mixture **of Leuconostoc mesenteroides** bacterial suspension in physiological saline and 0.5% **garlic** aqueous extract (in 2: 1 ratio, respectively)	10^6 CFU of bacterial suspension + Garlic aqueous extract 0.5% (w/v) (in 0.2ml)

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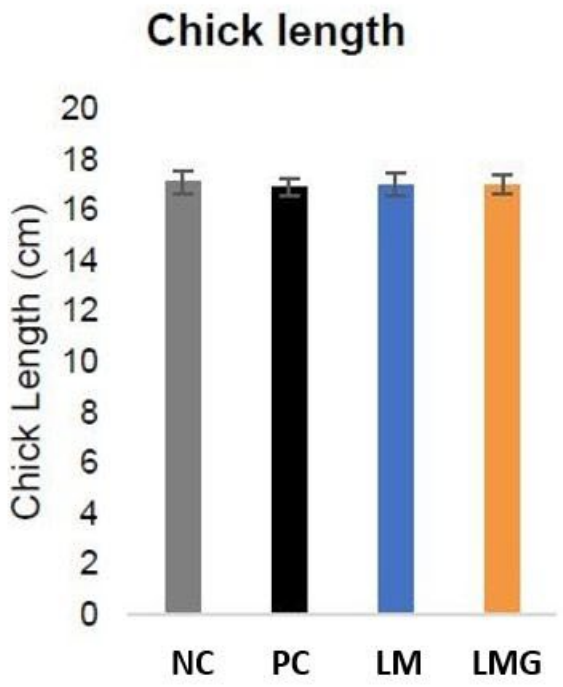
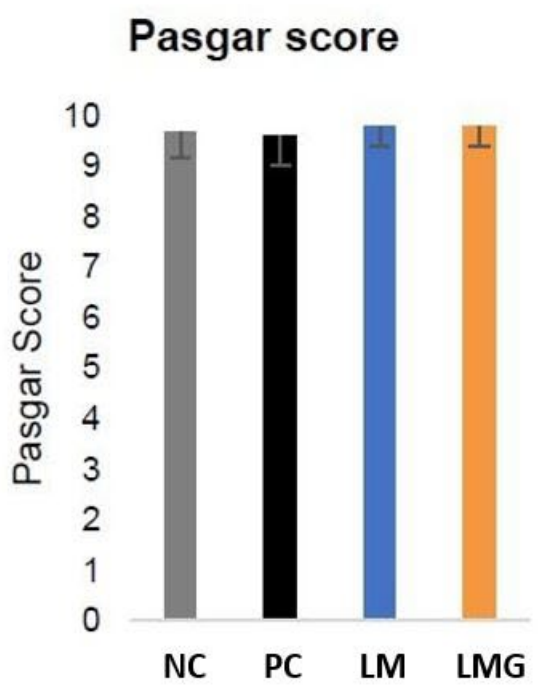
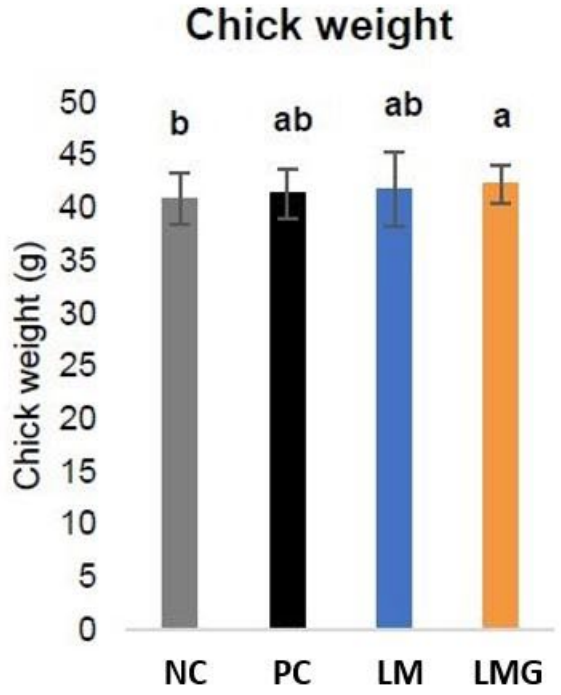
Campylobacter challenge – chick quality







Chick length, Pasgar score and chick weight of the experimental groups. Error bars: ± SD. Homogenous means have been indicated by similar letters (in descending order) ($p < 0.05$). NC: Negative control, PC: Positive Control, LM: Probiotic *Leuconostoc mesenteroides* and LMG: Prophybiotic: *Leuconostoc mesenteroides* + Garlic aeqous extract

