Altering *in ovo* the chicken microbiome - the concept and future

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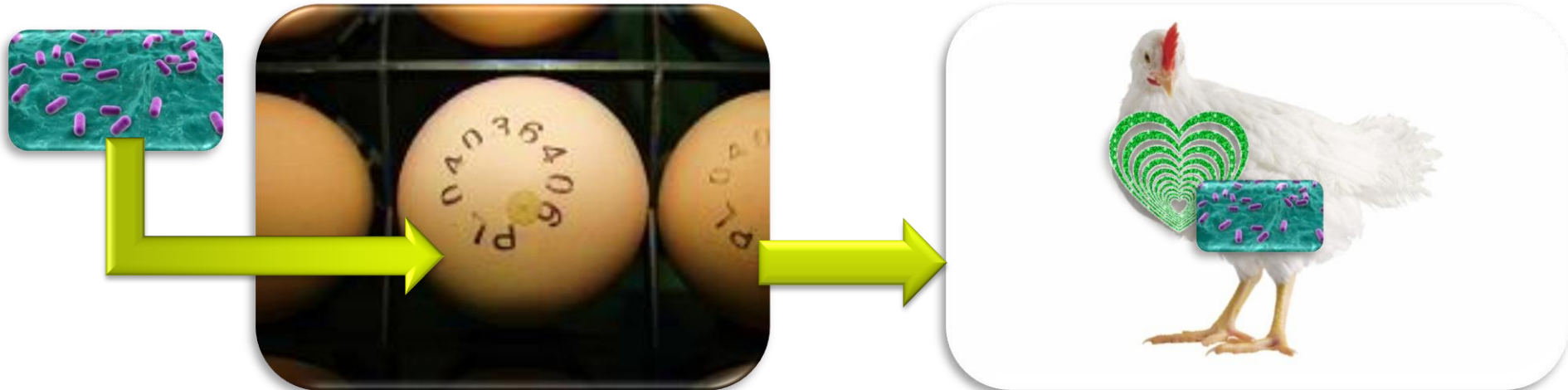


Idea and proof of the *in ovo* concept -the scope of research

DEPARTMENT OF ANIMAL BIOCHEMISTRY AND BIOTECHNOLOGY

•RAFFINOSE FAMILY OLIGOSACCHARIDES (LUPIN)
•INULIN
•Bi²tos (Clasado Ltd.)
•DiNovo (Bioatlantis Ltd.)
•Lavipan (JHJ Sp.z o.o.)

PREBIOTIC
SYNBIOTIC

AUTOMATIC SYSTEM FOR INJECTION IN OVO

DIFFERENT BROILER LINES:
•ROSS,
•COBB,
•HUBBARD

DIFFERENT MODE OF ADMINISTRATION:
•IN OVO
•IN OVO & IN WATER
•IN WATER ONLY

•HIGH TOLERANCE OF EMBRYOS FOR TREATMENT

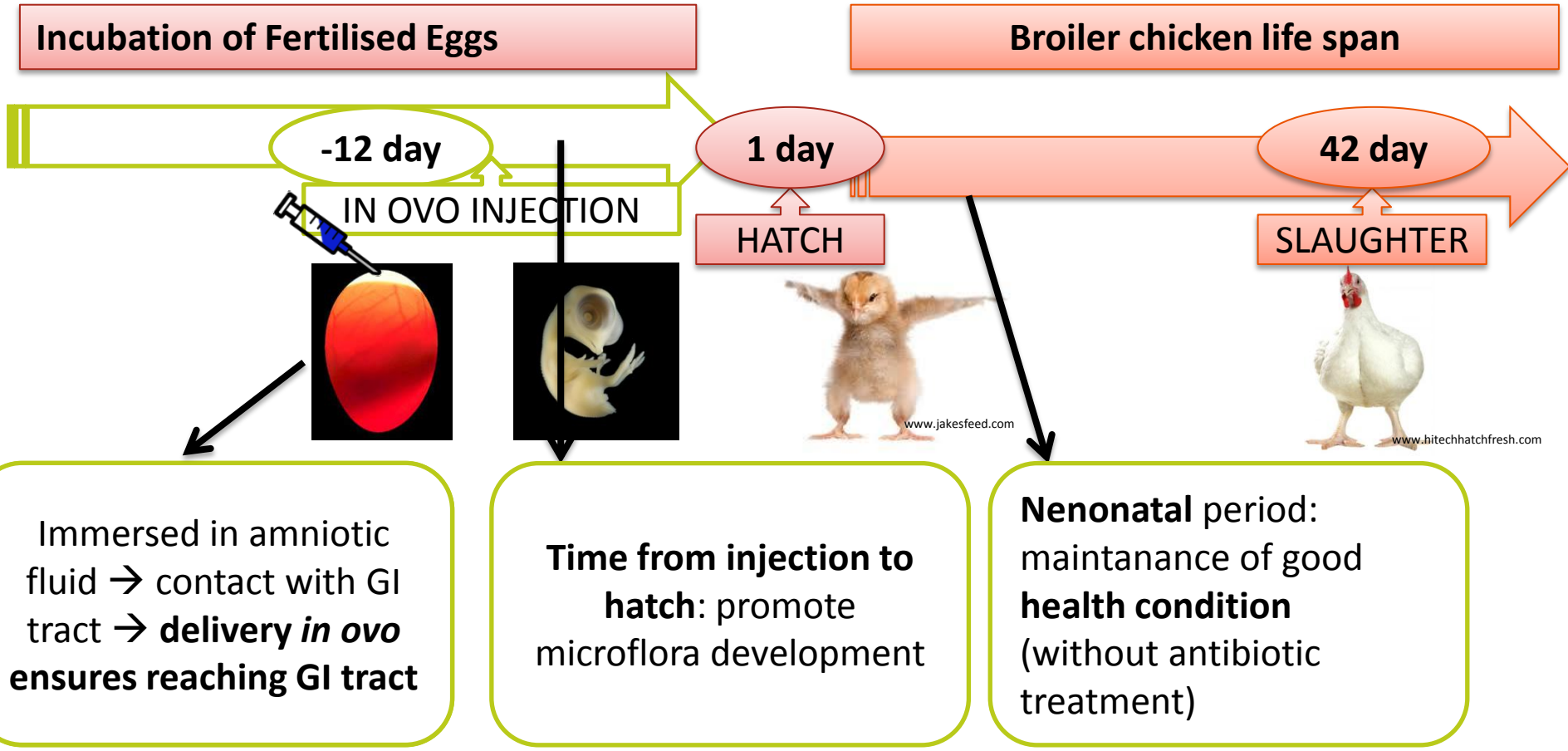
•HIGH HATCHABILITY RATES AFTER INJECTION

