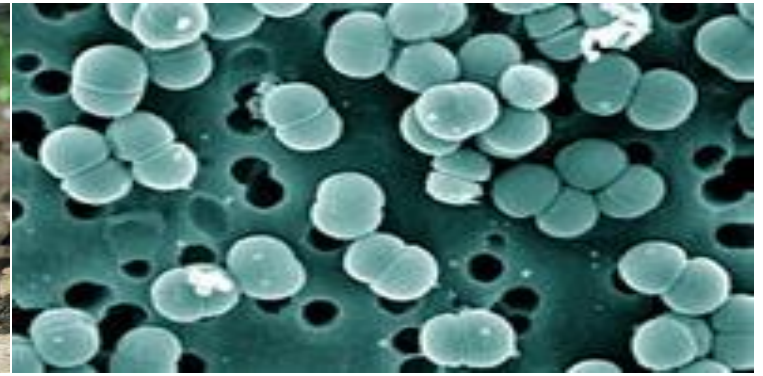
Effect of *in ovo* microbiome stimulation on immune responses in different chicken breeds

Anna Slawinska



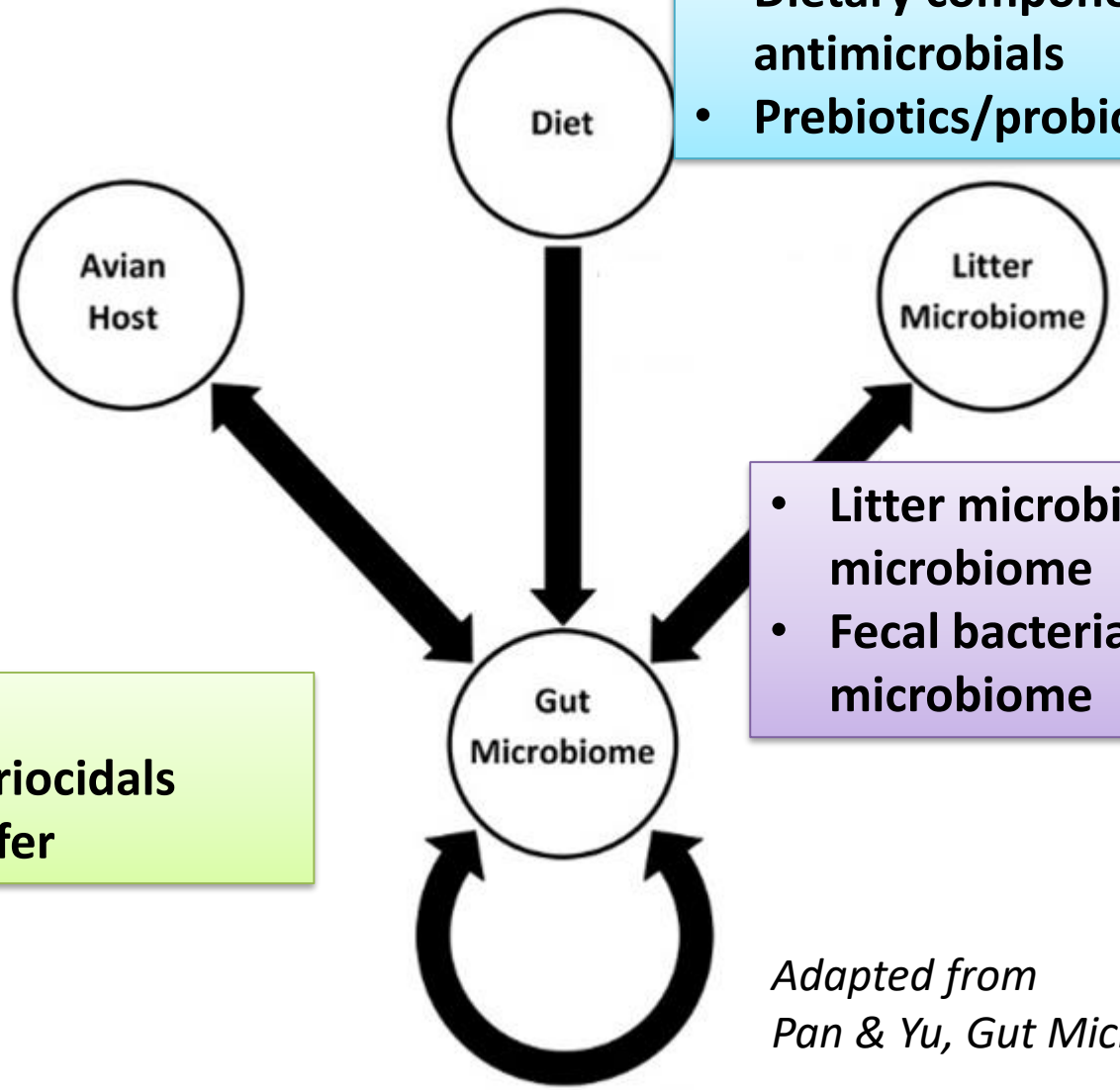


UTP University of Science and Technology, Faculty of Animal Breeding and Biology
Bydgoszcz, Poland

Avian host-diet-litter-microbiome interactions

- Dietary components and antimicrobials
- Prebiotics/probiotics/synbiotics

- Nutritional interactions
- Intestinal morphology and physiology
- Immune system



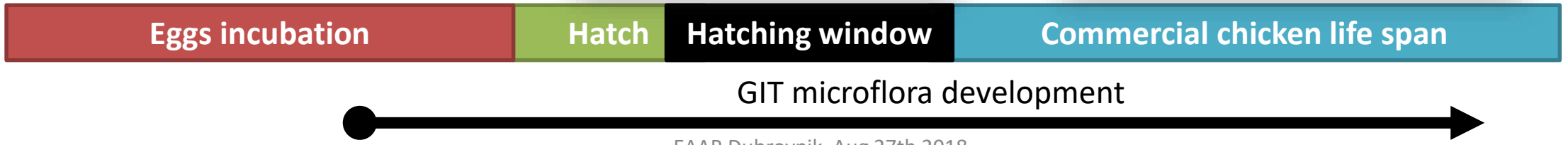
- Litter microbiome affects gut microbiome
- Fecal bacteria affect litter microbiome

- Competition