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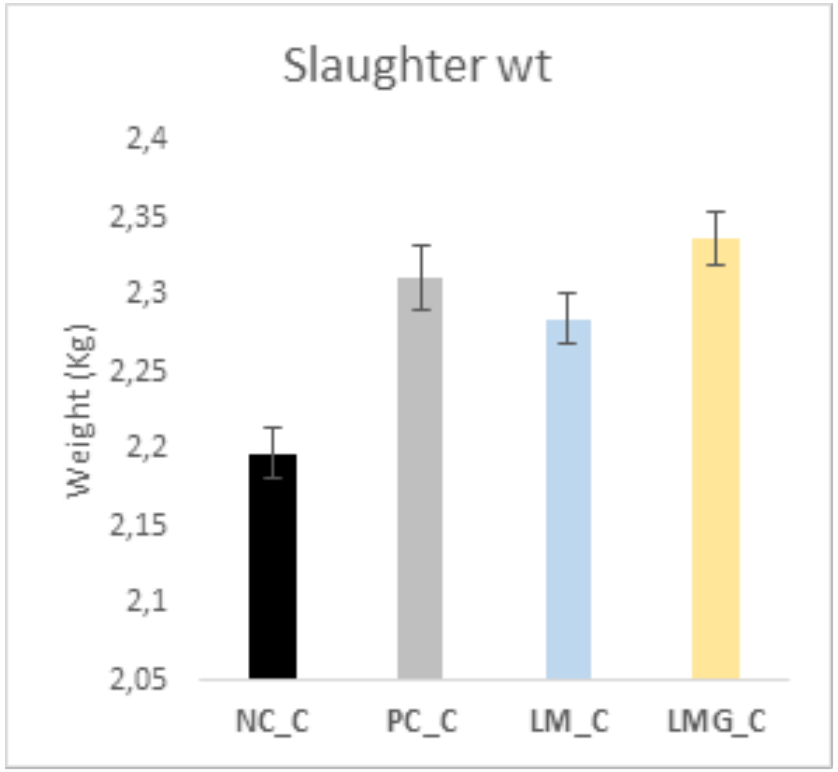
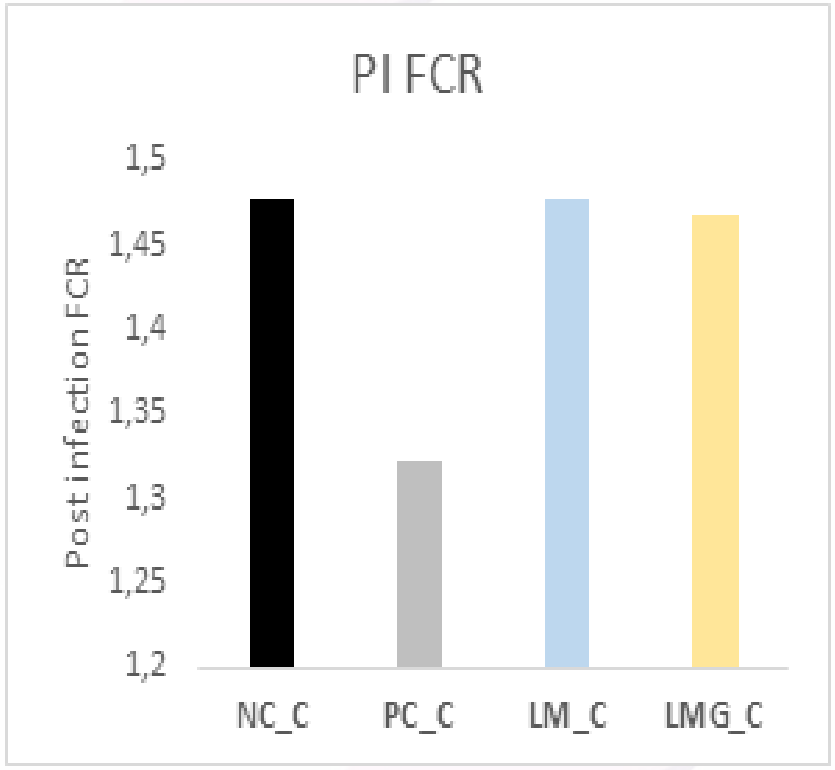
Campylobacter challenge – production parameters





Production parameters. A: Slaughter weight B: Post-infection feed conversion ratio