In field experiments on over 2 mln chicks

embryo,at 12d. of INCUBATION

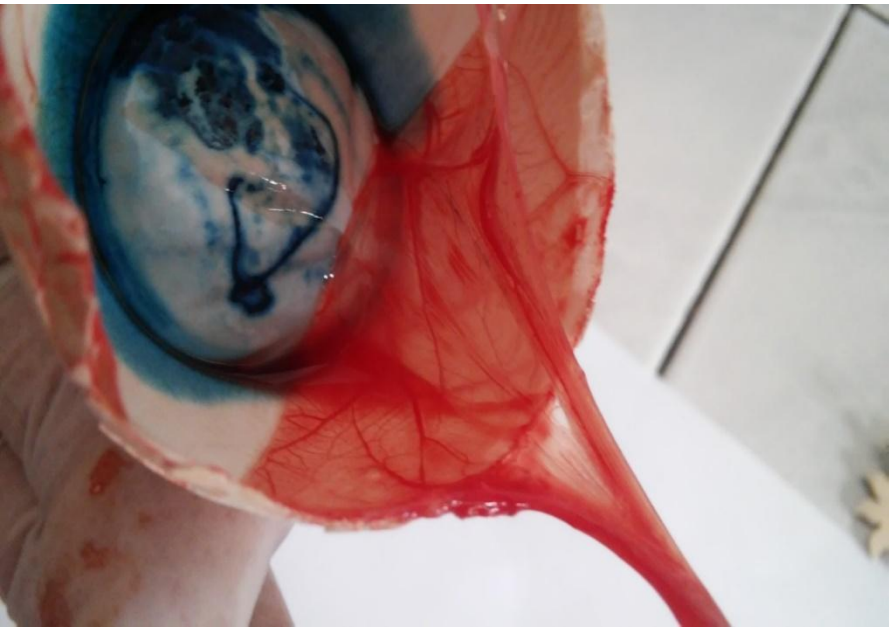
Why in ovo? Why on 12th day of egg incubation?

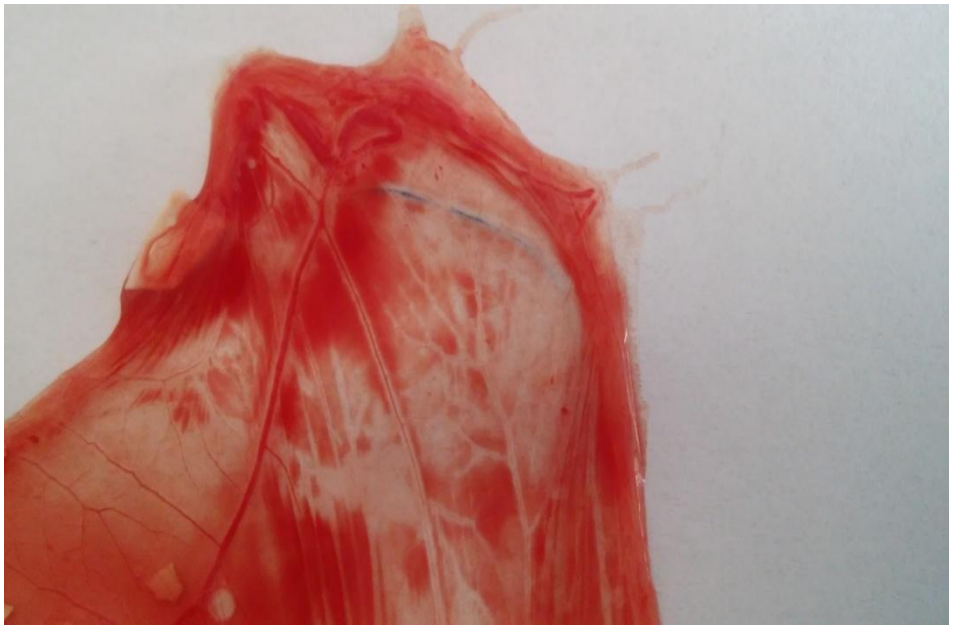
To ensure the best protection for the newly hatched individual, the external supplementation should be given **as early as possible (EVEN BEFORE HATCH!)**



Why in ovo on 12th day of egg incubation?

Confirmation of penetration of a prebiotic solution through the chorioallantoic membrane into the circulatory system of the chicken embryo





TECHNICAL ASPECT OF IN OVO DELIVERY

DEPARTMENT OF ANIMAL BIOCHEMISTRY AND BIOTECHNOLOGY

Optimization of prebiotic doses for in ovo delivery- manual injection

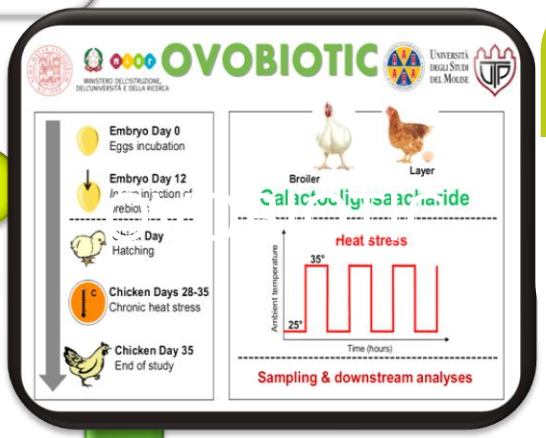
Production trial- improved system for automatic injection (THRIVE RITE PROJECT)

