



Perinatal management and dietary strategies improving embryonic and post-hatch development of broilers

Prof Dr Ir Nadia Everaert

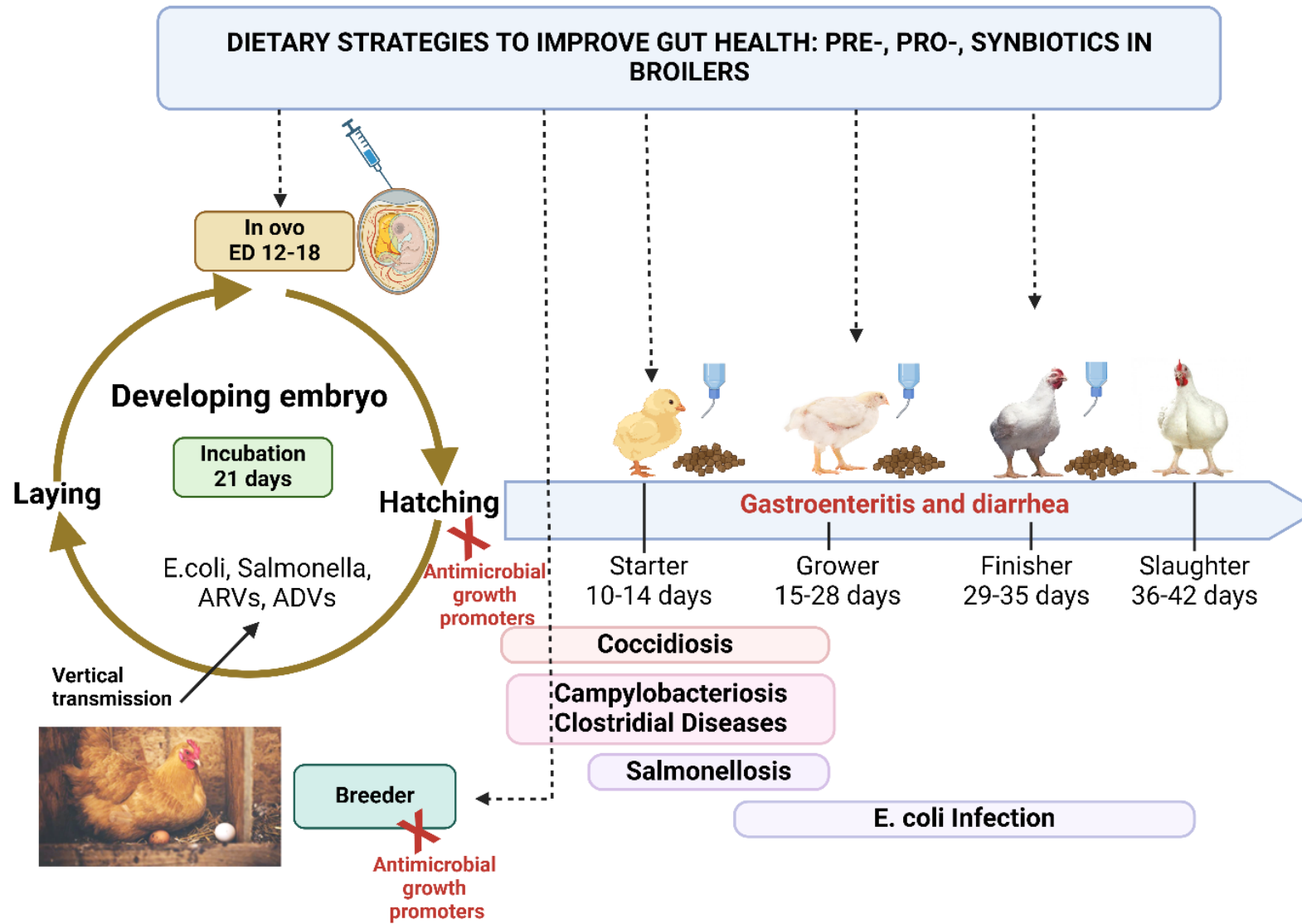
EAAP Annual Meeting, Florence, 4th of September 2024

Overview

- The gut microbiota and the immune system
- The mission
- In ovo strategies to obtain an optimal start
- The hatching window and access to feed
- Creating microbial diversity in early life

The gut microbiota and the immune system





No contact with adult hen post-hatch



- Nutrients and maternal antibodies are deposited in the egg before incubation
- A maternal core microbiota might not be completely transferred to hatching chicks

Microbiota in ileum and cecum on D7 and 35

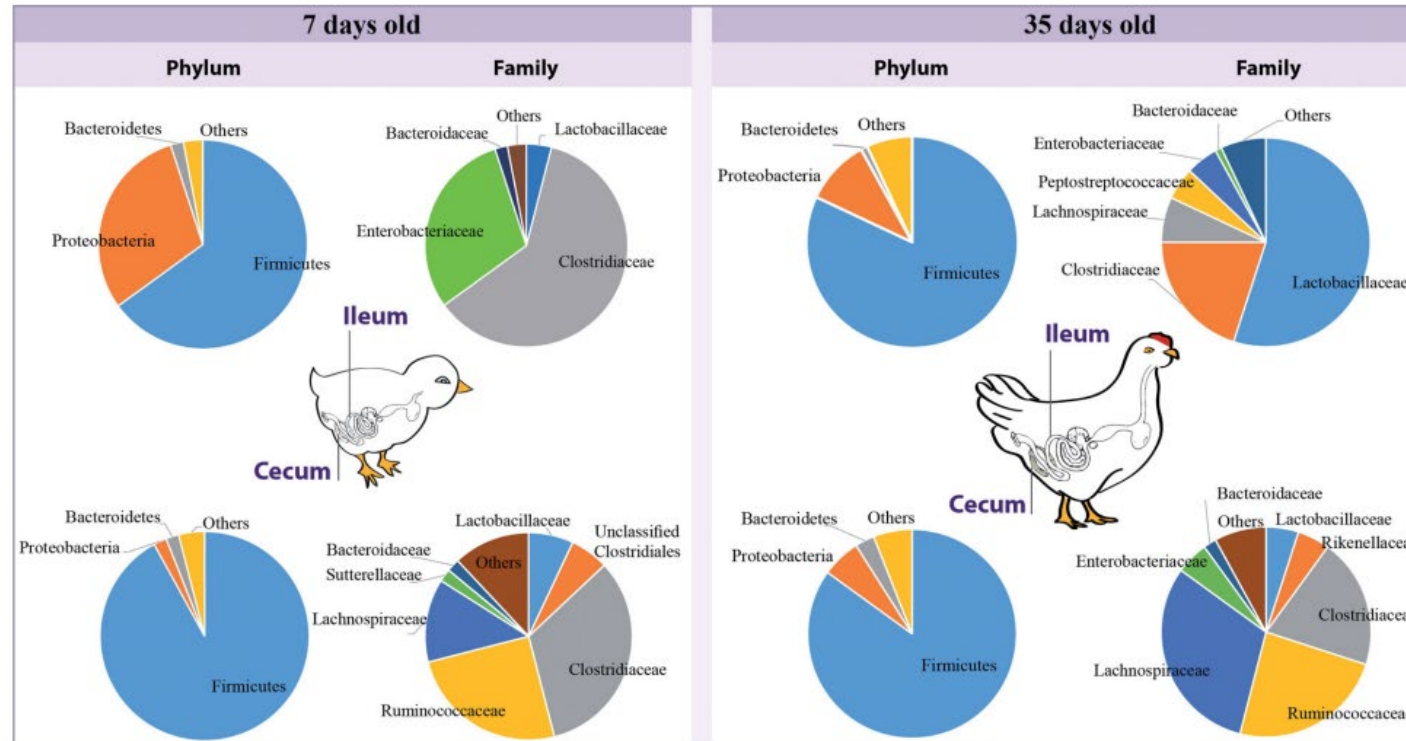


Figure 1. The chicken gut microbiome. The graphs provide an overview of the relative abundance of dominant bacterial phyla and families of the broiler chicken ileal (top level) and cecal (bottom level) microbiota in two different ages, 7 and 35 days. Data are compiled from three studies: Asrore et al. (2015) for ileum on day 7, Corrigan et al. (2015) for cecum on day 7 and Pourabedin et al. (2015) for ileum and cecum on day 35.

Hens act as an important source for certain genera

Table 1. Bacterial genera which were more abundant in microbiota of chicks raised in a contact with an adult hen than in the control chicks.

Phylum	Family	Genus	Control (%) [*]	Contact (%) [*]	Contact/ Control [#]
Actinobacteria	Bifidobacteriaceae	Bifidobacterium	0.067	1.714	25.6
Actinobacteria	Coriobacteriaceae	Olsenella	0.038	0.651	17.1
Bacteroidetes	Bacteroidaceae	Bacteroides	0.454	9.383	20.7
Bacteroidetes	Porphyromonadaceae	Barnesiella	0.085	10.023	118.3
Bacteroidetes	Porphyromonadaceae	Parabacteroides	0.071	1.731	24.4
Bacteroidetes	Prevotellaceae	Paraprevotella	0.068	2.140	31.5
Bacteroidetes	Prevotellaceae	Prevotella	0.047	1.993	42.8
Bacteroidetes	Rikenellaceae	Alistipes	0.032	2.756	86.3
Deferribacteres	Deferribacteraceae	Mucispirillum	0.014	0.505	36.9
Firmicutes	Ruminococcaceae	Faecalibacterium	0.191	6.687	35.0
Firmicutes	Acidaminococcaceae	Phascolarctobacterium	0.100	2.224	22.2
Firmicutes	Veillonellaceae	Megamonas	0.190	4.301	22.6
Proteobacteria	Desulfovibrionaceae	Desulfovibrio	0.079	0.675	8.5
Sum			1.44	44.78	31.2

^{*} average abundance of given genus in microbiota of control or contact chicks

[#] ratio of abundance in contact and control chick microbiota

Kubasova et al., 2019, PLoS One



Temporal dynamics of the microbiome

- Community richness increases rapidly over time, stabilizing at day 14
- Variation also increases over time

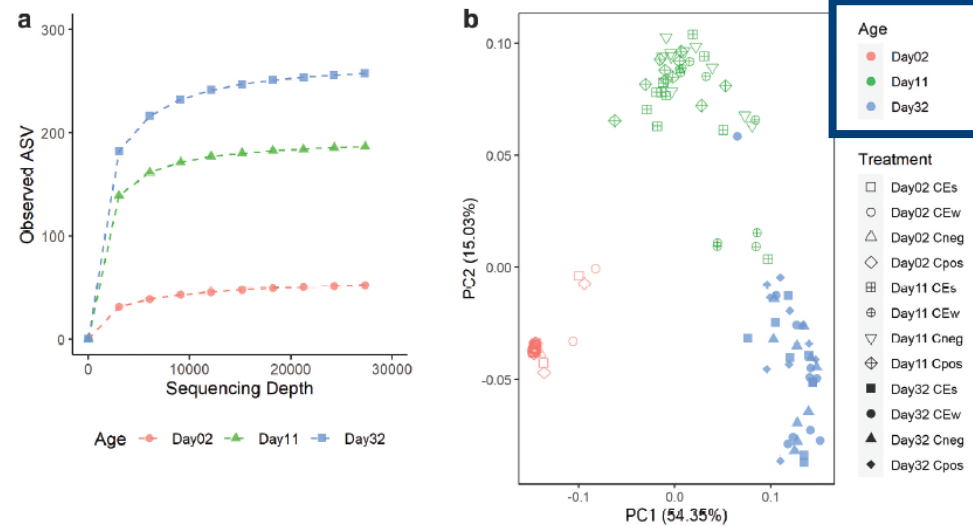
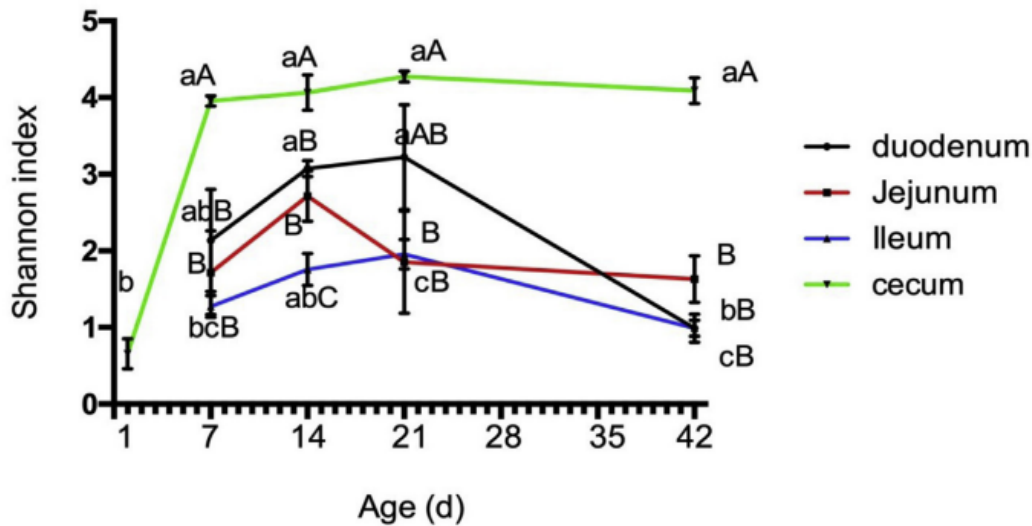


Figure 4. (a) Rarefaction curves of observed amplicon sequence variants (ASV) in caecal samples of different ages and (b) principal coordinate analysis (PCoA) plot showing differences in generalised UniFrac beta diversity at different treatments and ages. Treatments: chicks in the hatcher were given no access to feed and water (Cneg), access to feed and water (Cpos), access to feed, water, and a competitive exclusion (CE) product provided in the water (CEw), or access to feed, water and a CE product sprayed on the down of the newly hatched chicks (CEs). Different treatments are indicated by symbols, ages are indicated by colours.