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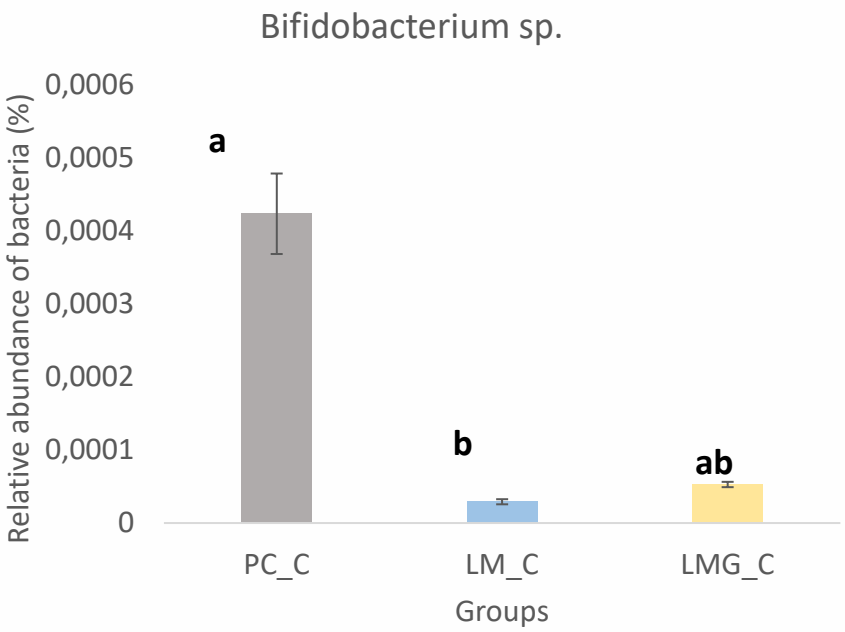
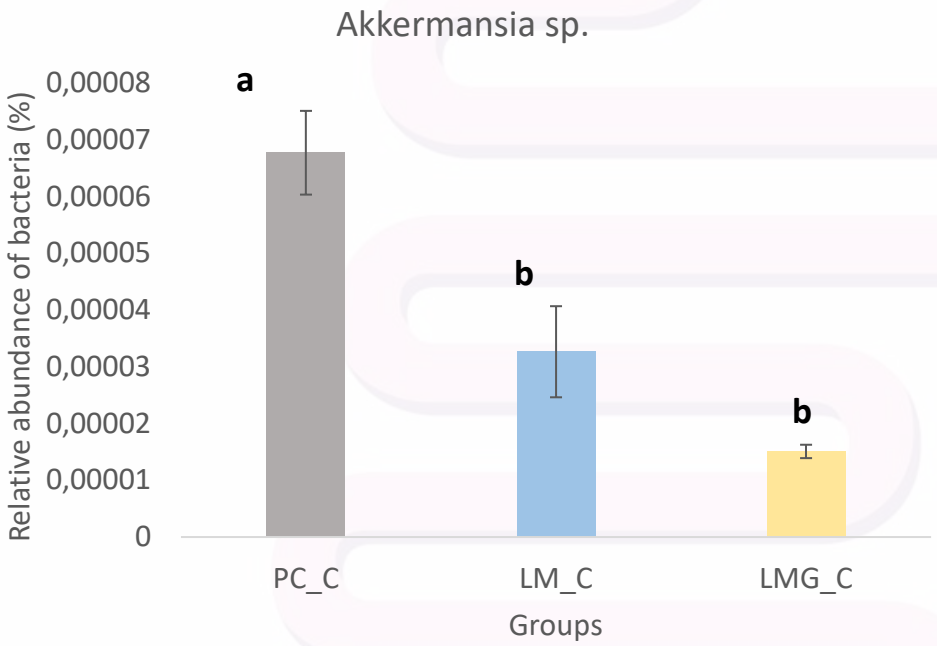
Campylobacter challenge – bacteria abundance