RESULTS OF RECENT AND ONGOING PROJECTS

Multi-effects of a single *in ovo* treatment

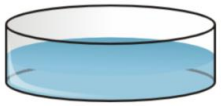
RECENT AND ONGOING PROJECTS

ECOFCE



TLR- National Science Centre

1. IN VITRO

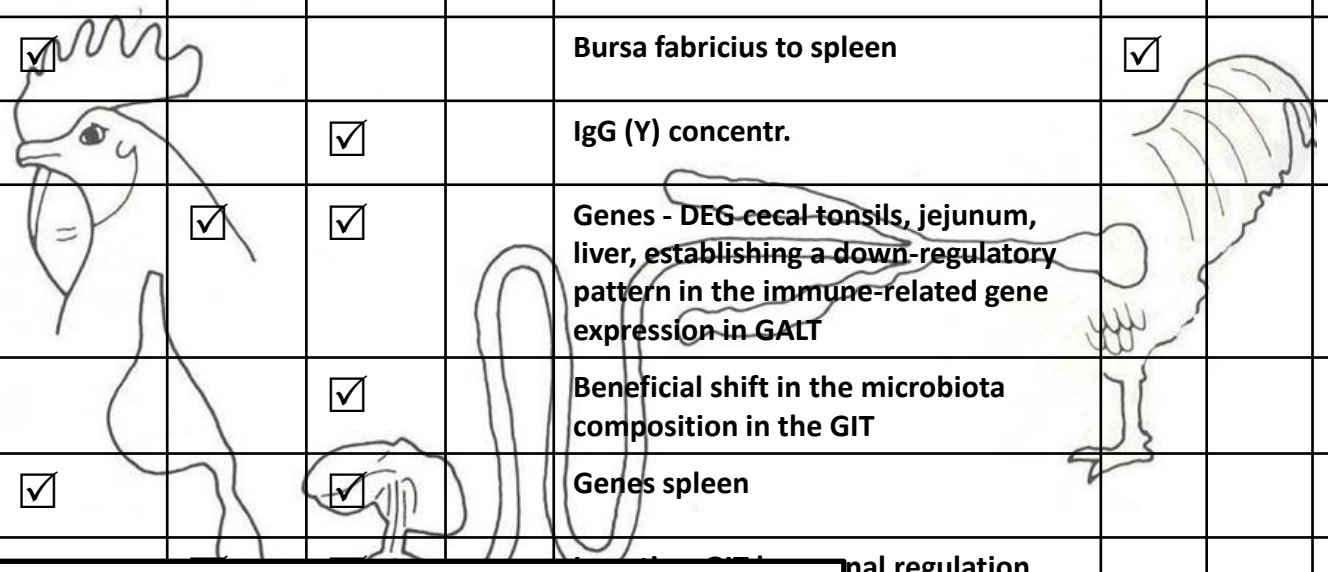


FUTURE APPROACH-

Based on *in ovo* model

- **New Synbiotics**
- Towards effectiveness (performance, health, meat)
- Towards realistic implementation (in water/in feed)

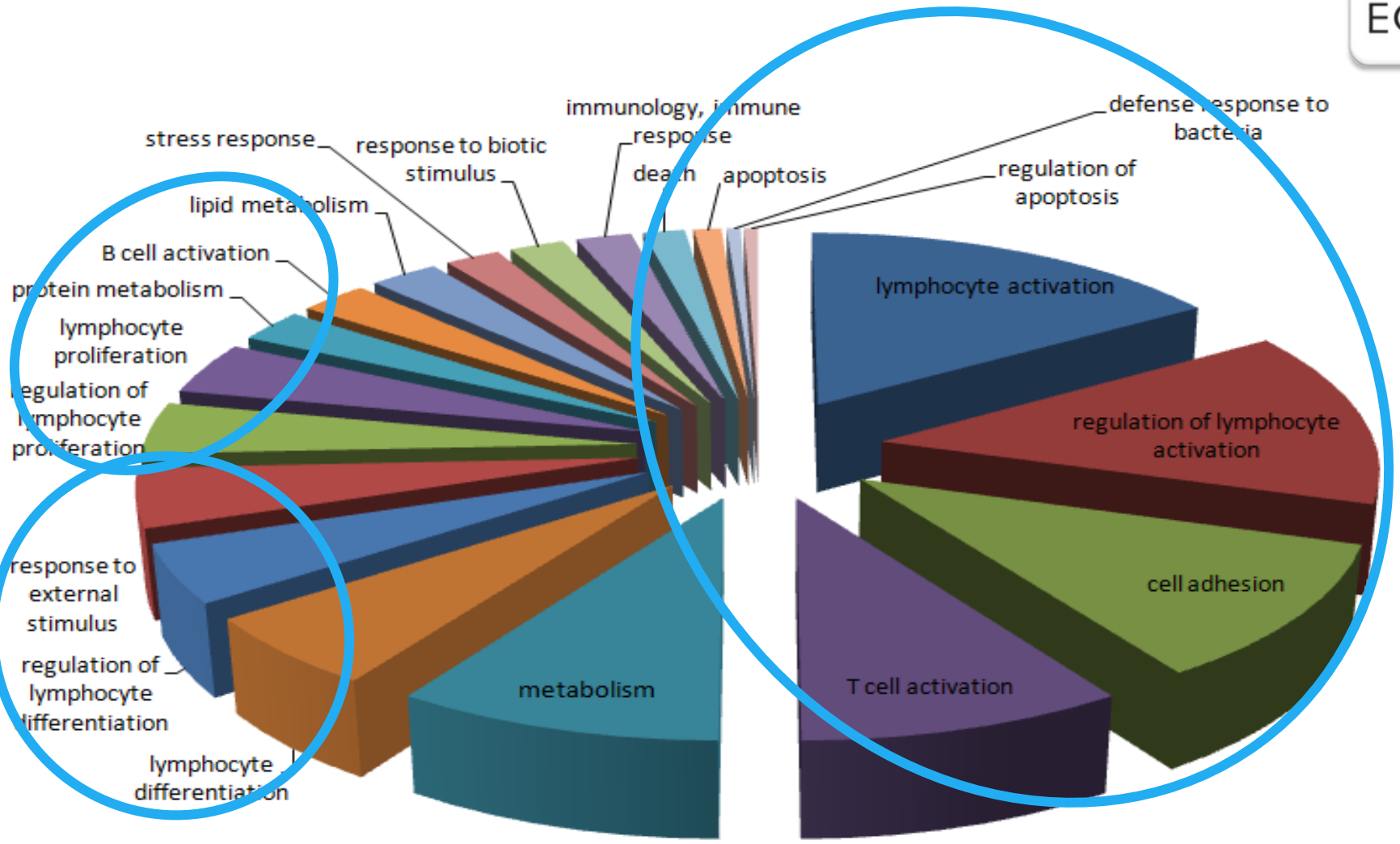
Inulin + L.Lactis ssp. lactis	Bi2tos + L.Lactis ssp. cremoris	RFO + L.Lactis ssp. cremoris	RFO+ L.plantarum	Bi2tos + L.salivarius	Duolac	ECOFCE / The National Centre for Research and Development	RFO	Inulin	Bi2tos	DiNovo
☑	☑					BW [1-3], BW [1-6], FINAL BW	☑		☑	☑
		☑	☑		☑	Breast muscle, stripping, TI, AI, P/S			☑	☑
	☑					Microvilli lenght, surface		☑	☑	
		☑				Bursa fabricius to spleen	☑			
				☑		IgG (Y) concentr.				
☑	☑		☑	☑		Genes - DEG cecal tonsils, jejunum, liver, establishing a down-regulatory pattern in the immune-related gene expression in GALT				
				☑		Beneficial shift in the microbiota composition in the GIT				
		☑		☑		Genes spleen				
						nal regulation				
☑	☑					tes B		☑	☑	
☑	☑					es B			☑	
☑	☑					ocytes		☑	☑	
☑	☑					LT for liver				