Passive immunity

- **IgY**: the mammalian IgG homolog
- During embryonic development, transported from Yolk to blood
- **Maternal IgA** is transported from albumen to chick's intestine before hatch, swallowed by embryo
- B cell population in the GALT starts at 4d posthatch, continues to increase during first 2 weeks

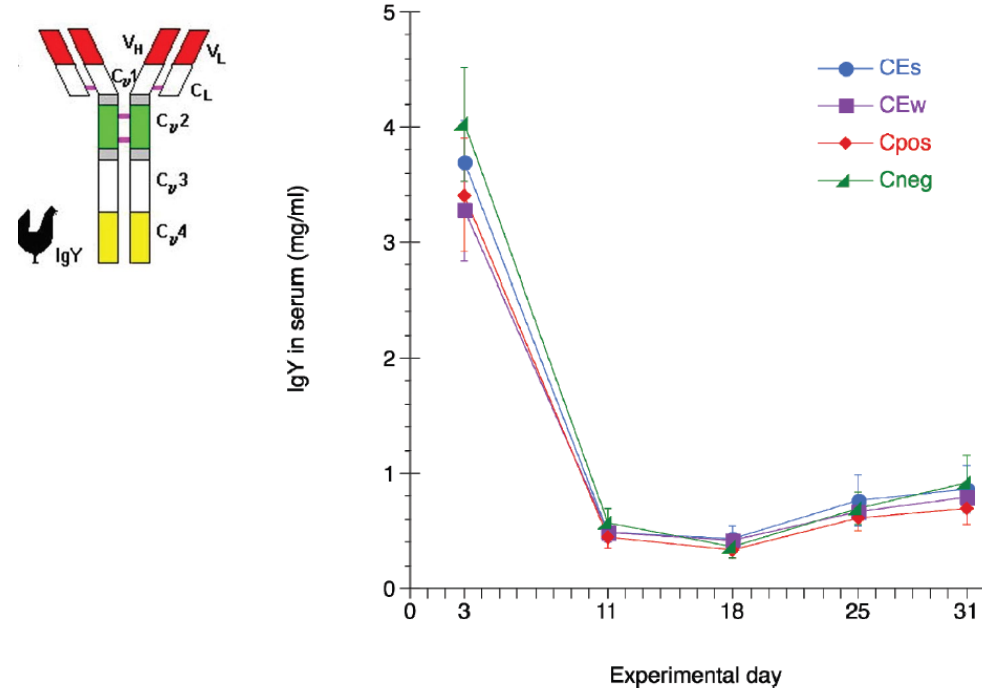
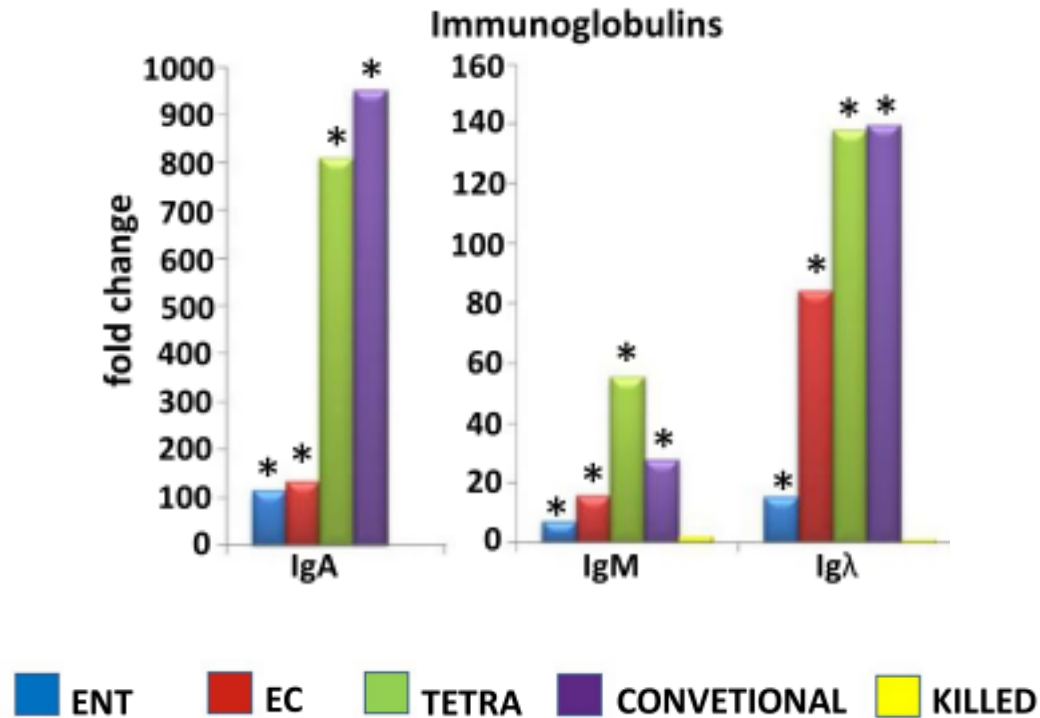


Figure 2. Total amounts of IgY in serum collected from chickens at 3, 11, 18, 25 and 31 d of age. Values are group mean $\pm$ 95% confidence interval. Treatments: chicks in the hatcher were given access to feed, water, and a competitive exclusion (CE) product sprayed on the down of the newly hatched chicks (CEs; circles), access to feed, water, and a CE product provided in the water (CEw; squares), access to feed and water only (Cpos; diamonds), or no access to feed and water (Cneg; triangles).

Boyner et al., 2023, Br Poult Sci

The need of living gut bacteria to synthesize Ig's

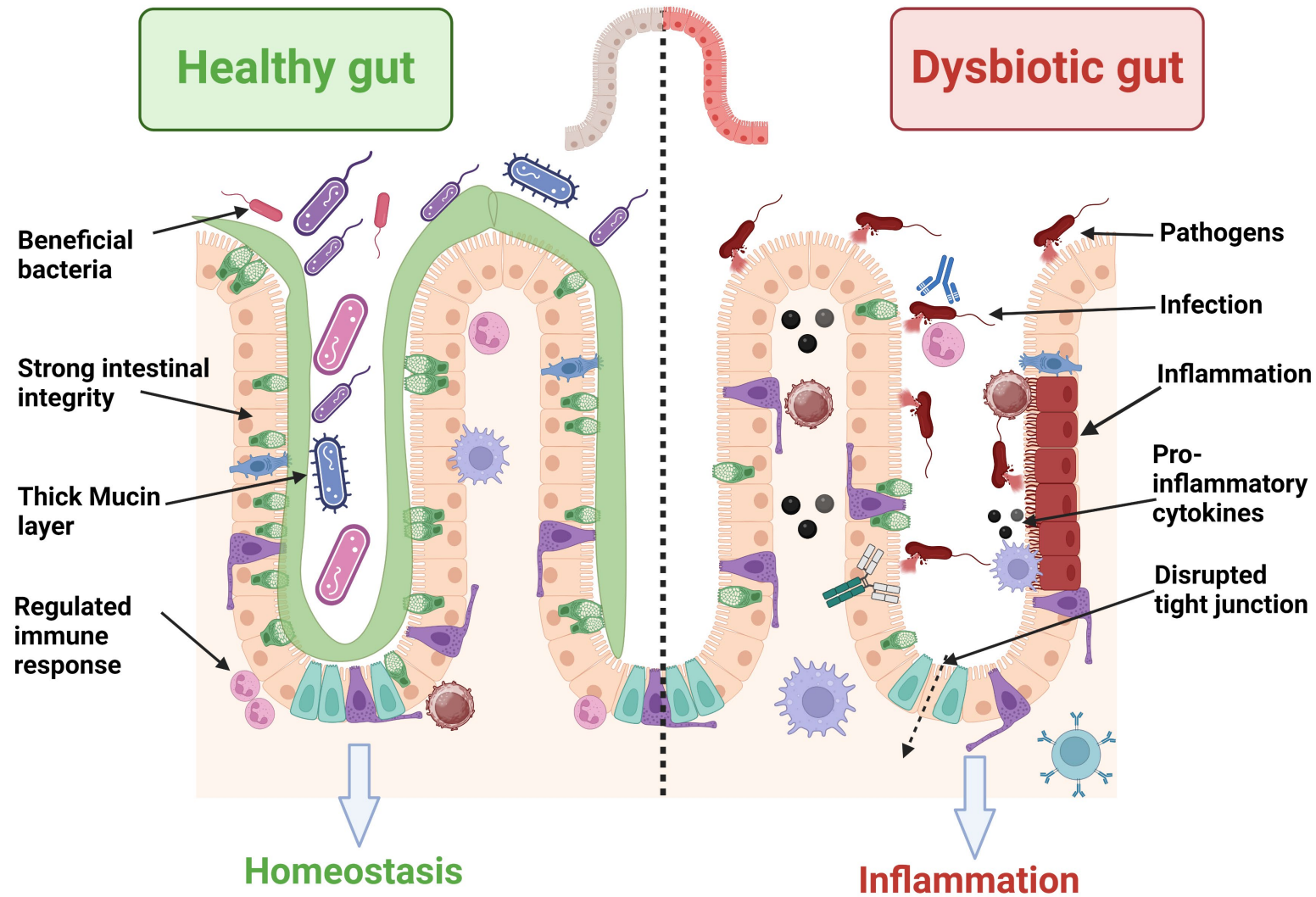
- Day 56



Volf et al., 2017, Vet Res

The mission

Targeting a health gut



Based on <https://doi.org/10.1038/s41392-022-00974-4>

Created with BioRender.com



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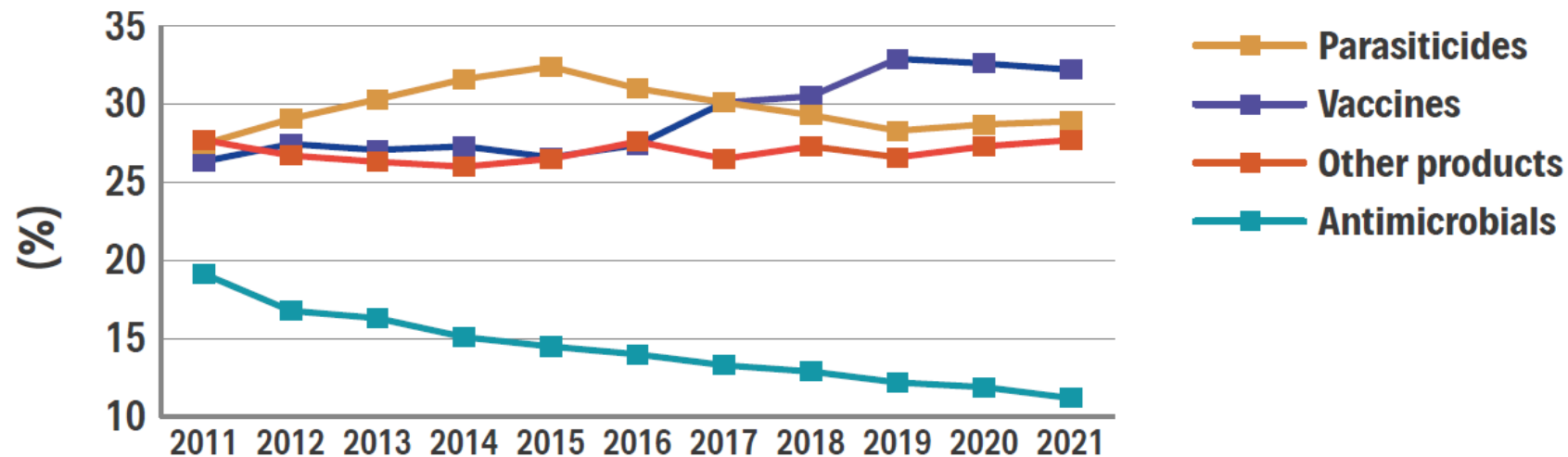


NAMES Lab
Nutrition & Animal
Microbiota EcoSystems

Decrease in antimicrobials, increase in alternative strategies

Sales of prevention products are increasing which can reduce the need for antibiotics