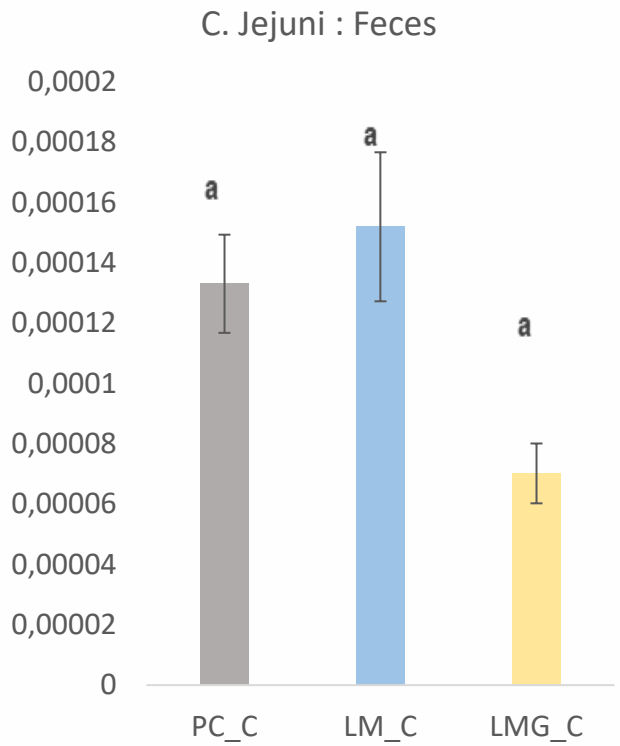


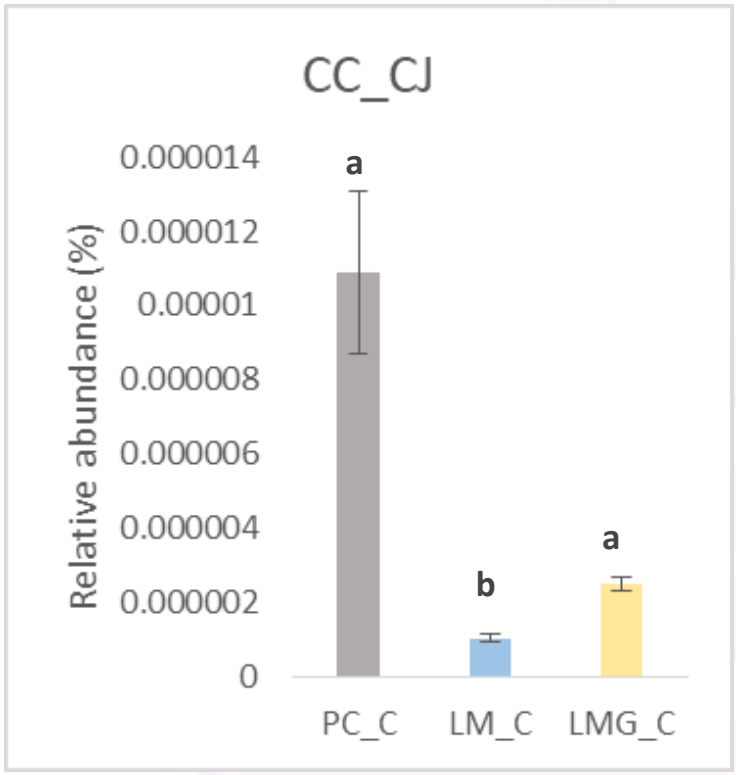


Relative abundance (%) of beneficial bacterial communities in the ceca of Campylobacter jejuni infected chickens. A: *Bifidobacterium sp.* B: *Akkermansia sp.*

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.

Campylobacter challenge experiment – bacteria





Results of *Campylobacter jejuni* relative abundance (%) in A: Feces on day 28 (one week post infection) and B: Cecal content on day 34 (2 weeks post infection). Error bars: ± SE. Homogenous means have been indicated by similar letters (in descending order) ($p < 0.05$). PC_C: Positive control_infected, LM_C: Probiotic_infected, LMG_C: Prophybiotic_infected

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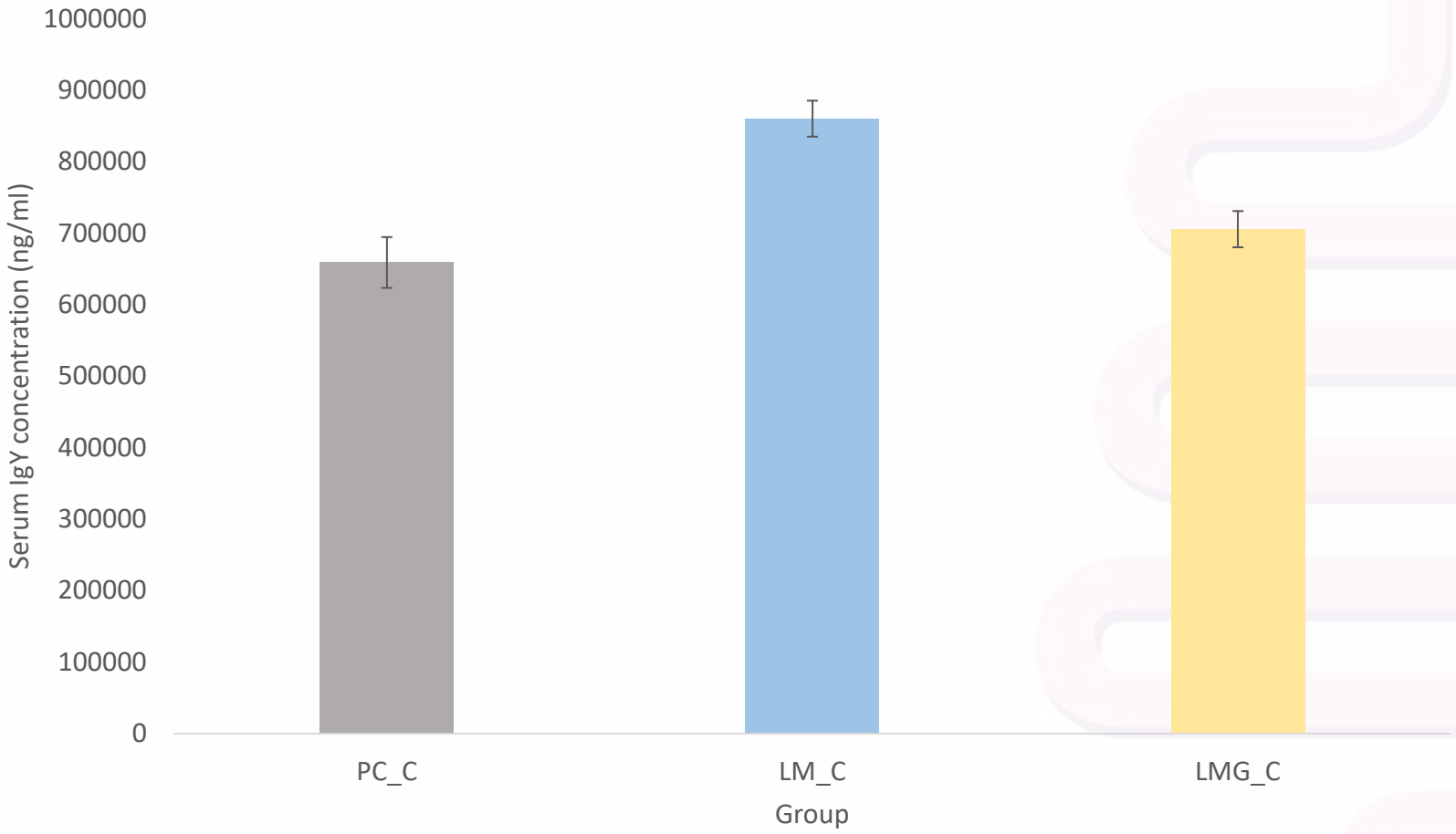
Campylobacter challenge – IgY