- PREBIOTICS: IMPROVED BW, MEAT QUALITY (STRIPING)
- SYNBIOTICS: GOOD BW & FCE
- SYNBIOTICS: ACTIVATION OF GALT PATHWAYS , EFFECT ON MICROBIOME

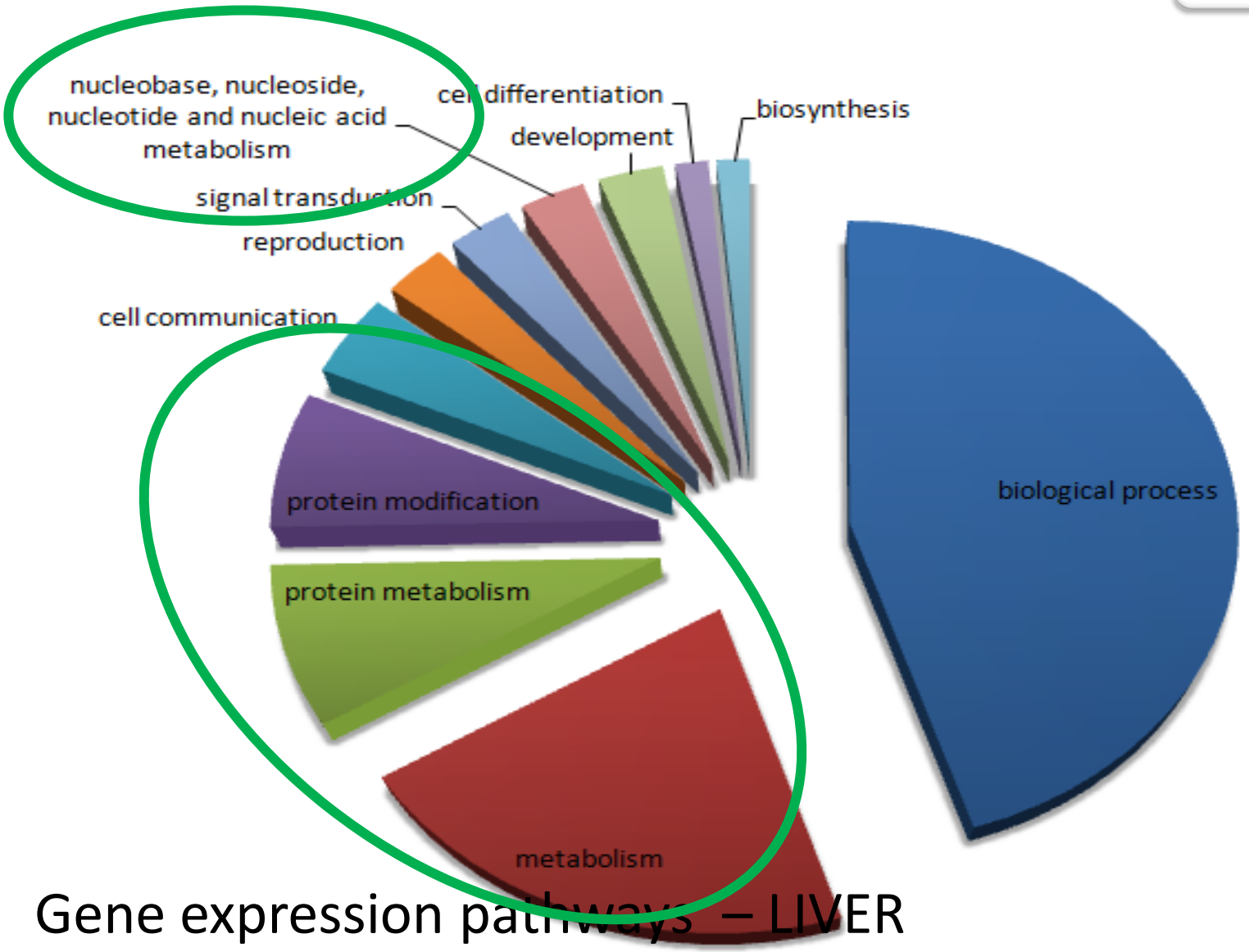
Lb. salivarius 3154 + Bi²tos (Syn1)