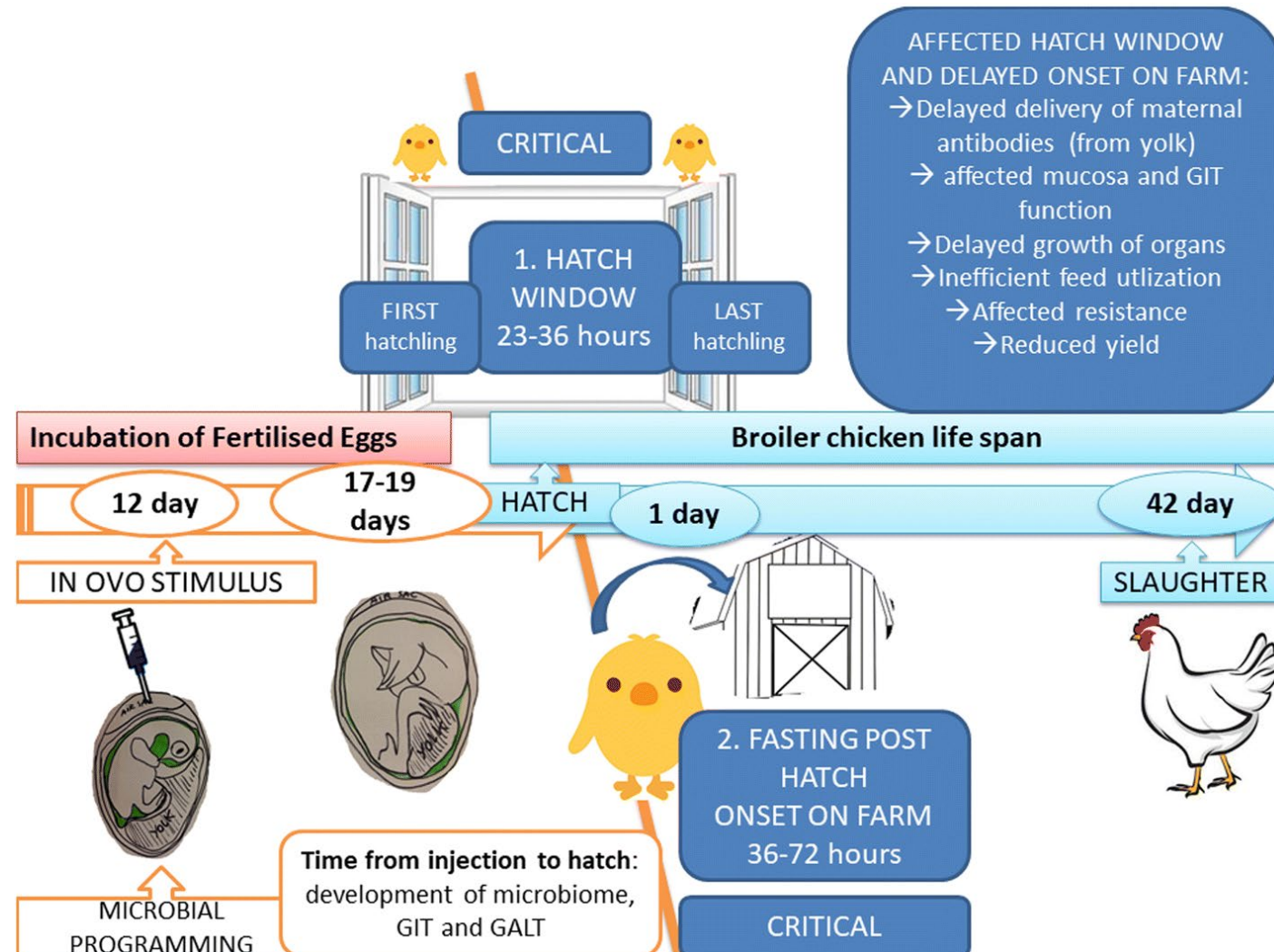
Sales per product category (%) (2011–2021)



Sales data from leading animal health companies demonstrate how the increased focus on prevention is reducing the need for antimicrobials. Since 2011, sales of vaccines and parasiticides combined have grown from 53.8% to 61.1% of the product portfolio while antimicrobials fell from 19.4% to 11.2% (a relative reduction of around 42%).



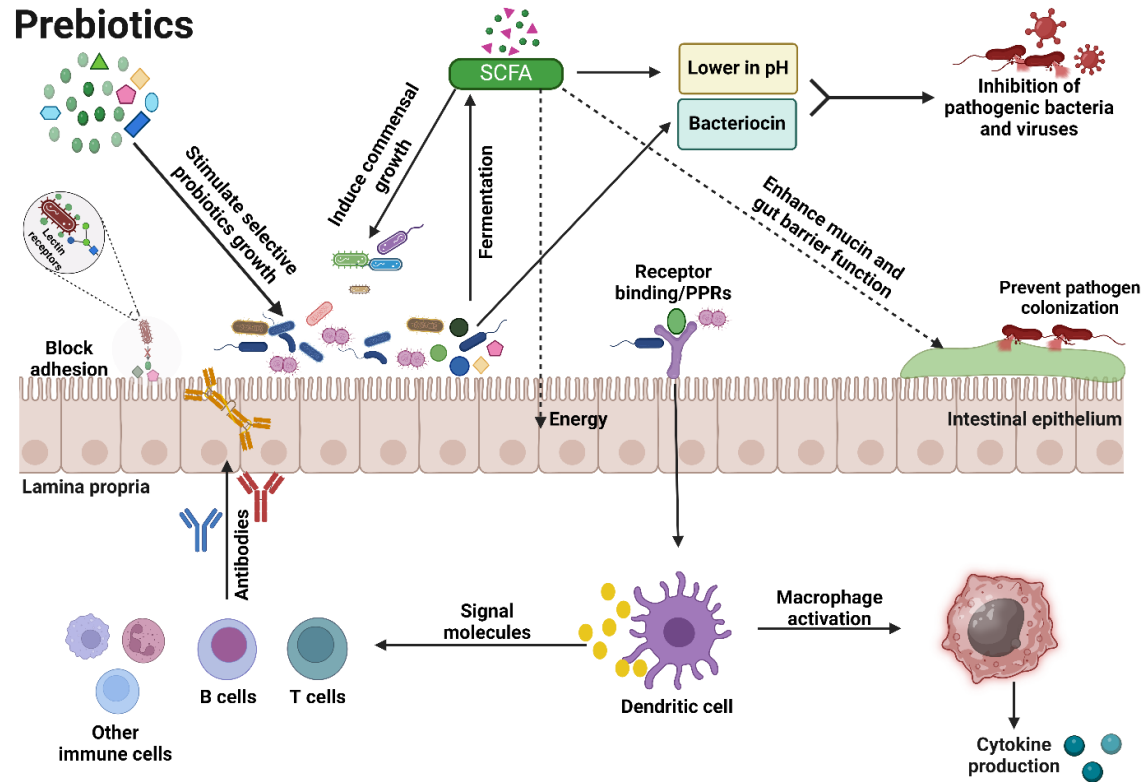
Different strategies to obtain an optimal start



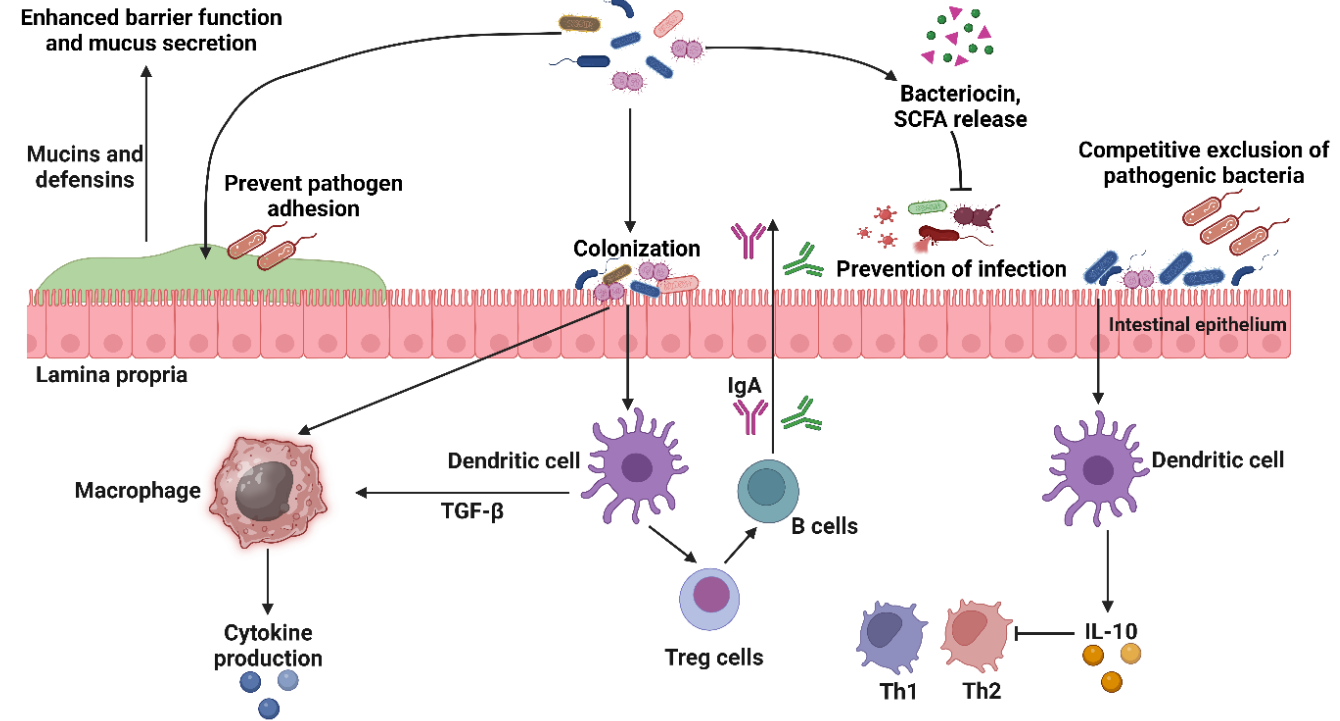
Siwek et al., 2018 BMC Vet Res

Alternative strategies: pre- and probiotics

Prebiotics



Probiotics



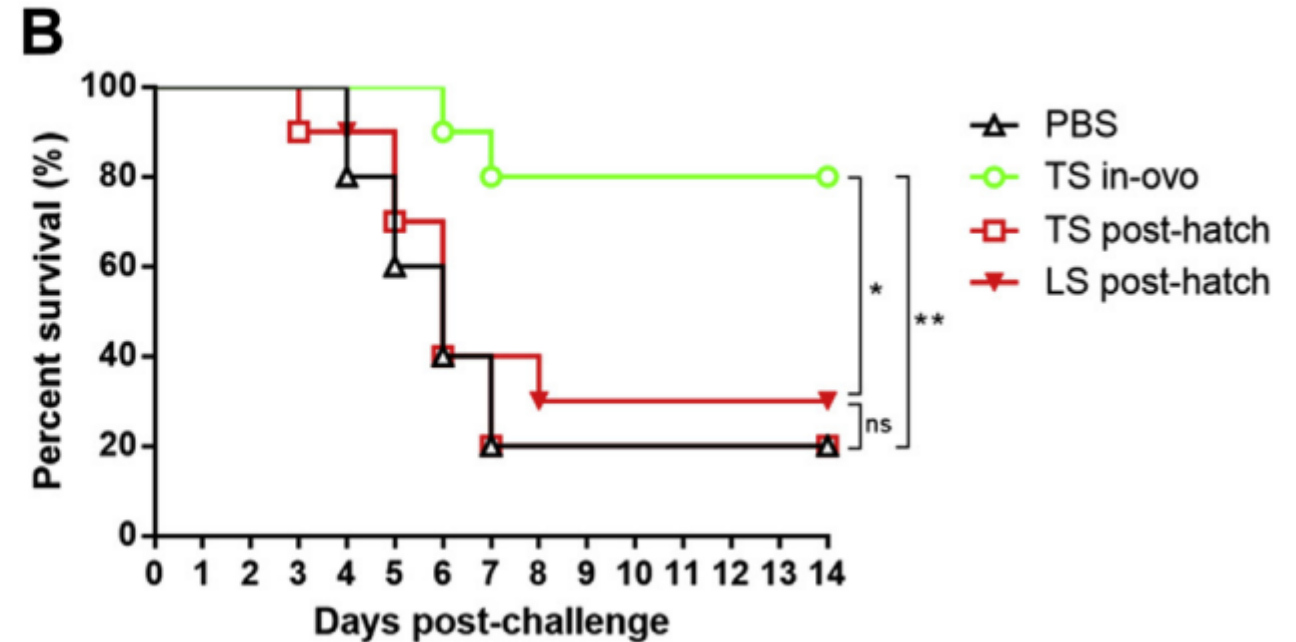
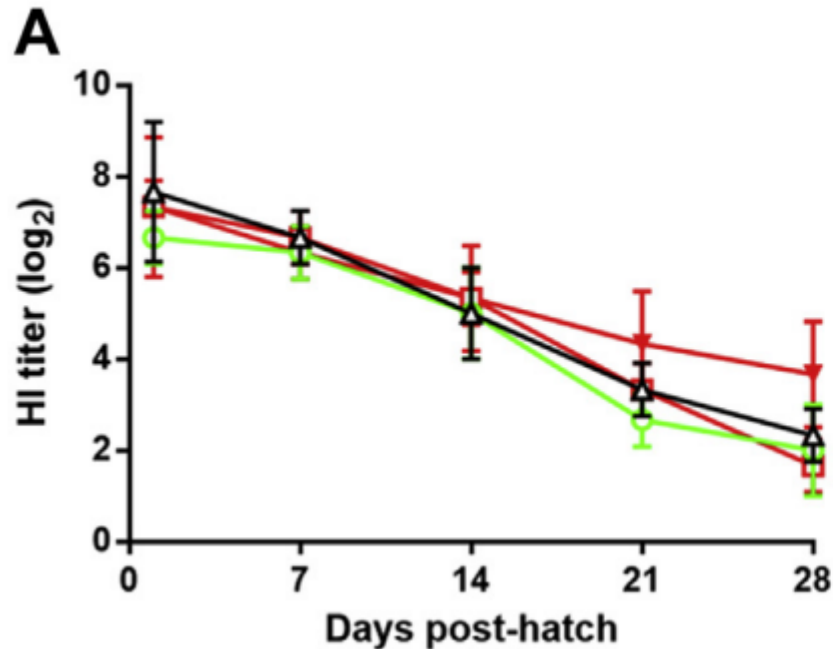
In ovo strategies to obtain an optimal start