- Bacteriostatics/bactoriocidals
- Horizontal gene transfer

Adapted from
Pan & Yu, Gut Microbes 2014

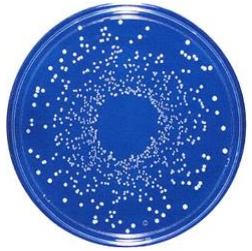
Perinatal (*in ovo*) stimulation of microflora development in chickens

- *In ovo* delivery of prebiotic/probiotic/synbiotic on day 12 ED to stimulate development of the embryonic microbiome
- Chicks hatch with developed gut microbiota
- Beneficial consequences for the life span condition
- Omitting „hatching window” (industrial application)

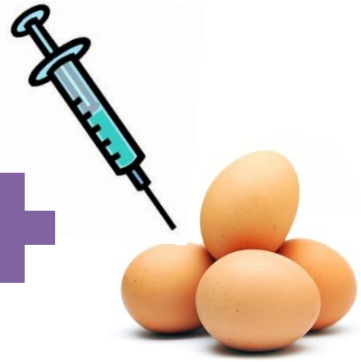


The goal of the study

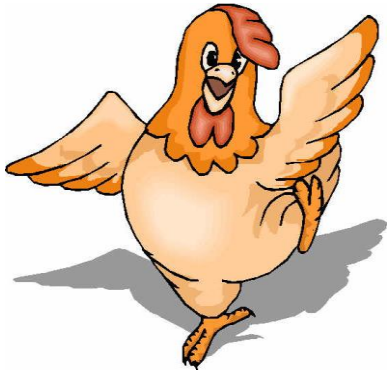
Prebiotic



Probiotic



Embryo



Phenotype

Host genetics ???