Serum immunoglobulin Y content of the *Campylobacter jejuni* infected chickens.

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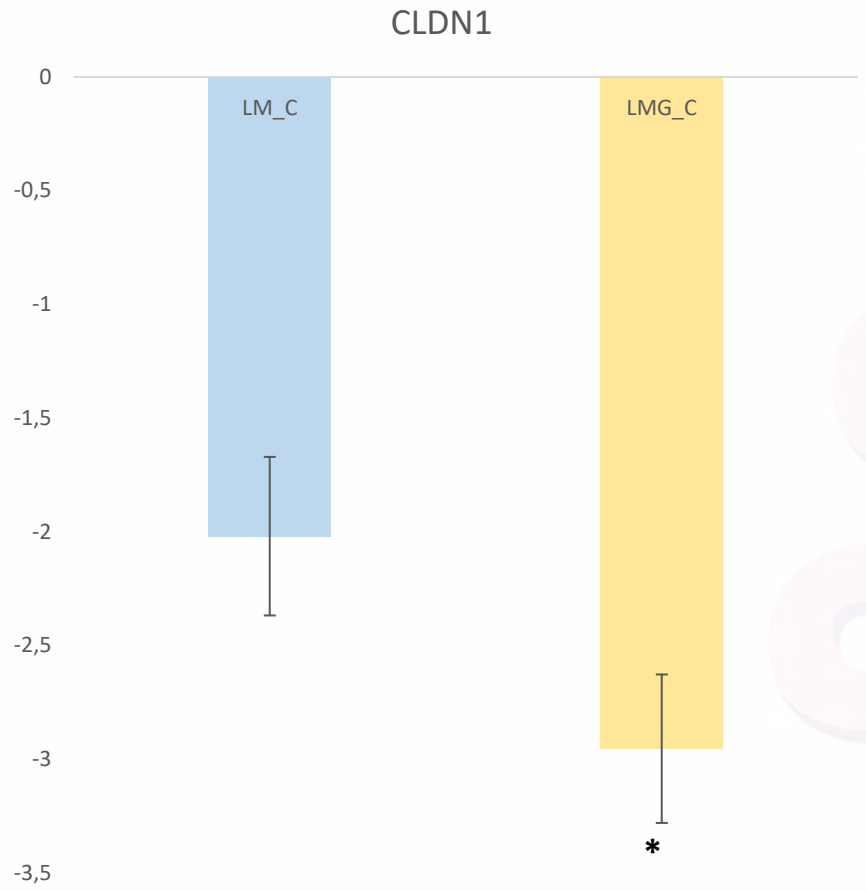
Campylobacter challenge – gene expression cecal mucosa