Preventive strategy: in ovo vaccination



In ovo vaccination against Newcastle disease

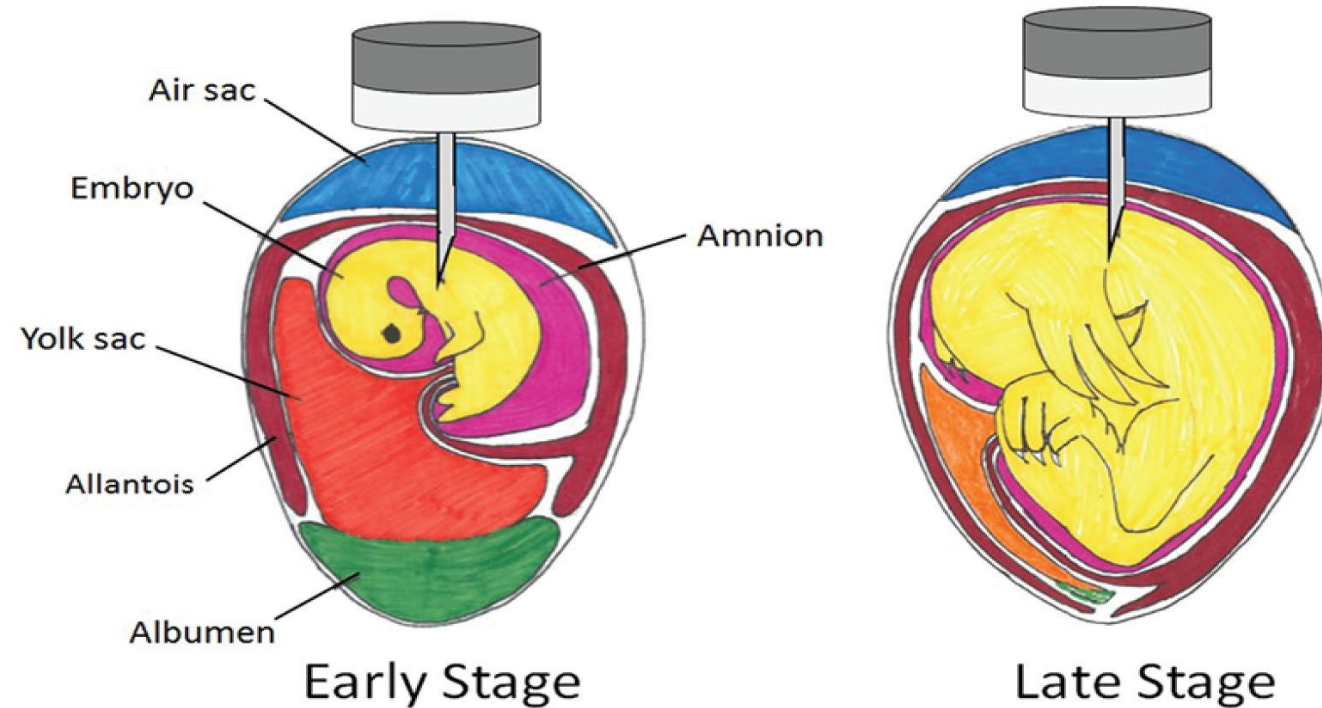
- Despite low antibody response, in ovo vaccination protects against NDV



Fan et al., 2020, Poult Sci

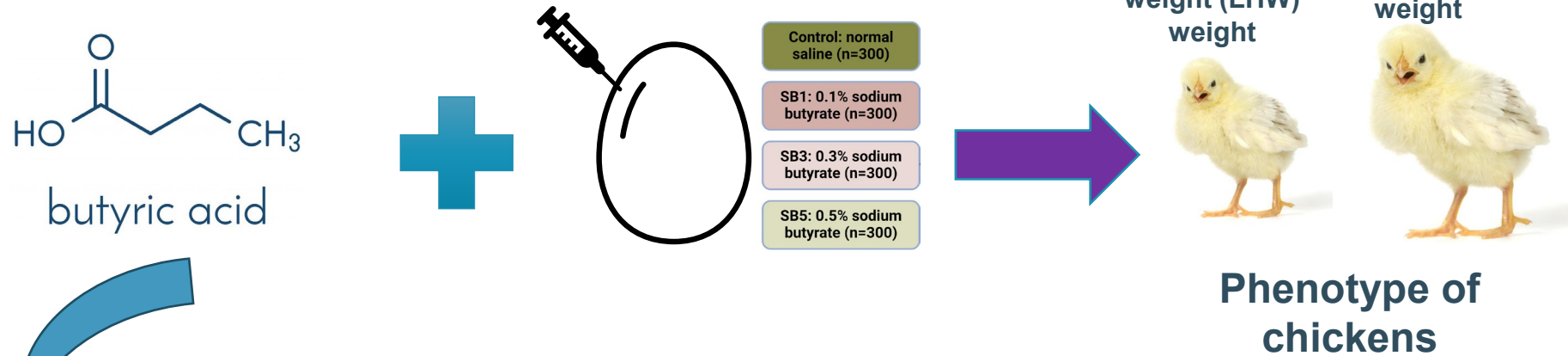
In ovo technology

- Injection on embryonic day (ED)12:
 - **In ovo stimulation**
 - In air cell
 - Delivery of pre- and synbiotics
 - Stimulate native egg microbiota
- Injection on ED17-18 (at transfer):
 - **In ovo feeding**
 - In amnion or embryo
 - May reduce hatchability



Siwek et al., 2018 BMC Vet Res; Abd El-Azeem et al., 2023 WPSJ

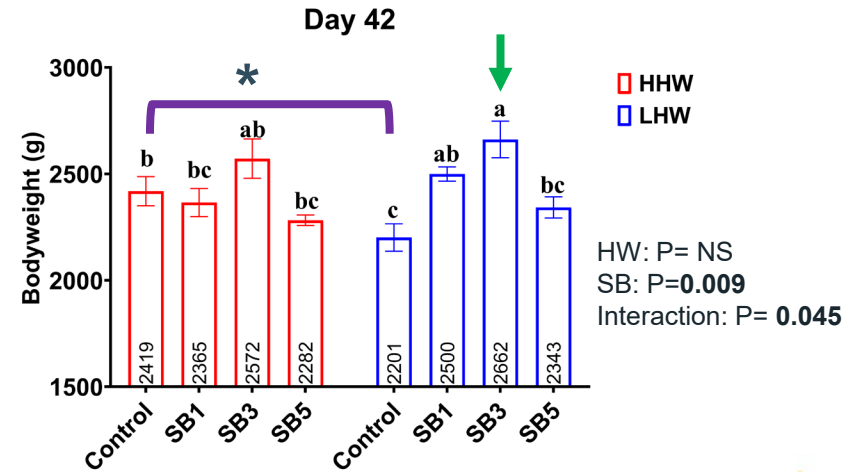
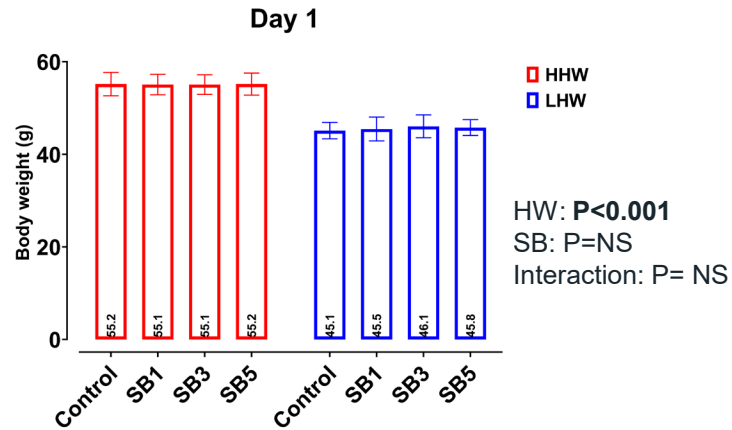
Effects of in ovo sodium butyrate administration in chickens



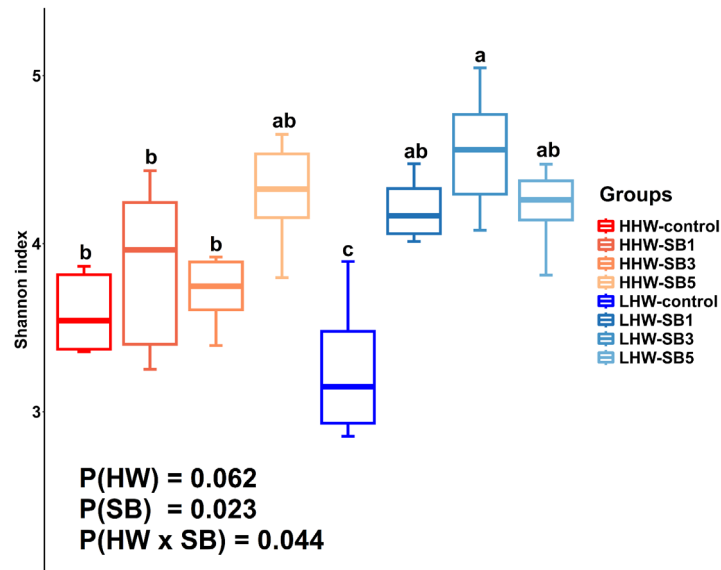
4 x 2 factorial design

To examine the effect of in ovo sodium butyrate (SB) injection on growth performance, intestinal development and gut microbiota of chickens with different hatch weight (HW)

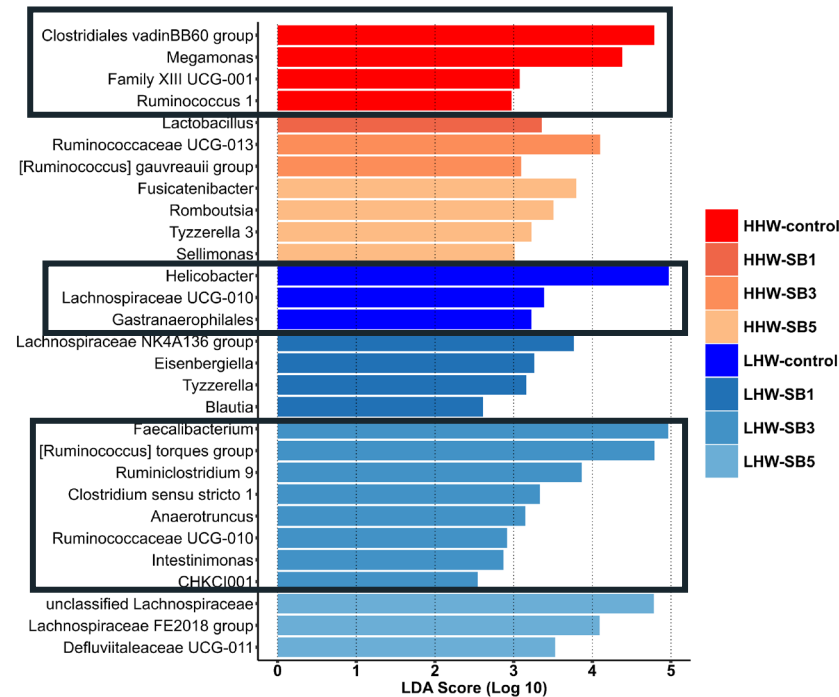
Results



Alpha diversity



Differential abundance analysis at genus level



- HW had a **positive effect** on subsequent **growth performance** and gut-related parameters.
- LHW chicks** relatively benefited more from **0.3% SB**, showing improvements in growth and a more favorable **gut microbiota characteristics**.



In ovo feeding

- Various nutrients have shown positive effects on growth and immune system development:
 - Vitamins, amino acids, glucose, prebiotics
- In ovo **inulin**:
 - Increased villus height at hatch
 - Increased mucin production
 - Proliferation of T cells
- In ovo **XOS** and **MOS**
 - Increased VH/CD at hatch
 - Higher cecal SCFA (acetate and/or butyrate) on d28
 - MOS4: higher OTUs and more differentially abundant taxa on d 28

Jha et al., Front Vet Sci, 2019; Singh et al., 2022 J. Anim. Sci. Biotechnol.



In ovo feeding (ED 18) of bioluminescent bacteria

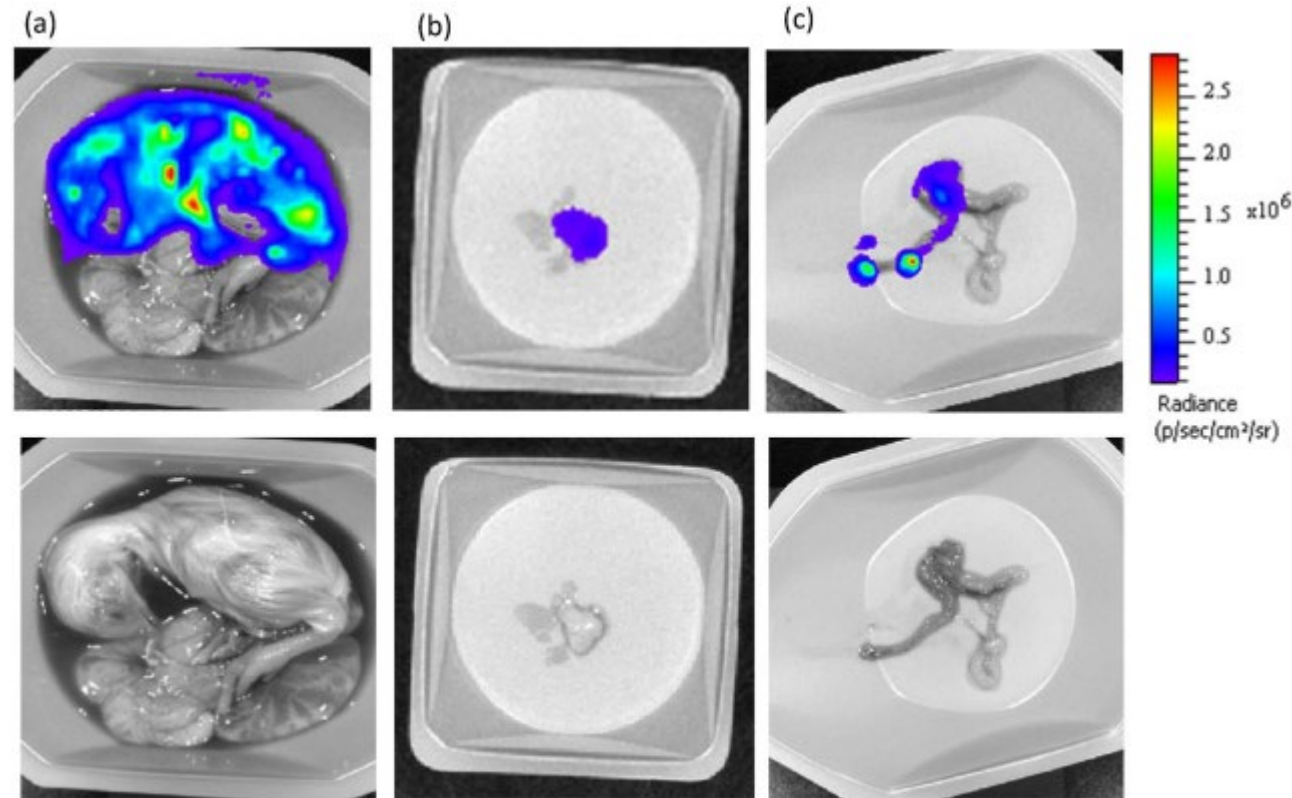


Figure 2. IVIS spectrum detection of bioluminescence from fertile hatching eggs that were injected with 10^6 CFU/mL of *E. coli* DH5 α pBS-slpGFP_{luxABCDE} on d18 of incubation. (a) top image represents an embryo from an in ovo injected egg covered in its amnion and bioluminescent bacteria; bottom image represents same image in a gray/white scale (b) bursa collected from an embryo of an egg in ovo injected with bioluminescent *E. coli* (c) Gastrointestinal tract collected from an embryo of an egg in ovo injected with bioluminescent *E. coli*.