ACTIVATES IMMUNOGENIC PATHWAYS



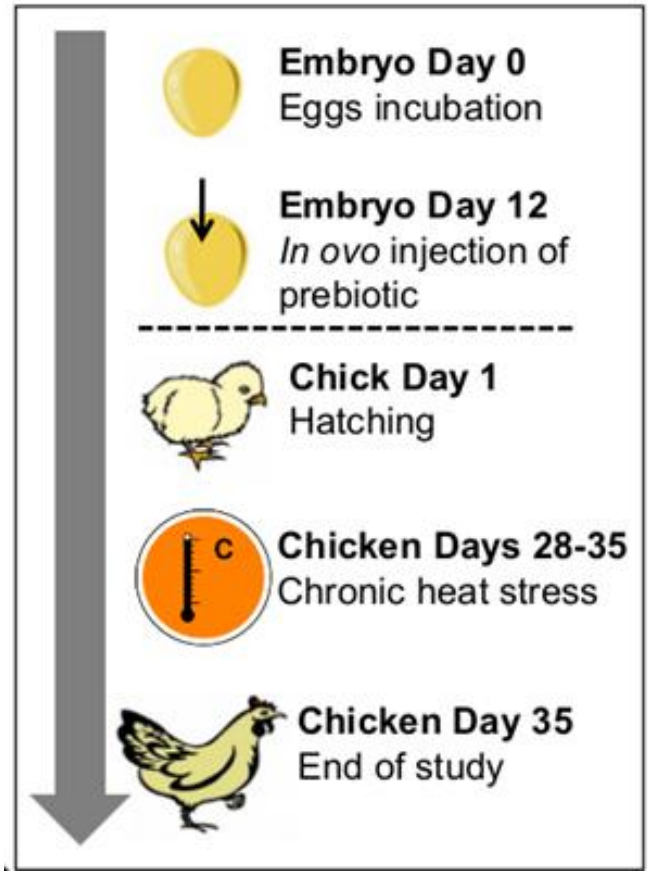
Gene expression pathways – **CECAL TONSILS**

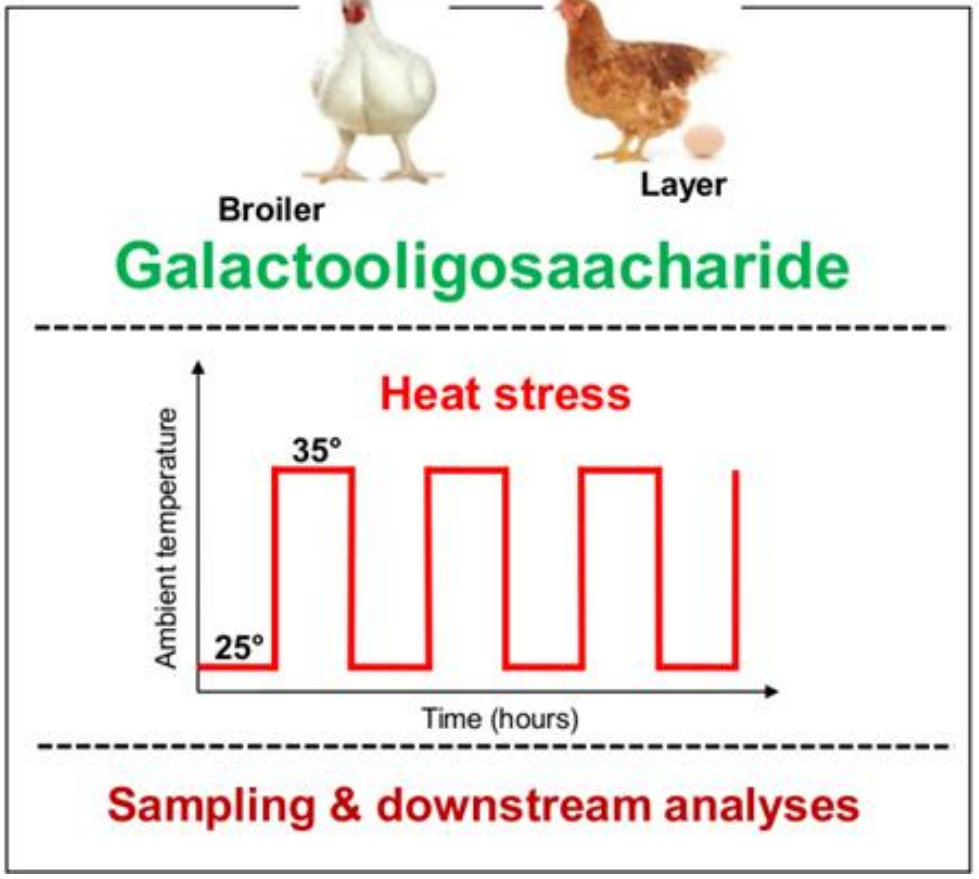
Lb. plantarum 3036 + RFO (Syn2)
ACTIVATES METABOLIC PATHWAYS



Gene expression pathways – LIVER

ONGOING: Project 1. OVOBIOTIC



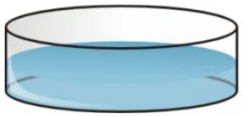


In ovo delivery of prebiotic **in commercial and indigenous Italian chicken breeds** provides a contribution to healthy food production under a heat stress (MIUR, Italy)