Significant changes in CLDN1 gene expression in the cecal mucosa

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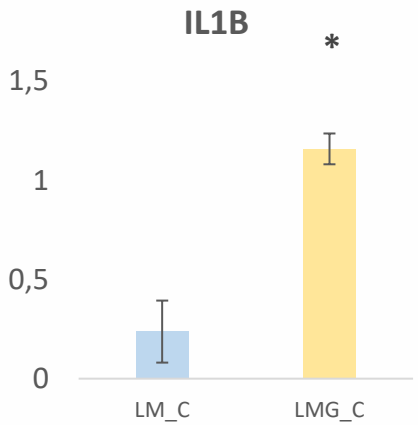
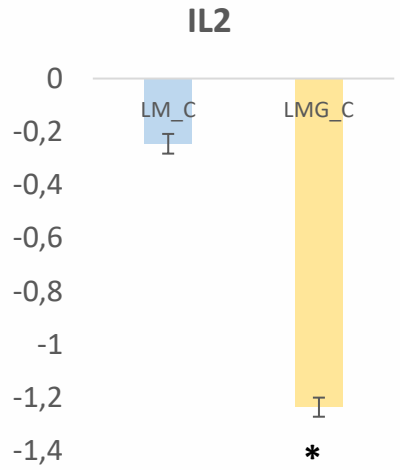
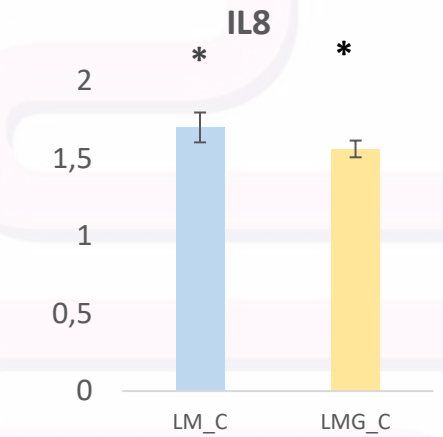
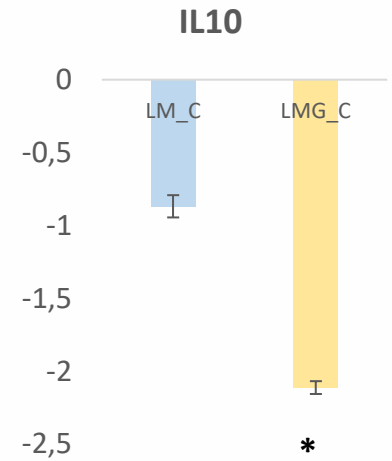
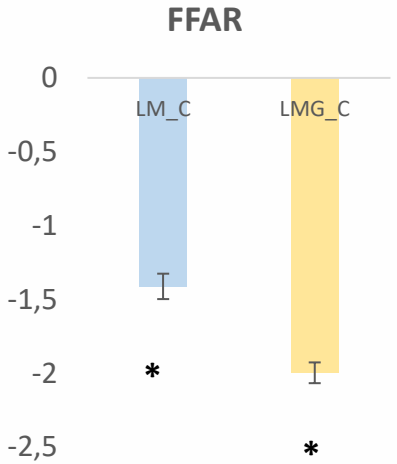
Campylobacter challenge – gene expression cecal tonsils

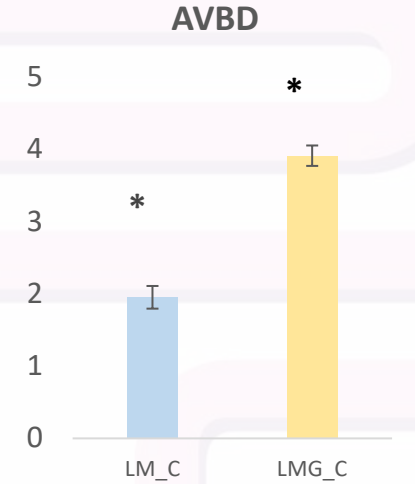












Significant changes in the gene expression of cecal tonsils of *Campylobacter jejuni* infected broiler chickens. *A: IL1B B: IL2 C: IL8 D: IL10 E: FFAR2 F:AVBD1*

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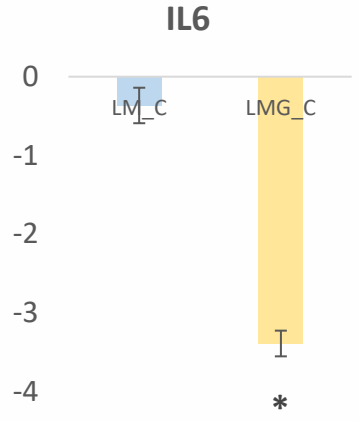
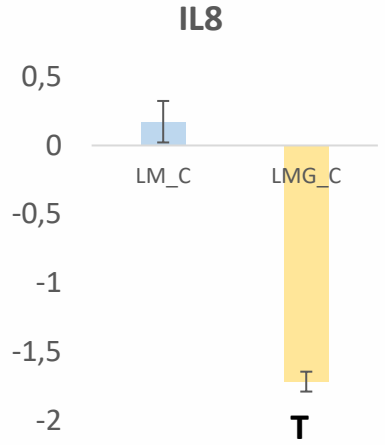
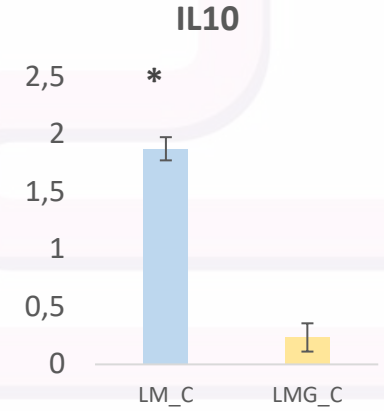
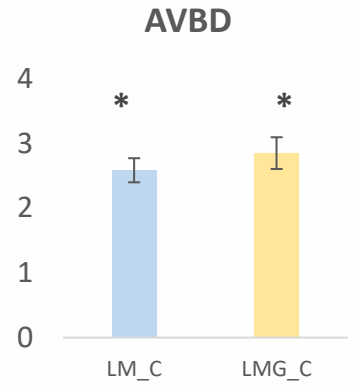
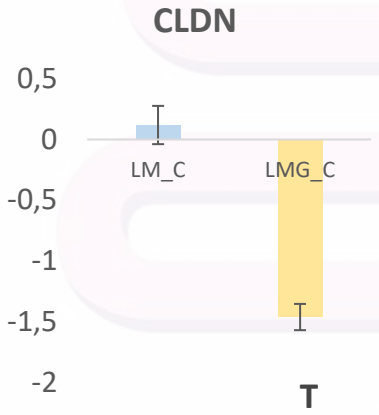
Campylobacter challenge - gene expression spleen