Castañeda et al., 2019; Poult. Sci.

Injection of probiotic *Enterococcus faecium*

- 6 probiotics were tested in ovo to evaluate hatchability
- When? ED 17.5 in ovo injection and dietary supplementation
- Decreased proportion of *Salmonella enteritidis* possibly by competitive exclusion

Item	Positive		Negative		P-value
	n	%	n	%	Compared with NC
Negative control	29	81	7	19	—
Positive control	1	3	35	97	<0.0001
PRB7	26	81	6	19	0.942
PRB7_IO	31	86	5	14	0.527
PRB5	28	78	8	22	0.772
PRB5_IO	19	53	17	47	0.012

¹n = number of birds out of 36 total. NC = negative control; PC = positive control.

The hatching window and access to feed



The hatching window per sex

- 48h hatching window
- Females hatch earlier than males

Interaction of broiler hatching time and feed access

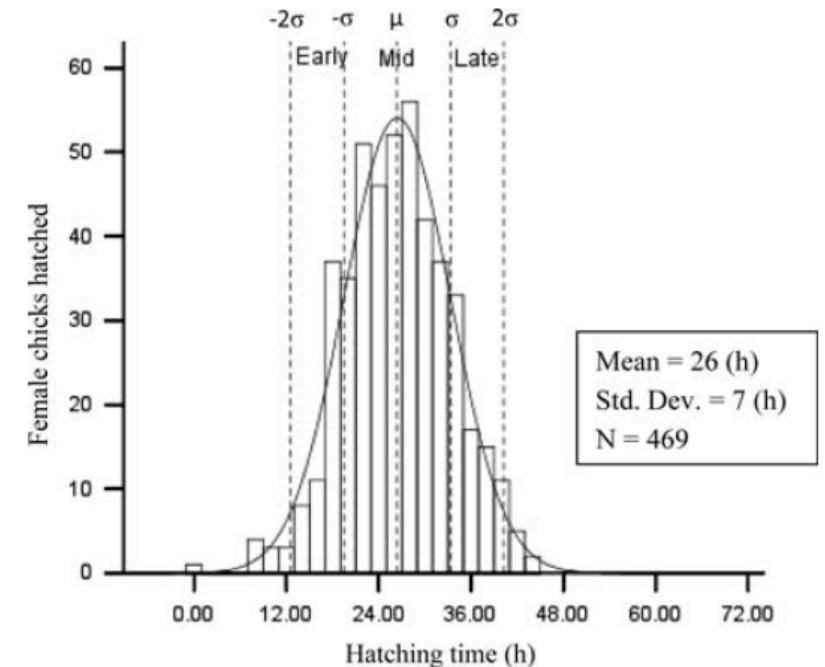


Figure 1 Hatching curve, early, middle and late hatching and spread of hatch of female chicks ($n = 469$). Distance between two close dotted lines is σ .

Factors explaining hatching window

- T_3 stimulates hatching
- Early hatchers have less energy left

Table 4 *Effect of hatching time on residual yolk weight, liver glycogen content, hatching muscle glycogen content, plasma glucose and T_3 levels at hatch (BA day 0)*

Performance parameters	Hatching period			s.e.m.	P-value
	Early	Middle	Late		
Residual yolk (g)	8.47	8.08	8.98	0.38	0.64
Liver glycogen ($\mu\text{g}/\text{mg}$)	1.82	3.04	3.84	0.37	0.09
HM glycogen ($\mu\text{g}/\text{g}$)	179.1	279.1	301.8	23.3	0.07
Plasma glucose (mg/dl)	205.4	208.6	209.4	3.0	0.87
Plasma T_3 (ng/ml)	4.68 ^a	2.69 ^b	2.90 ^b	0.37	0.04

BA = biological age; HM = hatching muscle.

^{a,b}Values within the same parameter with different superscripts differ significantly at $P < 0.05$.

Growth in early-post hatch phase

- Late hatchers win the early post-hatch race when fed
- No difference when they were all feed deprived

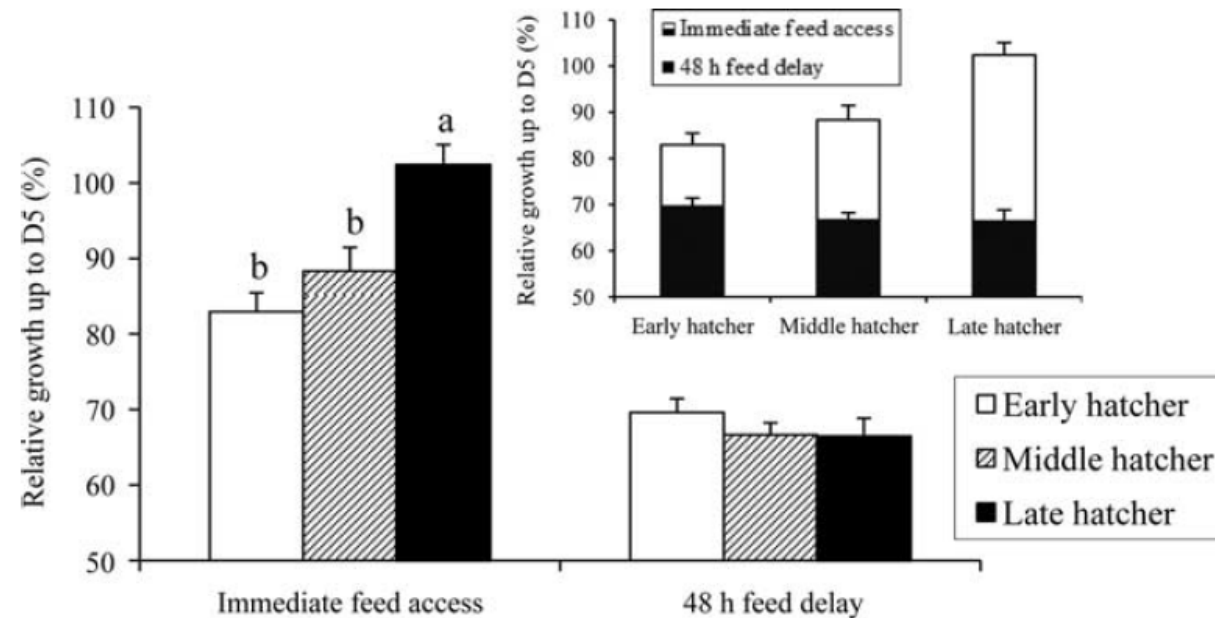
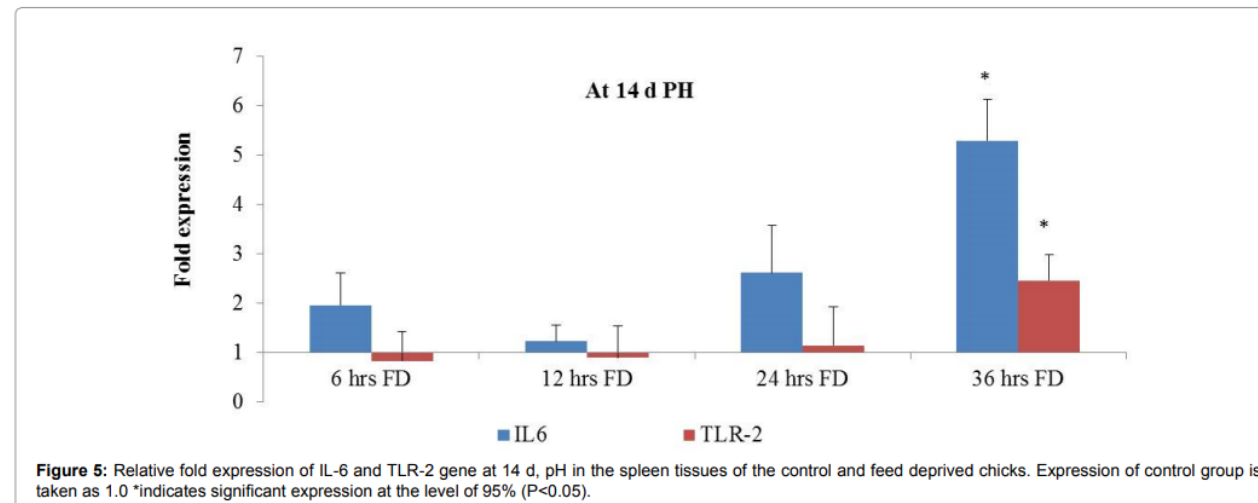
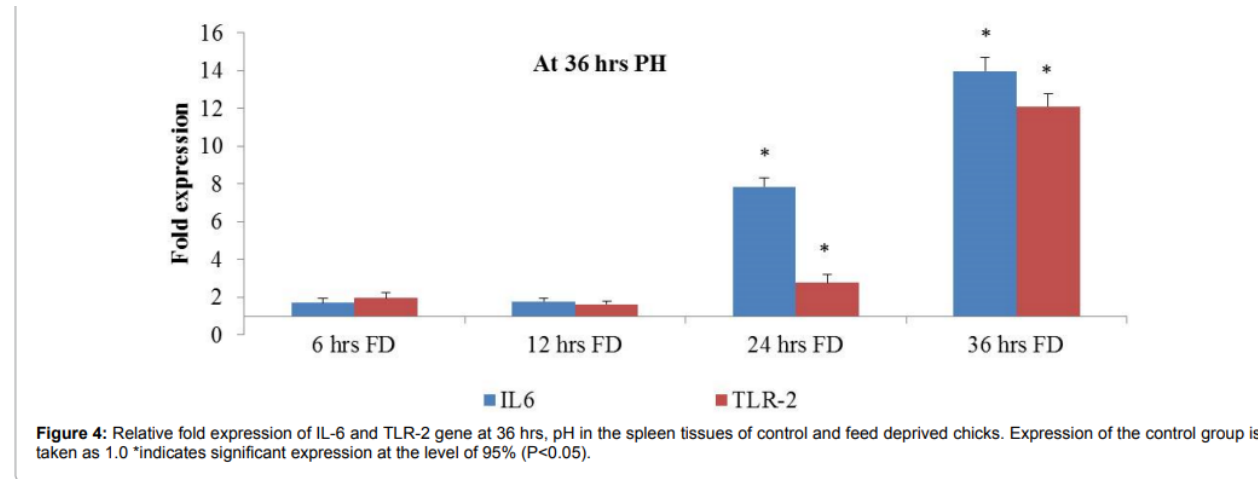


Figure 3 Effect of hatching time and feeding treatments (immediate access to feed and 48 h delay in feed access) on relative growth up to biological age day 5 ($n = 25$). Data sharing no common letter are different between hatching times within the same feeding treatment ($P < 0.05$). HT = hatching time; DF = delay in feed intake.

Impact of delayed feeding on immune system

- spleen



Shinde et al., 2015 J. Nutr. Food Sci.



Delayed feeding: affects chick's response to an immune challenge

- DF-F: Strongest sickness response and poorest performance

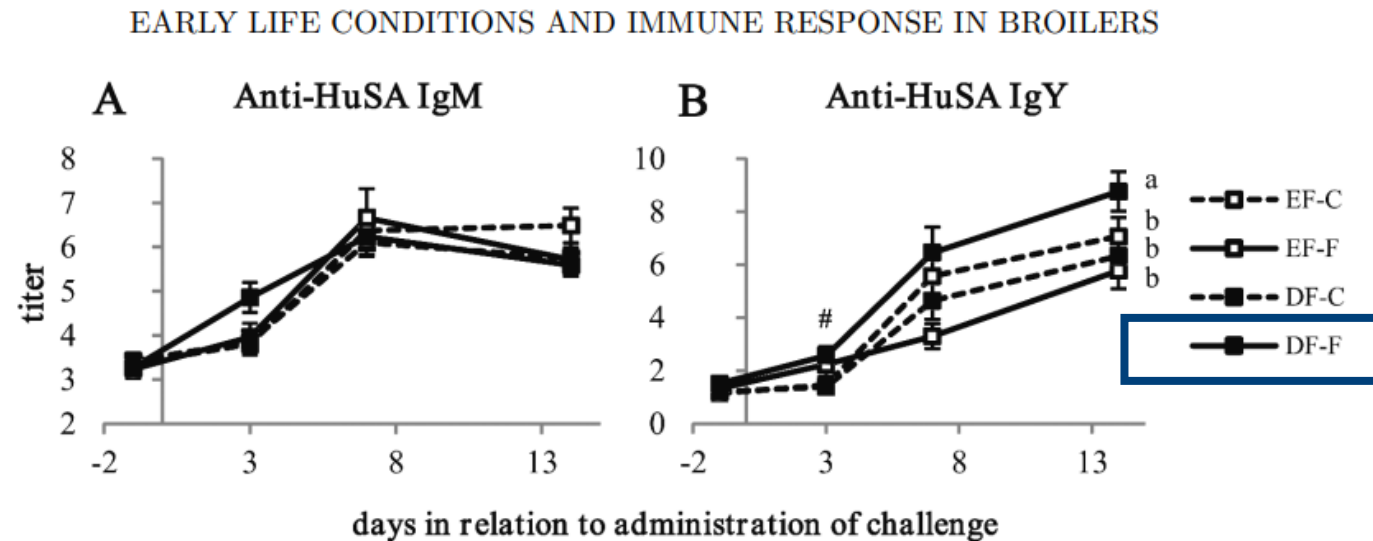


Figure 1. Specific antibody titers against HuSA of LPSA/HuSA challenged birds. A: Anti-HuSA IgM, B: Anti-HuSA IgY. Treatments were combinations of early life feeding strategy (EF = early feeding, DF = delayed feeding) and early life housing conditions (F = floor-housing, C = cage-housing). Data are displayed as means and corresponding SE. # indicates significant differences ($P \leq 0.01$) between F birds and C birds within a time point. Treatment combinations lacking a common superscript differ significantly ($P \leq 0.05$).