To determine the effect of the **avian host** on response to microbiome stimulation *in ovo*

Avian host: commercial vs. native chicken

Ross 308

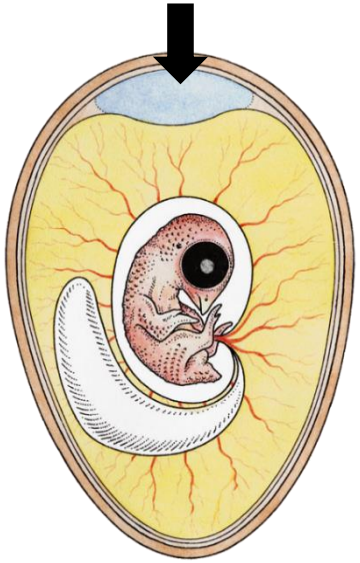
VS.

Green-legged Partridgelike (GP)

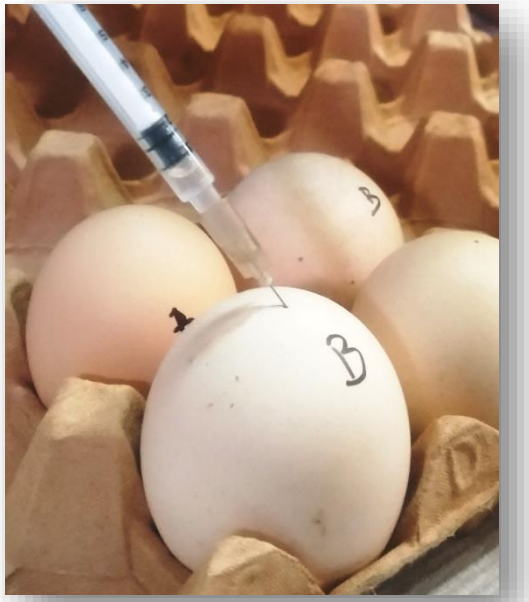
Gut microbiome stimulation *in ovo*

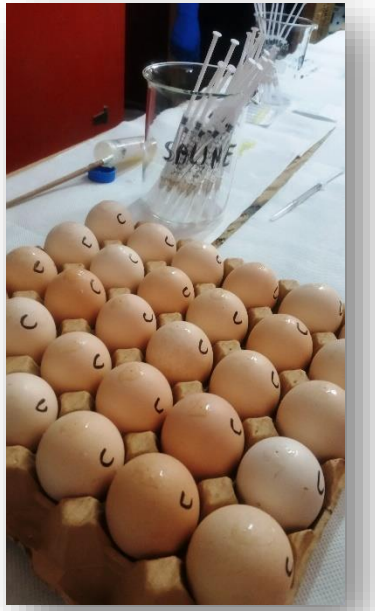
- **Prebiotic** – Galactooligosaccharides (GOS)
- **Probiotic** – *L. lactis* subsp. *cremoris* IBB477
- **Synbiotic** – GOS + *L. lactis* subsp. *cremoris* IBB477

Pre/Pro/Synbiotic
vs. saline (C)