ONGOING Project 2.
TLR

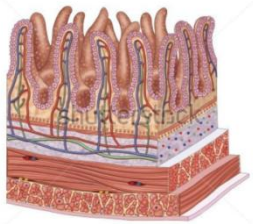
Synbiotics-activated TLR-signaling as a measure of improved immunity in chickens

1. IN VITRO



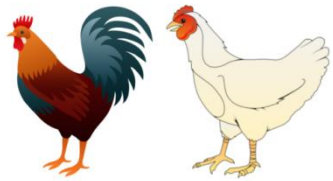
To detect gene expression signatures (by RNAseq) that correspond to the specific TLR pathways **(HD11, BMDC)**

2. EX VIVO



To validate gene expression signatures in intestinal tissue (at the protein level, WB) **(BMDC)**

3. IN VIVO



To map the gene expression signatures to the specific immune cell type (T/B), in GALT, immune organs and blood & correlate them with the immune parameters **(Ross, GP)**

TLR: expected from *in vivo* trial Measures of improved immunity

1. **Spleen & CT** – colonization with T and B cells (*in ovo* injected chickens) **IHC**
2. **Spleen & CT** – immune response to LPS/LTA (*in ovo* injected chickens) **WB**
3. **Blood** - immune response to LPS/LTA (*in ovo* injected chickens) **ELISA**

To answer the questions

1. **Can we prove the hypothesis?**
2. **Can we track back the biomarkers of improved immunity from *in vitro* to in vivo?**

NEW KNOWLEDGE GAINED ON THE MECHANISMS OF SYNBIOTIC ACTION

NEW KNOWLEDGE – SYNBIOTIC COMPOSITION

Optimization of synbiotic composition should be considered from two perspectives (Dunislawska et al., 2017):

Efficient use of prebiotic by probioitic (prebiotic a source of substrate for fermentation)

Synbiotic exerts synergisitc effect towards the probiotic

Positive influence of a prebiotic on the host organism (improvment of microbial balance in GIT)

Synbiotic exerts synergisitc effect towards the host

One of the proposed mechanisms explained by ECO FCE results:

- Prebiotic/synbiotic delivered *in ovo* at 12 day EI was able to **infiltrate** chorioallantoic membrane in the egg and
- stimulate/change the **growth of indigenous microbiota** in the embryonic GIT.
- Microbiota **boosted maturation** of gut-associated lymphoid tissue, which
- resulted in **enhanced tolerance** of the local immune system.
- **Transcriptomic effects** were triggered and change of the cellular and humoral immune responses.

PROSPECTIVE INVESTIGATION - AVAILABLE FOR THE COST ACTION

1. OPTIMIZATION OF NEW SYNBIOTICS
2. IMPLEMENTATION APPROACH
   Commercial implication for in ovo technology and its limitation
3. MEETING REQUIREMENTS TO OVERCOME CHALLENGES IN POULTRY PRODUCTION
4. TO ADDRESS UNANSWERED Questions USING IN OVO MODEL