Feed restriction during hatching practices

- Decreased intestinal villus height and area
- Impaired intestinal barrier function
- Impact on gut microbiota
 - Increasing *Lactobacillus*
 - Decreasing *Turicibacteraceae* and *Enterobacteriaceae*

Metzler-Zebeli et al., 2019 mSystems



Immediate access to feed



Hatch brood system, Hatchtech



One2Born system

Immediate access to feed



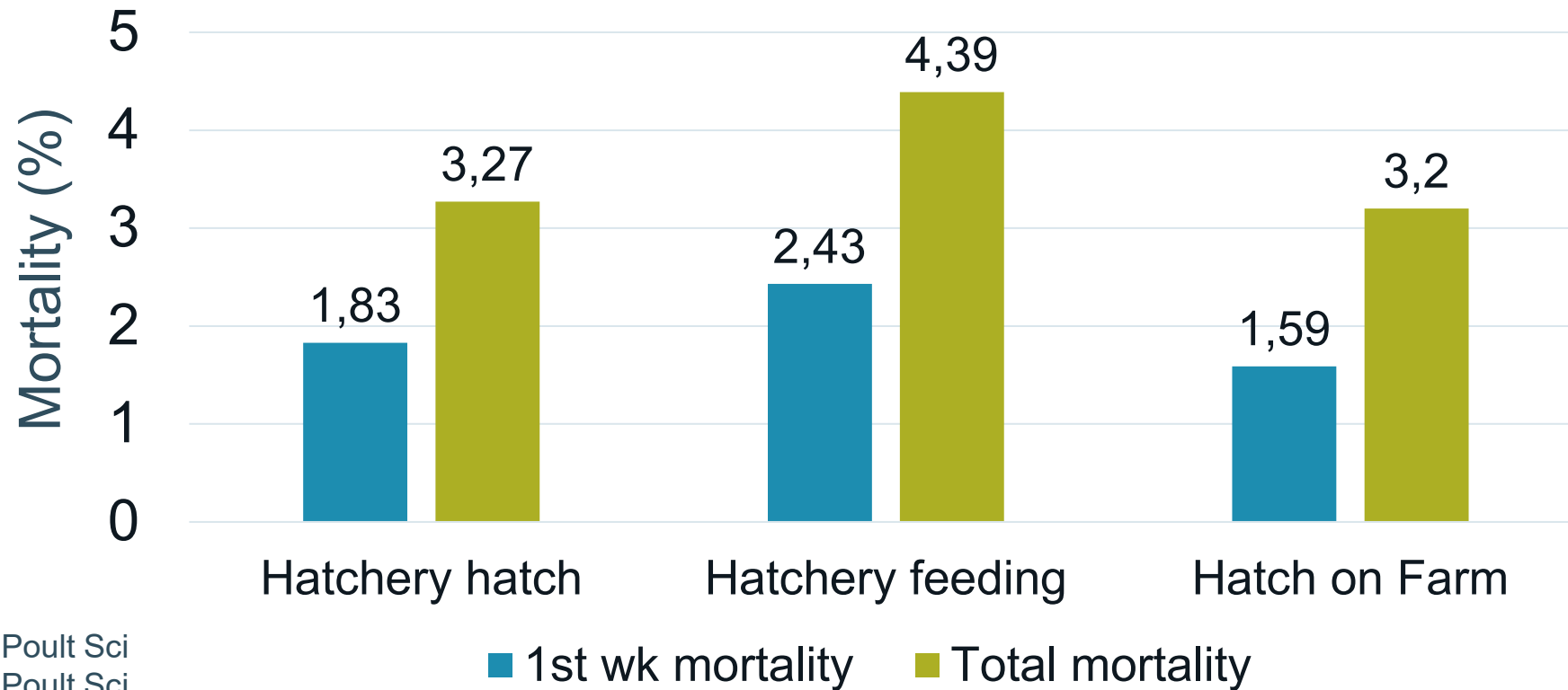
X-Treck system, Vencomatic



NestBorn system, Belgabroed

Immediately access to feed

- Beneficial for **growth** during first weeks
- Improved **litter quality** → less footpad dermatitis



Da Silva et al., 2021 Poult Sci
De Jong et al., 2020 Poult Sci



Early fed chicks have a higher body weight

Table 3. Predicted means for body weight $\pm$ SEM of hatchery-hatched (nonfed) (HH), hatchery-fed (HF), and on-farm hatched (OH) broiler chickens between D0 and D39.

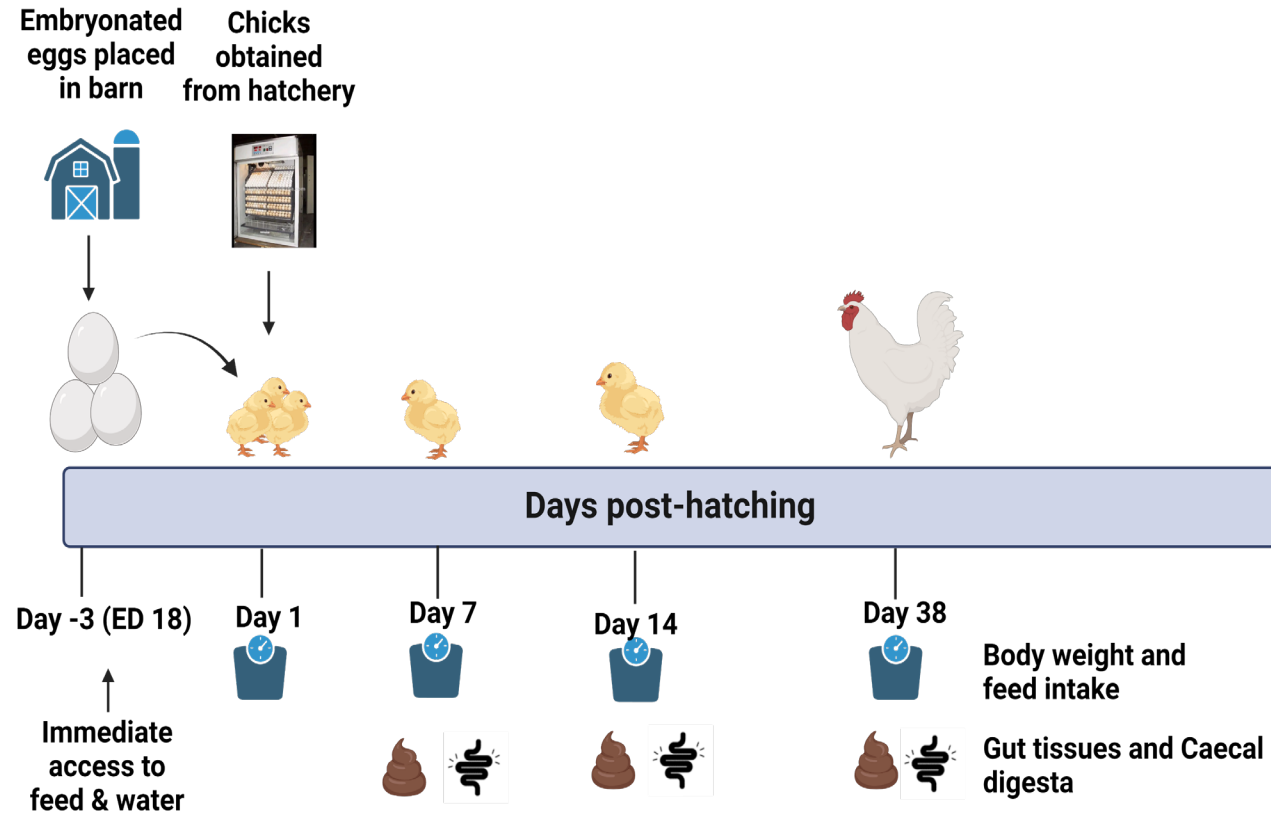
Body weight (g) ¹	Hatching system		
	HH	HF	OH
Day 0	36.9 $\pm$ 0.5 ^c	43.4 $\pm$ 0.5 ^b	46.6 $\pm$ 1.4 ^a
Day 7	167.0 $\pm$ 6.7 ^c	182.3 $\pm$ 6.2 ^b	188.4 $\pm$ 3.2 ^a
Day 14	481 $\pm$ 18 ^c	510 $\pm$ 18 ^b	530 $\pm$ 14 ^a
Day 21	971 $\pm$ 31 ^b	1,026 $\pm$ 37 ^a	1,045 $\pm$ 37 ^a
Day 32	1,944 $\pm$ 81 ^c	2,025 $\pm$ 84 ^b	2,082 $\pm$ 45 ^a
Day 39	2,634 $\pm$ 88 ^b	2,718 $\pm$ 78 ^a	2,750 $\pm$ 75 ^a
P-value			
P _{system}	<0.001		
P _{age}	<0.001		
P _{system*age}	<0.001		

^{a-c}LSmeans within a row lacking a common superscript differ ($P < 0.05$).

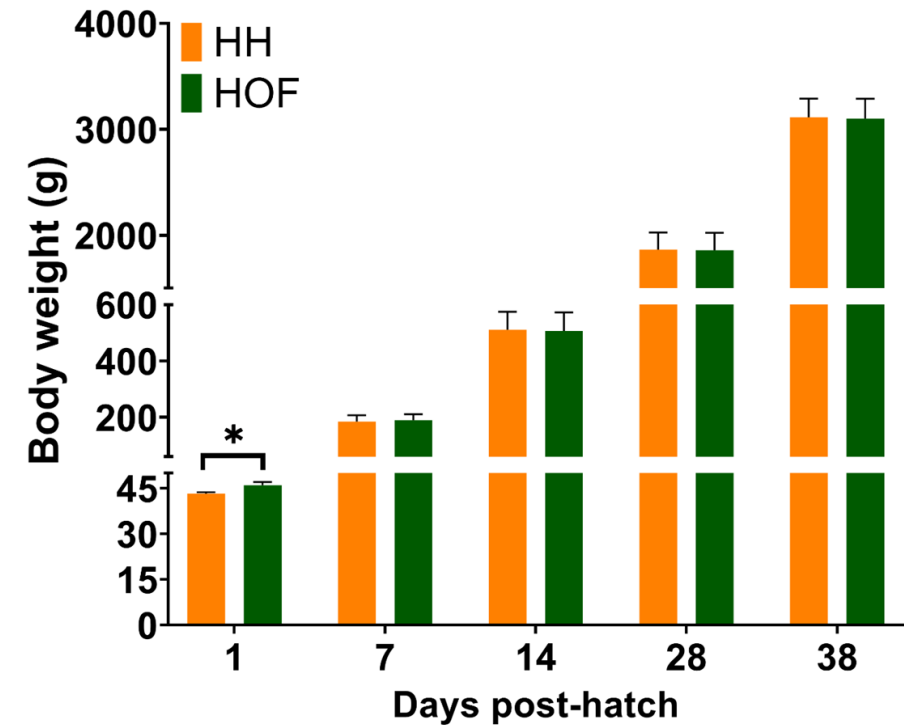
Bold indicates statistical significant differences ($P < 0.05$).

¹Body weights based on a sample of 50 broilers per pen, apart from day 32 and day 39, when 75 chickens were weighed.