Significant changes in the gene expression in the spleen of *Campylobacter jejuni* infected broiler chickens. *A: IL6 B: IL8 C: IL10 D: AVBD E: CLDN*

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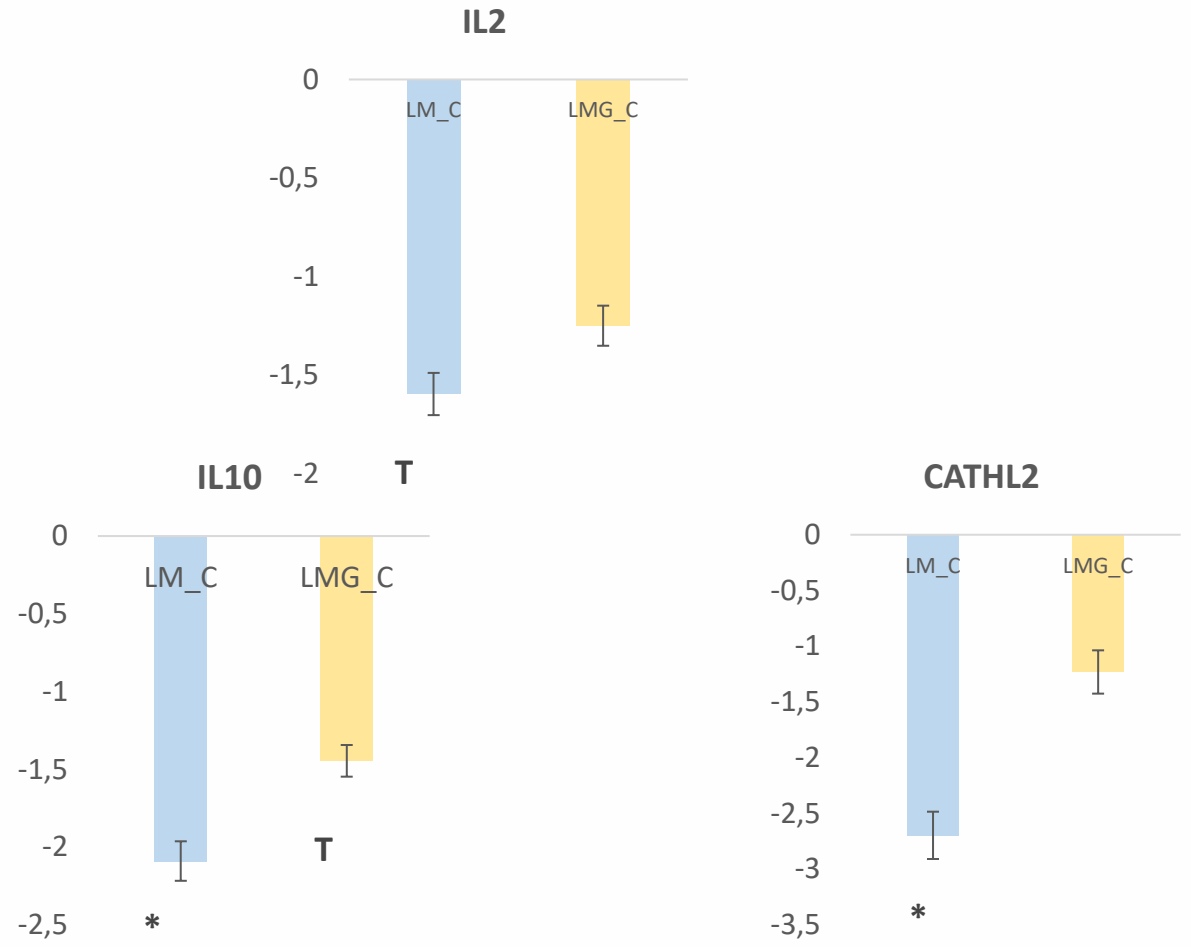
Campylobacter challenge – gene expression liver