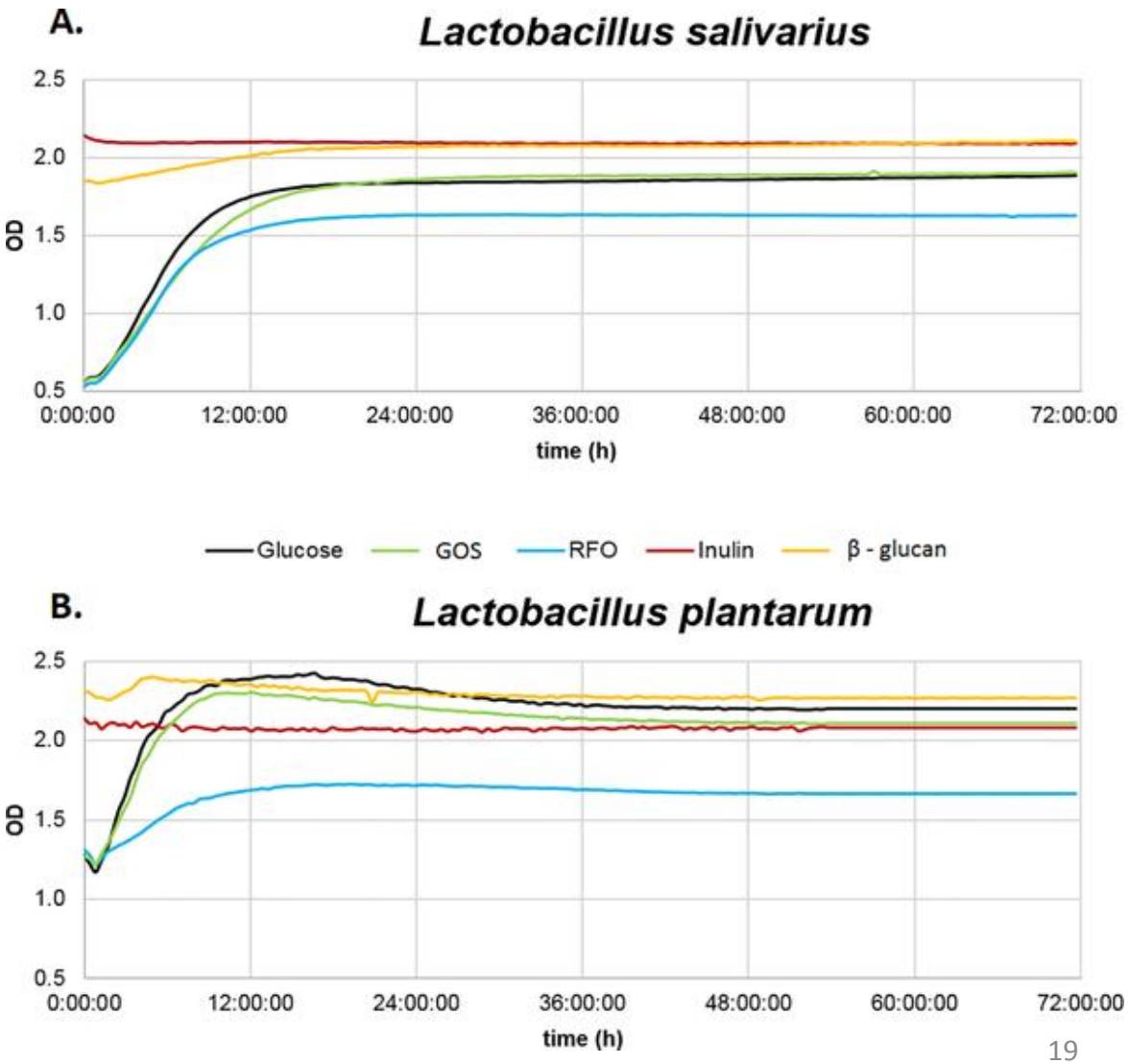
1. OPTIMISATION OF NEW SYNBIOTICS

- None of a gold synbiotic standard exists
- No synbiotic was developed *de novo* specifically for poultry

An unlimited number of pro/prebiotic combinations may be screened *in vitro* using relatively cheap bench trials

OPTIMISATION OF NEW SYNBIOTICS
A. BIOSCREENING

Changes in optical density (OD) of culture media during incubation of Lactobacillus bacteria with prebiotics.



OPTIMISATION OF NEW SYNBIOTICS
B. **TESTING FOR DOSAGES**

EXAMPLARY CHOICE OF A DOSE:
HATCHABILITY RATE

*E.g. 4 300 embryos in total;
3 repetitions per prebiotic

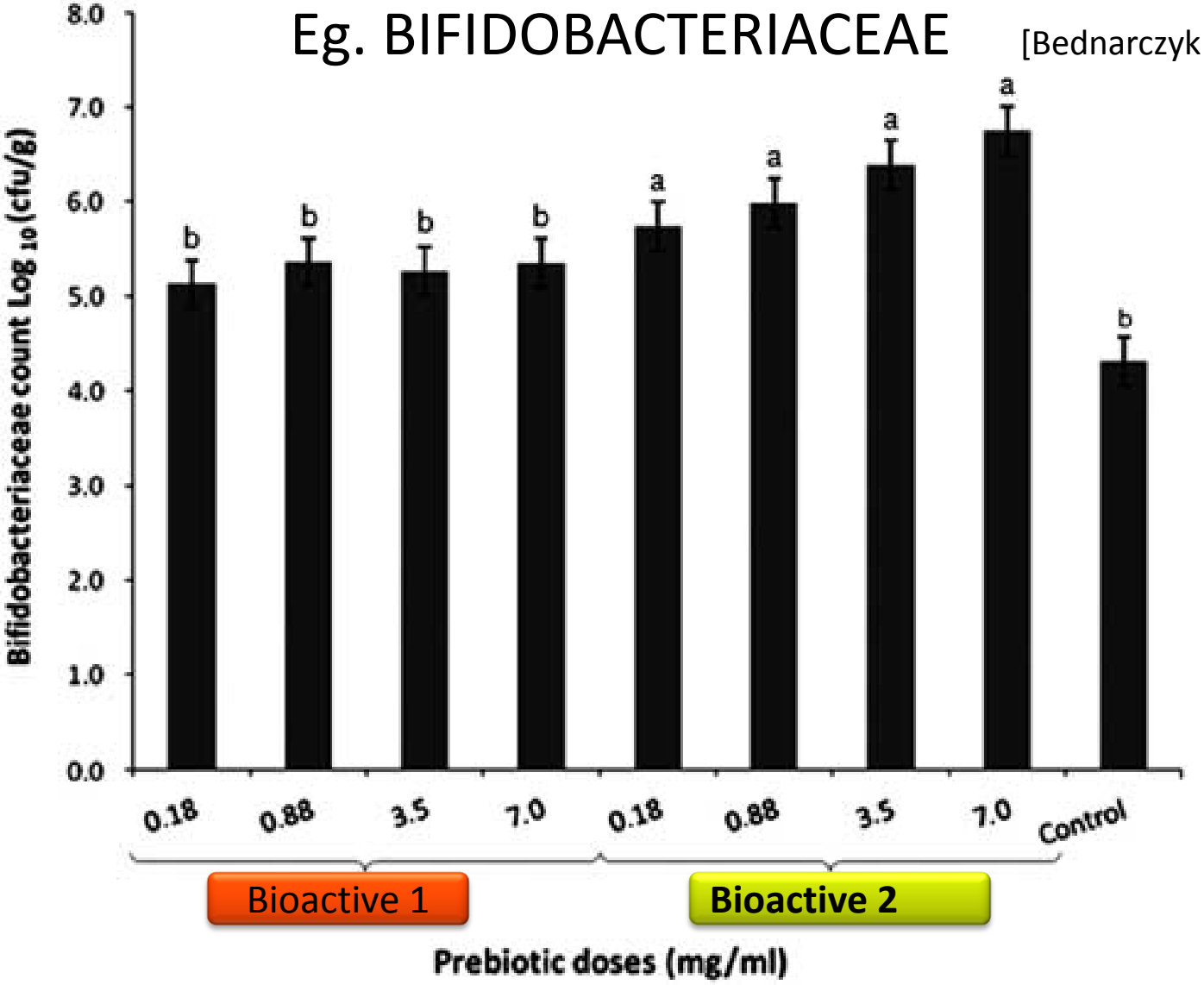
	Dose [mg/embryo]*	Number of embryos treated	Hatchability %
Bioactive 1	0.18	500	90.4 a
	0.88	499	89.2 a
	3.50	500	84.0 a
	7.00	499	56.5 b
Control	0.00	300	88.7 a
Bioactive 2	0.18	500	91.0 a
	0.88	500	92.6 a
	3.50	497	89.7 a
	7.00	500	71.4 a
Control	0.00	300	88.7 a

Data were analyzed by a chi square contingency test (Laughlin and Lundy, 1976).
Means with no common superscript differ significantly between groups ($P \leq 0.01$).