Hatch in hatchery vs. Hatch on-farm

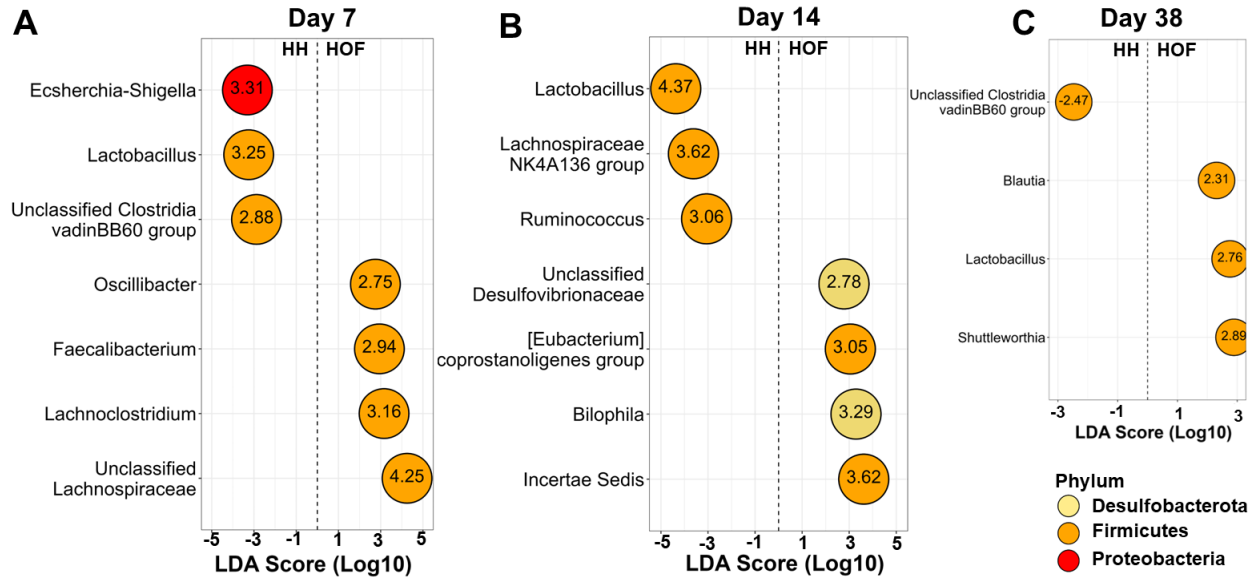


Body weight

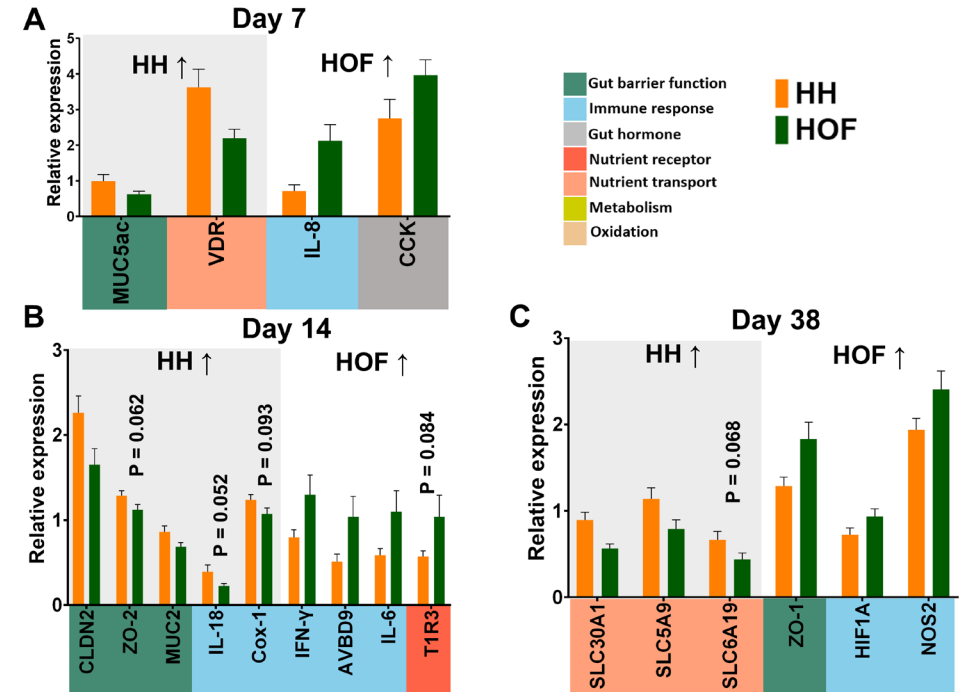


Hatch in hatchery vs. Hatch on-farm

Caecal microbiota



Ileal gene expression



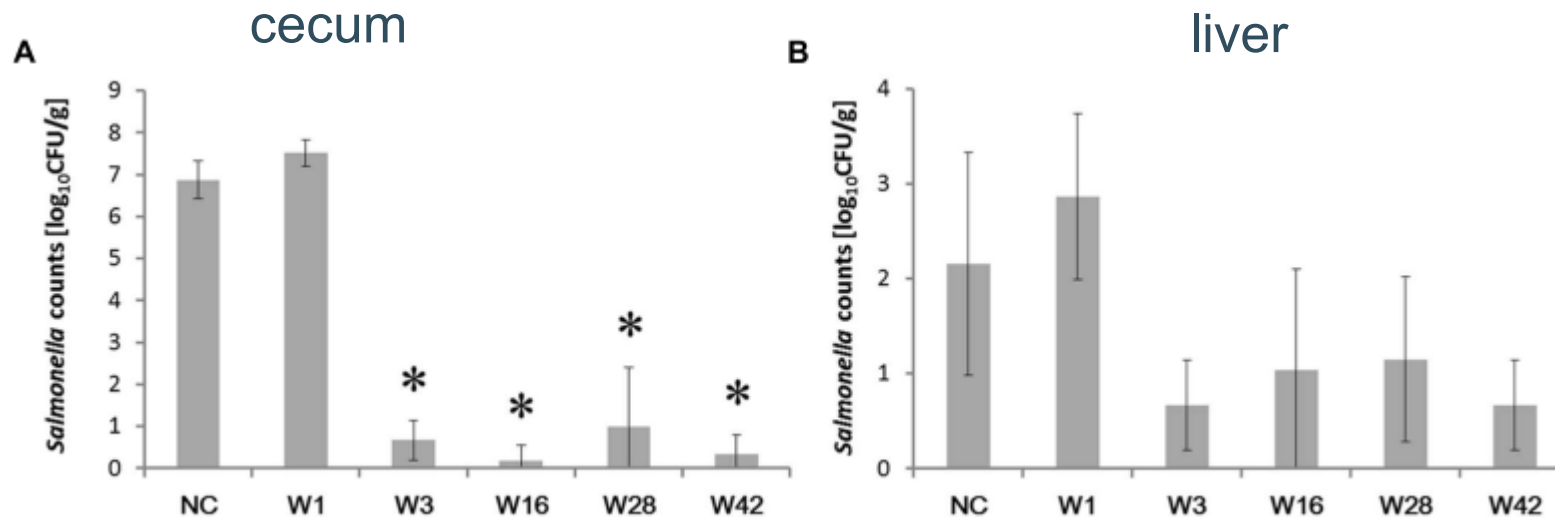
- HOF system shows **short-term growth benefits**, mainly in the first week.
- Hatching system affects **early gut microbiota**, but differences reduce by slaughter age.
- Early feeding via HOF increases **expression of immune-related genes** in chickens.
- A **good start** and a **better immune system** in chicks are both potential benefits for production efficiency.

Creating microbial diversity in early life



Chickens receiving cecal microbiota from hens of different age

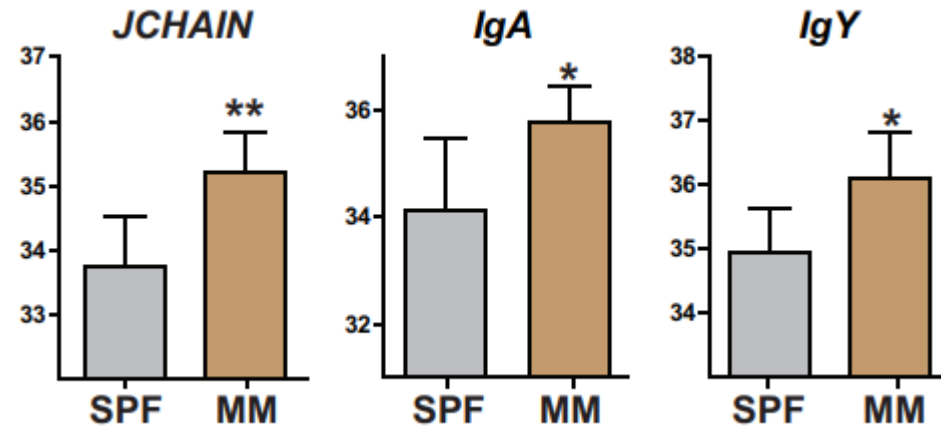
- Promotes resistance against *Salmonella enteritidis*



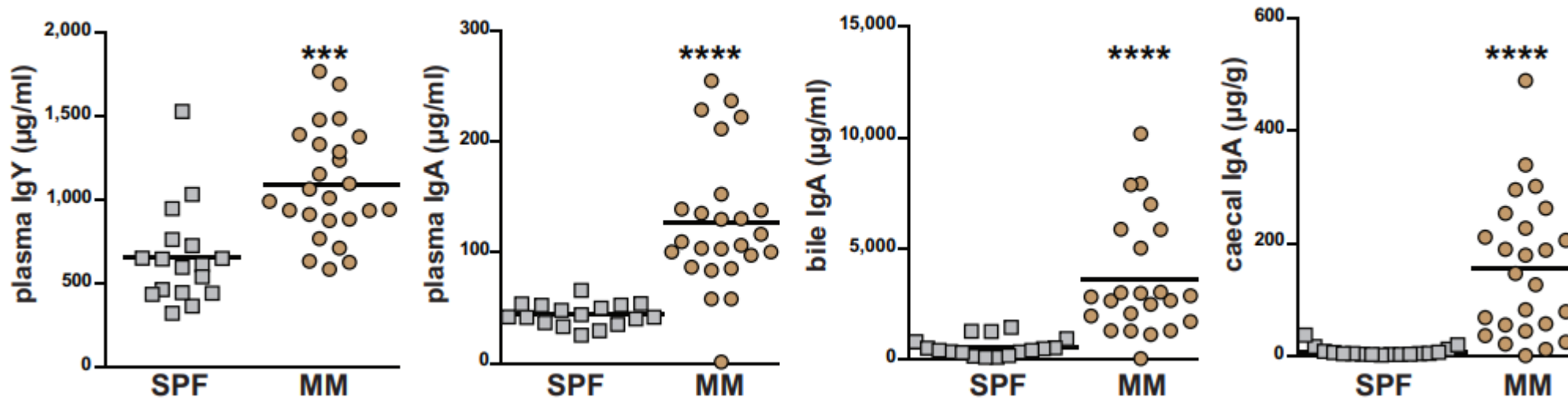
Day 4 post-infection

Varmuzova et al., 2016, Front. Microbiol.

Maternal microbiota: passive colonization with feces from hens



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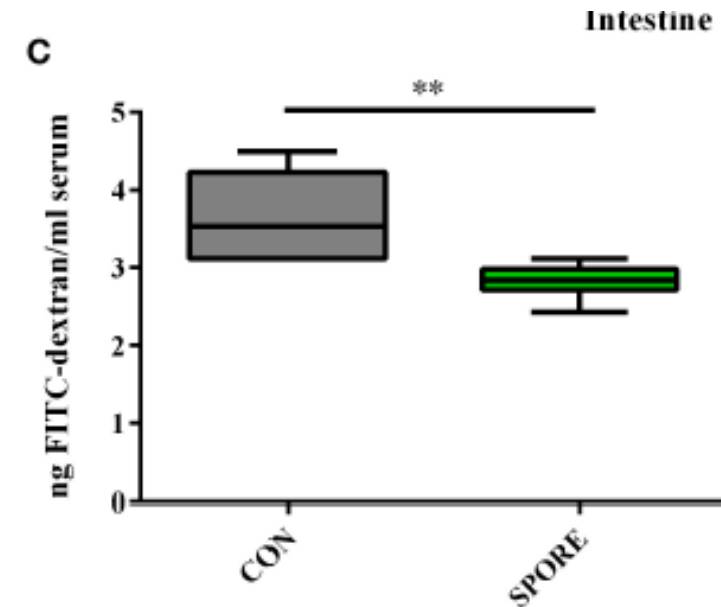


Zenner et al., 2021, mSystems



Early life probiotic administration

- **Inoculation of day-old chicks with ileal spores**
 - Modulation of **innate** immunity (TLR, JAK-STAT pathway)
 - Modulation of **acquired** immunity (T/B cell receptors T_H17 differentiation)
 - Increased bactericidal effect on Salmonella on d7-14
 - Improved gut integrity
 - → improved gut health



In conclusion

- Early life events:
 - Gut and immune system maturation
 - Microbiota colonization
 - No help of the mother hen
- Promising new technologies under development
 - Pre- and post hatch help





Thank you!



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www.nameslab.be



Names Lab

SAVE THE DATE

ICPD 2025

**ONE HEALTH
ONE WELFARE**

08 → 11 JULY
Leuven, Belgium

19th International
Conference of Production
Diseases in Farm Animals



Thank you!



Nutrition and Animal-Microbiota Ecosystems Lab



Competitive exclusion product, evaluating the antibody response after vaccination

(Boyner's PhD)

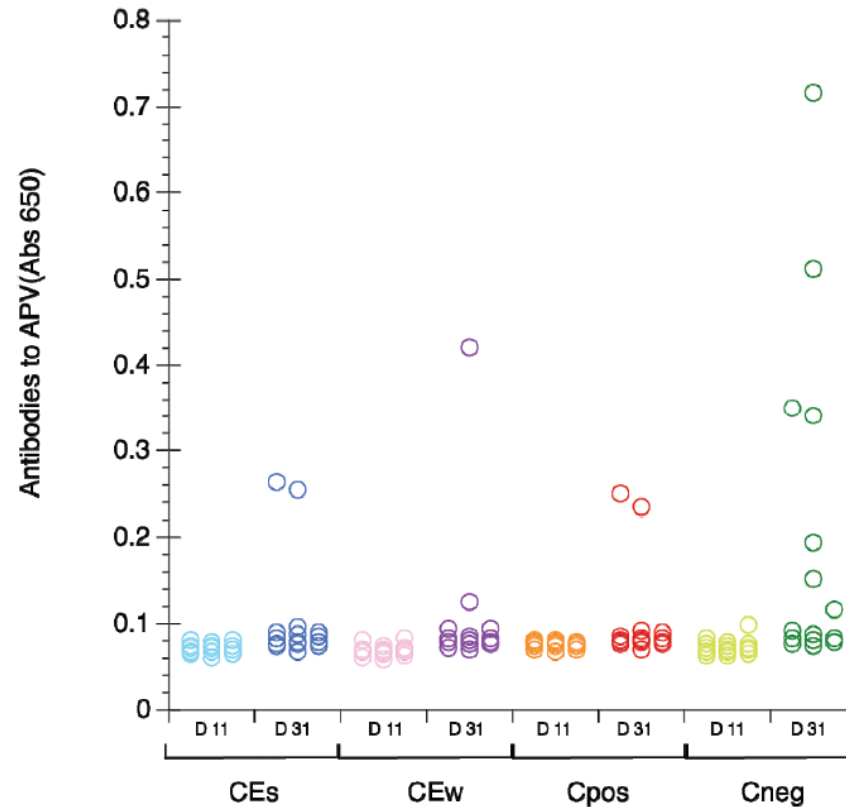


Figure 3. Antibodies to avian pneumovirus (APV) in serum samples collected before vaccination against APV at d 11 and 20 after vaccination at d 31. Results shown are absorbance 650 nm values for individual chickens in the four treatment groups. The cut-off value for samples testing positive for antibodies to APV was calculated to be 0.086 (for details, see Materials and Methods). Treatments: chicks in the hatcher were given access to feed, water, and a competitive exclusion (CE) product sprayed on the down of the newly hatched chicks (CEs), access to feed, water, and a CE product provided in the water (CEw), access to feed and water only (Cpos), or no access to feed and water (Cneg).