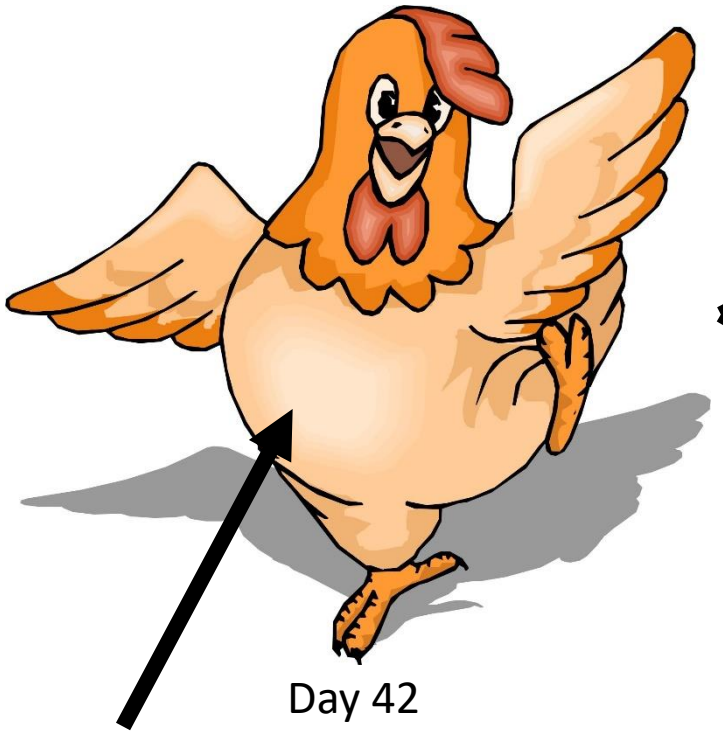


***In ovo* stimulation**





Phenotype: immune response to antigens



2 hrs post-injection

Cecal Tonsil
local gut immunity

Spleen
systemic immunity

Phenotype
Relative gene expression of interleukins (RT-qPCR):
IL1B – pro-inflammatory
IL2 – Th1
IL4 – Th2
IL6 – pro-inflammatory
IL10 – anti-inflammatory
IL12 – pro-inflammatory
IL17 – Th17

Injection of: **LTA (lipoteichoic acid)** or **LPS (lipopolysaccharide)**

Mimics G-positive bacteria
e.g. Lactobacillus

Mimics G-negative bacteria
e.g. Salmonella

Analysis

- 3x2 Experimental design (3 *in ovo* treatments x 2 breeds)
- 2-way ANOVA (linear model)
- Effects:
  - **Breed (*P* < 0.0001)**
  - *In ovo* treatment (NS)
  - Breed x *In ovo* treatment (NS)

Effect of genotype !!!!

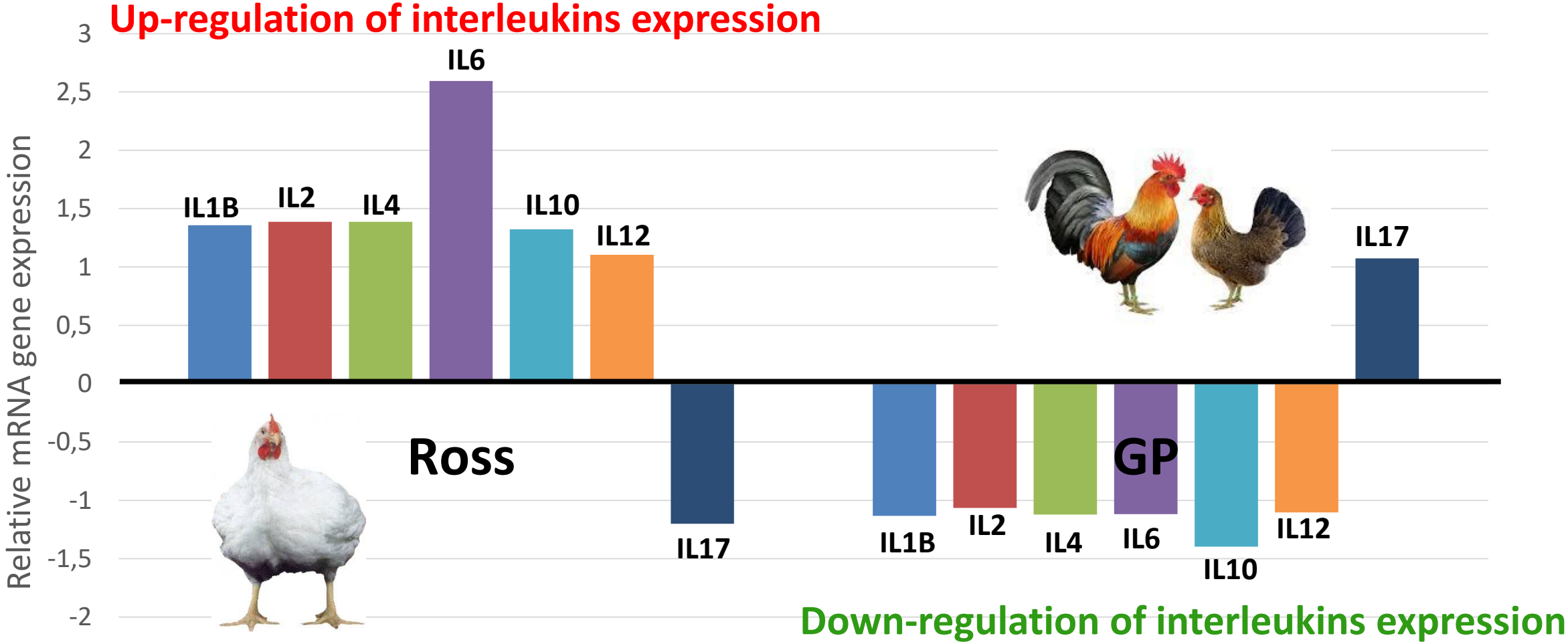
vs.