Significant changes in gene expression in the liver of *Campylobacter jejuni* infected broiler chickens. A: *IL2* B: *IL6* C: *IL10* D: *CATHL2* E: *AVBD1*

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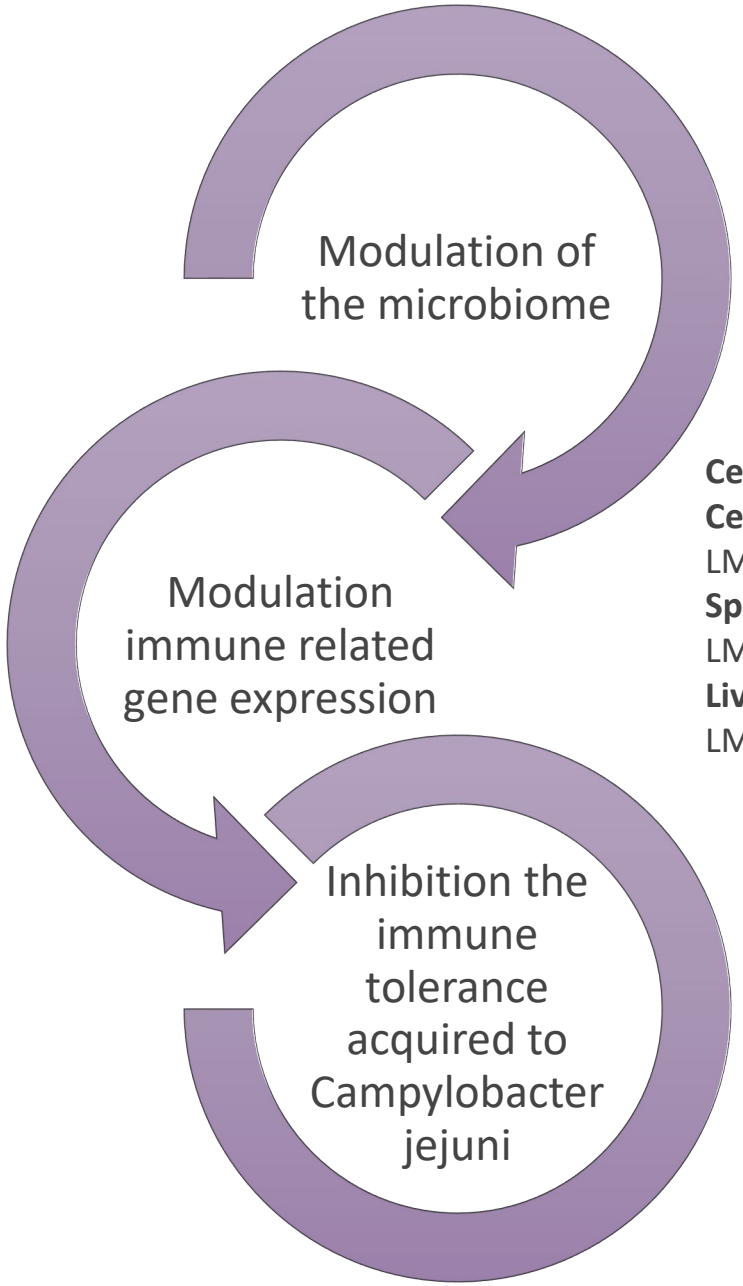

Akkermansia sp. ↓

Bifidobacterium sp. ↓

Cecal mucosa = CLDN ↓

Cecal tonsils = LMG group : IL1B, IL6, AVBD ↑ IL2, IL10, FFAR2. ↓

LM group: IL8, AVBD1 ↑ FFAR2 ↓

Spleen = LMG group: AVBD ↑ IL6, IL8, CLDN ↓

LM group: AVBD, IL10 ↑

Liver = LMG group: IL10 ↓

LM group: IL2, IL6, IL10, CATHL2, AVBD ↓

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Conclusions

Selected **probiotic** (*Leuconostoc mesenteroides* B/00288) and a **prophybiotic** (*Leuconostoc mesenteroides* B/00288 + garlic aqueous extract) administered *in ovo* mitigated *Campylobacter jejuni* colonization in ROS308 broiler chickens without compromising the production parameters

In ovo stimulation with **probiotic and prophybiotic** triggered genes related to innate immunity in cecal tonsils and spleen.

LIMITATION !!!!!! → Only one strain of *Campylobacter jejuni* was used in the current study and the colonization potential and mechanisms differ in different strains.

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TEAM WORK !

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THANK YOU

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