Table 4. Predicted means $\pm$ SEM for performance indicators of hatchery-hatched (nonfed) (HH), hatchery-fed (HF), and on-farm hatched (OH) broiler chickens over the whole rearing period (D0-39).

Indicator ¹	Hatching system			SEM	P _{system}
	HH	HF	OH		
First wk mortality (%)	1.83	2.43	1.59	0.39	0.32
Total mortality (%) ²	3.27	4.39	3.20	0.47	0.17
Total found dead (%) ²	1.88	2.09	1.62	0.25	0.45
Total culled (%) ²	1.39 ^b	2.30 ^a	1.57 ^b	0.25	0.05
Cumulative feed intake (g/chicken)	3,823 ^c	3,962 ^b	4,045 ^a	23.8	<0.001
FCR total ³	1.46	1.46	1.48	0.009	0.28
Cumulative water intake (ml/chicken)	7,346 ^b	7,323 ^b	7,477 ^a	33.7	0.01
Cumulative water-feed ratio	1.92 ^a	1.84 ^b	1.83 ^b	0.013	0.001
Variation coefficient of body weight at D39 (%)	12.7 ^a	13.1 ^a	11.6 ^b	0.3	0.01
EPEF ⁴	426.9	434.8	442.0	4.3	0.08

^{a-c}LSmeans within a row lacking a common superscript differ ($P < 0.05$).

Bold indicates statistical significant differences ($P < 0.05$).

¹FCR total, variation coefficient of BW at D39, and EPEF are based on BW of 75 chickens per pen.

²Total mortality is the sum of total culled and total found dead. Total found dead represents all chickens that were found dead during inspection of the pens; total culled represents the proportion of chickens that were euthanized because of compromised health or being extremely small.

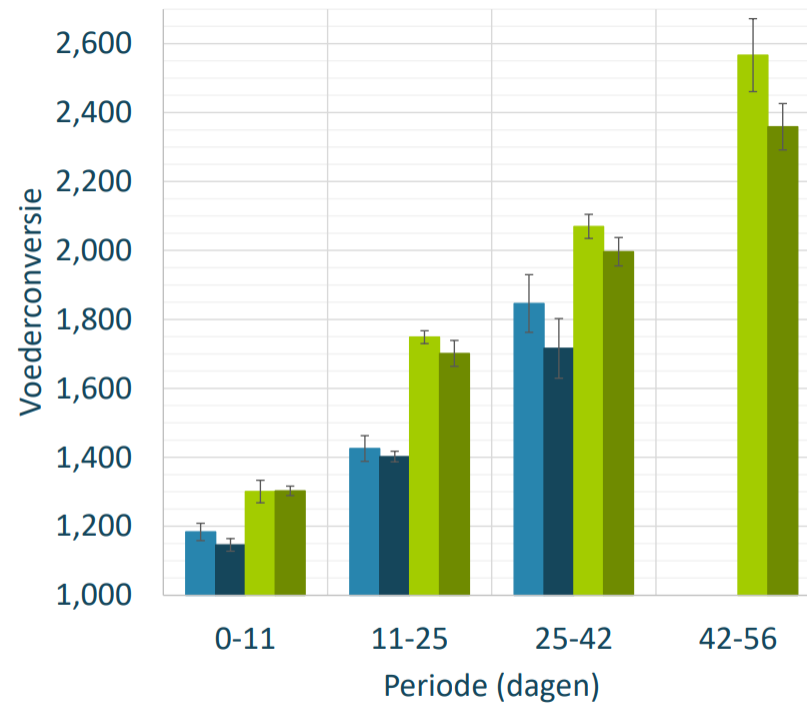
³FCR total: feed conversion ratio calculated over the whole production period between D0 and D39.

⁴European Production Efficiency Factor (EPEF) = $(1 - \% \text{ mortality}) \times \text{mean bird weight} / \text{mean length cycle} / \text{net feed conversion} \times 10$.



Feed conversion ratio

ILVO



Reguliere - V : 1,638 → 1,587
Reguliere - M : 1,553
Traaggroeieters - V : 2,102 → 2,056
Traaggroeieters - M : 2,010

0-42d en 0-56d	P-waarde
Concept*Geslacht	NS
Concept	<0,001
Geslacht	<0,01

Feed conversion ratio

Resultaten ForFarmers	Beste 2 koppels afgesloten dec 2018		Kwartaal 4, 2018	
	26.300 kuikens	54.720 kuikens	beste 25%	gemiddelde
Groeidagen	40,4	40,3	39,1	38,6
Gemiddeld aflevergewicht	3.086	2.922	2566	2456
Groei	76	73	66	64
Doorgroei na uitladen	129	118	99	98
Netto VC	1.545	1,552	1,537	1,562
VC 1.500	0.911	0,983	1,111	1,180
VC 2.300	1.388	1,428	1,484	1,531
Voetzoolscore	17	38	45	39
Productiegetal	468	451	412	393

VC 2.300 als kengetal

In 2018 introduceerde ForFarmers een nieuw kengetal, VC 2.300, als alternatief voor de VC 1.500. Deze waarde voor voederconversie heeft een slachtgewicht van 2.300 gram als uitgangspunt, wat beter aansluit bij de huidige slachtgewichten van vleeskuikens. Daarnaast blijkt uit praktijkgegevens dat een correctie van 2 punten per 100 gram beter is (in tegenstelling tot 4 punten per 100 gram bij de VC 1.500). De gecorrigeerde VC 2.300 leidt zo tot een meer realistische voederconversie en is een betere maatstaf om koppels, stallen of voeders onderling te beoordelen of te vergelijken.



Hatching time and delayed feeding

Table 2 Effects of hatching time and delay in feed intake on proportional breast muscle weight, residual yolk, hatching muscle glycogen content, plasma glucose and T_3 levels on BA day 2

Performance parameters	Feeding treatment	Hatching period			s.e.m.	P-value		
		Early	Middle	Late		HT	DF	HT × DF
Proportional BM (%)	Immediate	0.62 ^{bc}	0.62 ^c	0.74 ^a	0.01	0.02	<0.01	0.03
	Delayed	0.72 ^{ab}	0.71 ^{abc}	0.71 ^{abc}				
Residual yolk (g)	Immediate	3.40	3.38	2.44	0.13	<0.01	0.92	0.78
	Delayed	3.58	3.16	2.56				
HM glycogen (μg/g)	Immediate	115.0 ^{ab}	149.7 ^{ab}	197.2 ^a	11.6	0.02	<0.001	0.30
	Delayed	29.6 ^c	99.4 ^{bc}	75.1 ^{bc}				
Glucose (mg/dl)	Immediate	229.5 ^a	227.3 ^a	217.0 ^{ab}	3.0	0.04	<0.001	0.99
	Delayed	197.0 ^{bc}	192.3 ^c	185.7 ^c				
Plasma T_3 (ng/ml)	Immediate	1.89 ^a	1.70 ^a	1.68 ^a	0.07	0.28	<0.001	0.53
	Delayed	0.93 ^b	0.97 ^b	0.82 ^b				

BA = biological age; HT = hatching; DF = delay in feed intake; BM = breast muscle; Immediate = fed immediately; Delayed = delay in feed intake; HM = hatching muscle; Glucose = plasma glucose.

^{a-c}Values within the same parameter with different superscripts differ significantly at $P < 0.05$.



Eggshell temperature of eggs in different hatching systems

6

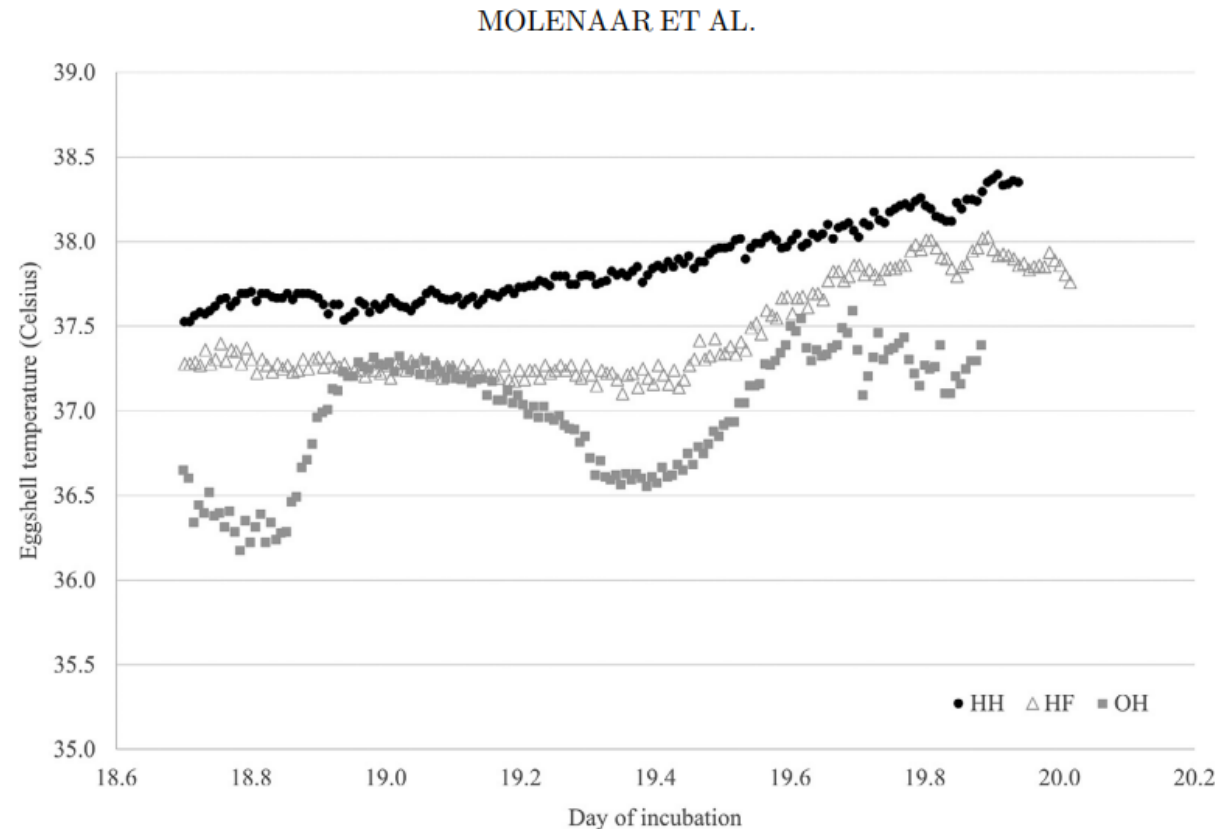


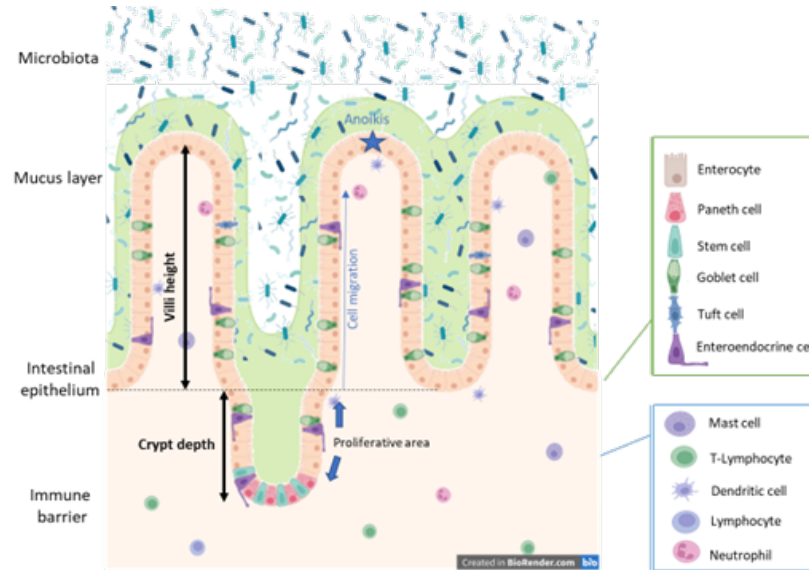
Figure 1. Mean eggshell temperature from day 18.7 until 20.0 of incubation of eggs that were hatchery-hatched (HH), hatchery-fed (HF), or on-farm (OH) hatched (n = 6 eggs per treatment group).



Development of immune system in the gut

- Expression of antimicrobial peptides (**AMPs**) and **Toll-like receptors** during embryonic development
- **MUC-2** expression is stimulated by colonization in the gut
- **IL-8 IL-17** increased gene expression first week post-hatch
→ maturation

Cross-talk between microbiota and immune cells



Rodrigues, 2022, chapter 11 in Gut microbiota, immunity and health in production animals