- The following slides present results from animals stimulated *in ovo* with synbiotic and challenged with LTA and LPS

Cecal tonsils

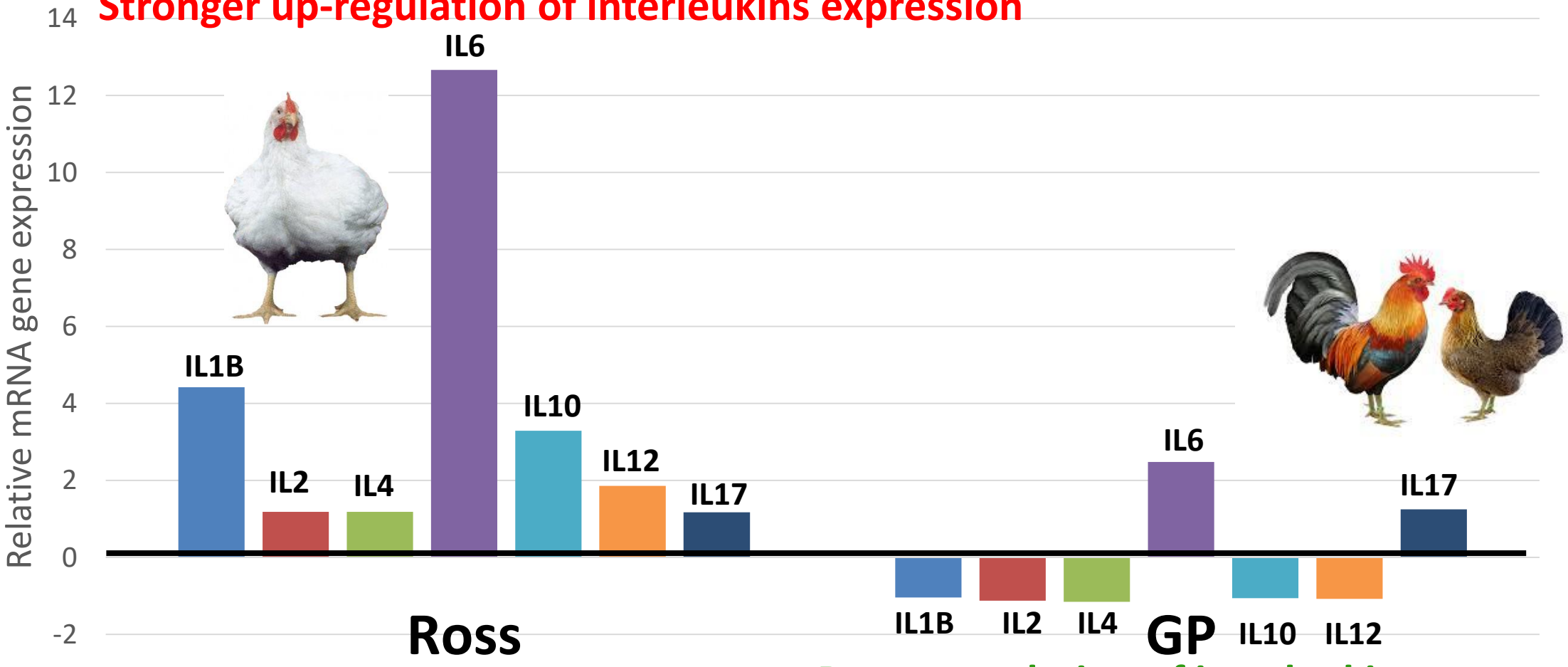
LOCAL IMMUNE RESPONSES

LTA: antigen from G-positive bacteria



LPS: antigen from G-negative bacteria

Stronger up-regulation of interleukins expression

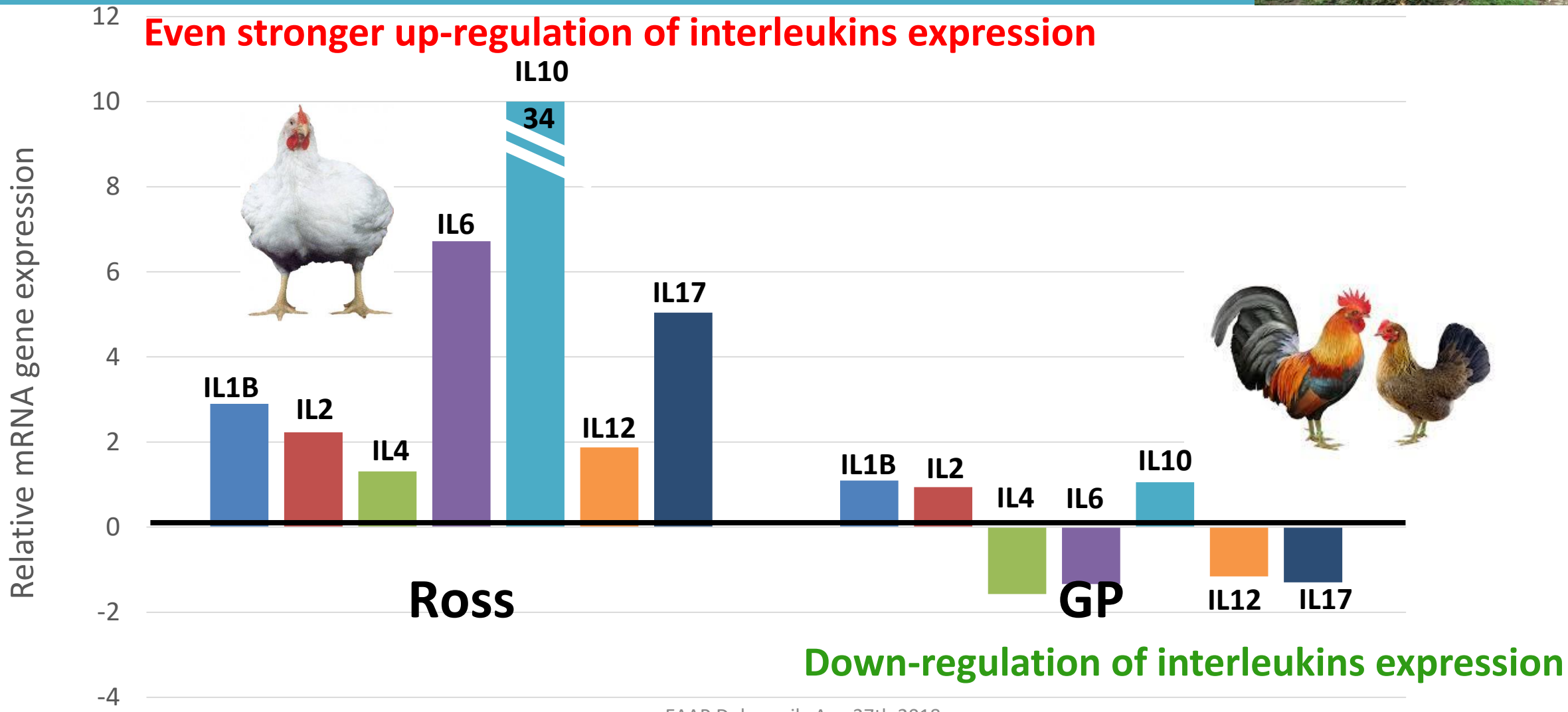


Down-regulation of interleukins expression

Spleen

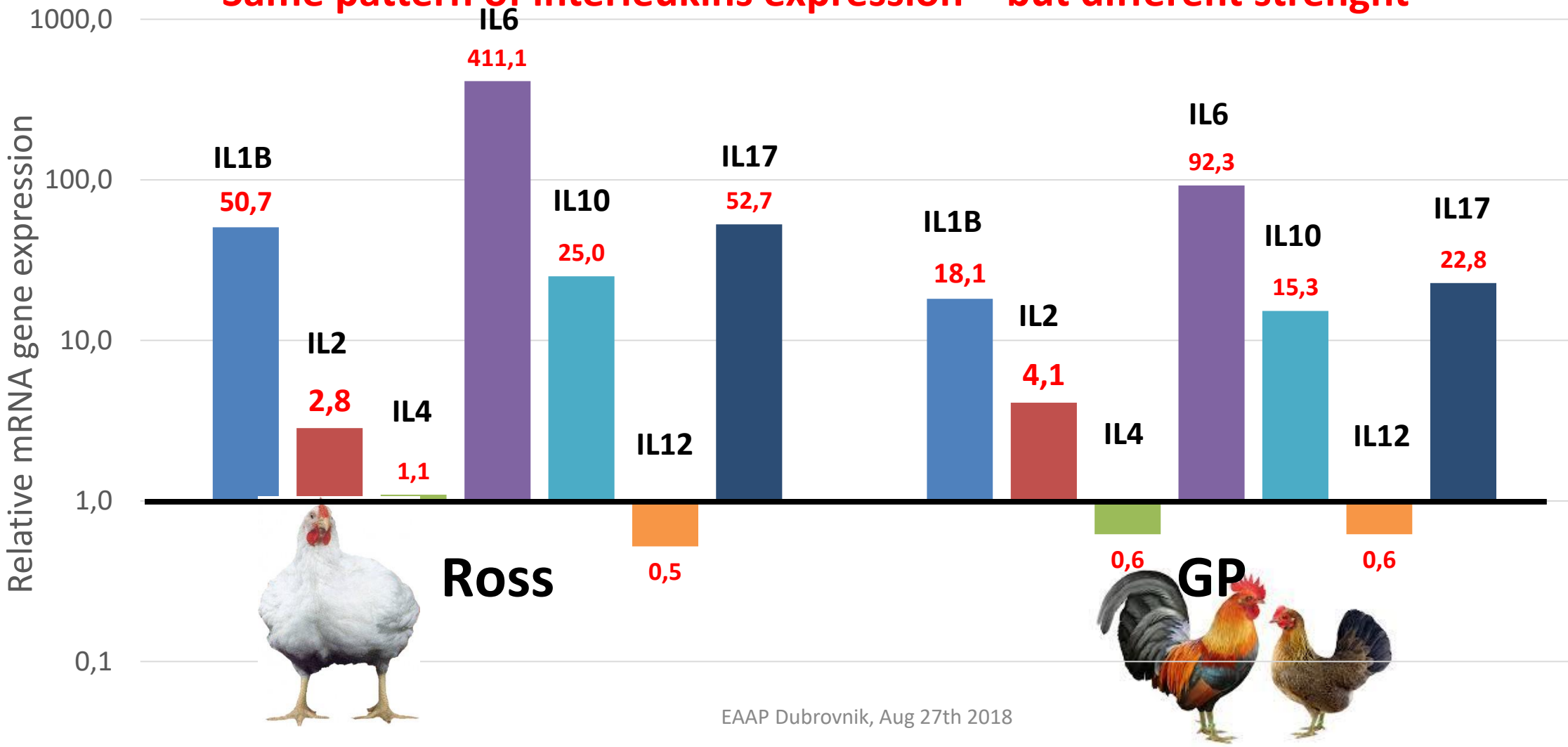
SYSTEMIC IMMUNE RESPONSES

LTA: antigen from G-positive bacteria



LPS: antigen from G-negative bacteria

Same pattern of interleukins expression – but different strenght



Conclusions & further goals

1. Genotype had stronger effect on the immune response than the bioactive substance injected *in ovo*
2. Immune responses triggered in chickens stimulated *in ovo* are breed-dependent ($P < 0.0001$)
3. Commercial broiler (Ross) reacted to antigenic challenge by triggering local and systemic immune responses
4. Native chicken (GP) did not response to challenge to LTA antigen; its systemic response to LPS antigen was significantly lower than in broiler chicken
5. Lower local immune responses indicate better tolerance of the immune system
6. Resilient animals express lower sensitivity to environmental antigens

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Thank you for your attention !!!