OPTIMISATION OF NEW SYNBIOTICS
C. BACTERIOLOGIAL COUNTS

Eg. BIFIDOBACTERIACEAE

[Bednarczyk et al. 2016, ANIMAL]



2. IMPLEMENTATION APPROACH **Commercial implication for in ovo technology and its limitation**

Advantages over in feed supplementation

- uniformity and precision in delivery to each embryo
- low usage of a bioactive compound
- optimal timing (12th day of embryo incubation)
- encouraging maturation of the immune system

Associated risks/limitation

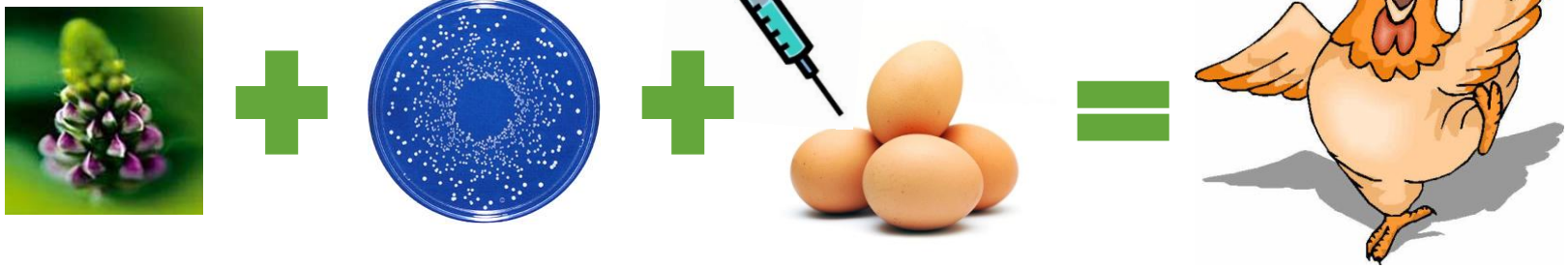
Potential impediment of viability and hatchability, due to:

- improper injection technique (optimized) ☑
- Improper dose of the bioactive compounds (to be optimized prior to field trials) ☑
- **compatible alignment** with hatchery production line X

3. MEETING REQUIREMENTS TO OVERCOME CHALLENGES IN POULTRY PRODUCTION

1. To mitigate harmful effects of climatic change
2. To fight enterogastric diseases (e.g. *Salmonella, Campylobacter*)

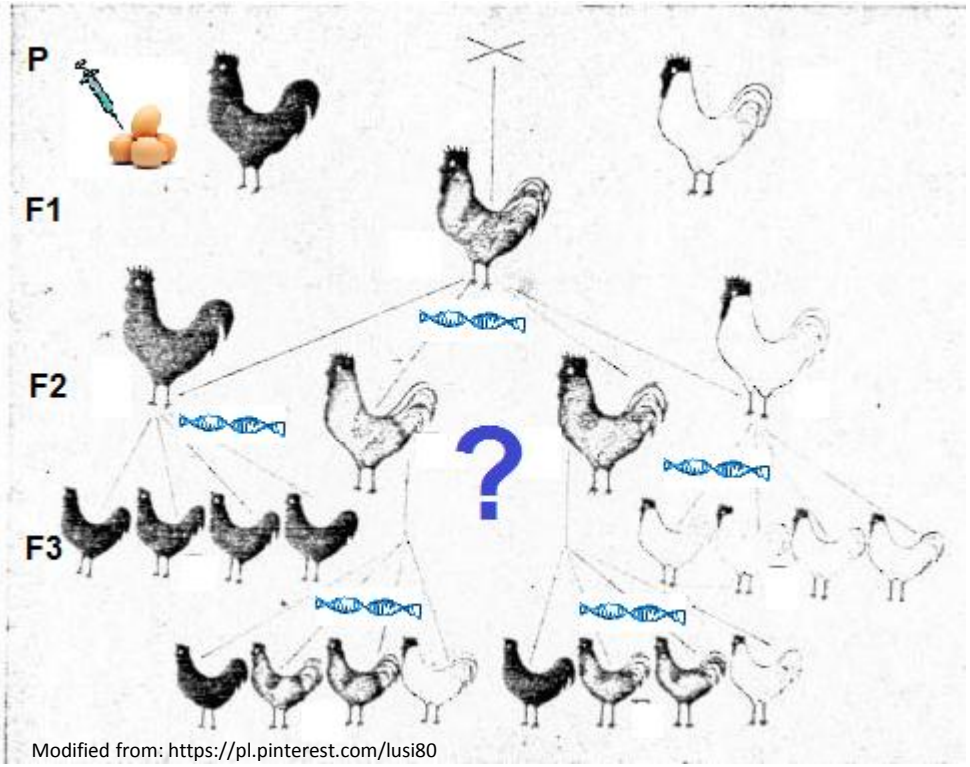
Healthy guts = Resilient animals
1. **Prevent dysbiosis**
2. **Compete with gut pathogens**



4. TO ADDRESS UNANSWERED Qs USING IN OVO MODEL

- Is the effect of *in ovo* treatment with bioactives imprinted in genotype and inherited/ permanent?

- Trials to trace intra generation effects



Modified from: https://pl.pinterest.com/lusi80

THANK YOU FOR THE ATTENTION

Acknowledgment to *in ovo*

Insitutional Partners…

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BioAtlantis Nature Working Naturally™

Clasado BioSciences Delivering wellness through knowledge

VET DIAGNOSTICA weterynaryjne laboratorium diagnostyczne

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