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INOCULATION OF FOUR COMMENSAL BACTERIA IN CHICKEN HAS AN IMPACT ON THE GUT MICROBIOTA COMPOSITION, SALMONELLA COLONISATION AND THE IMMUNE RESPONSE



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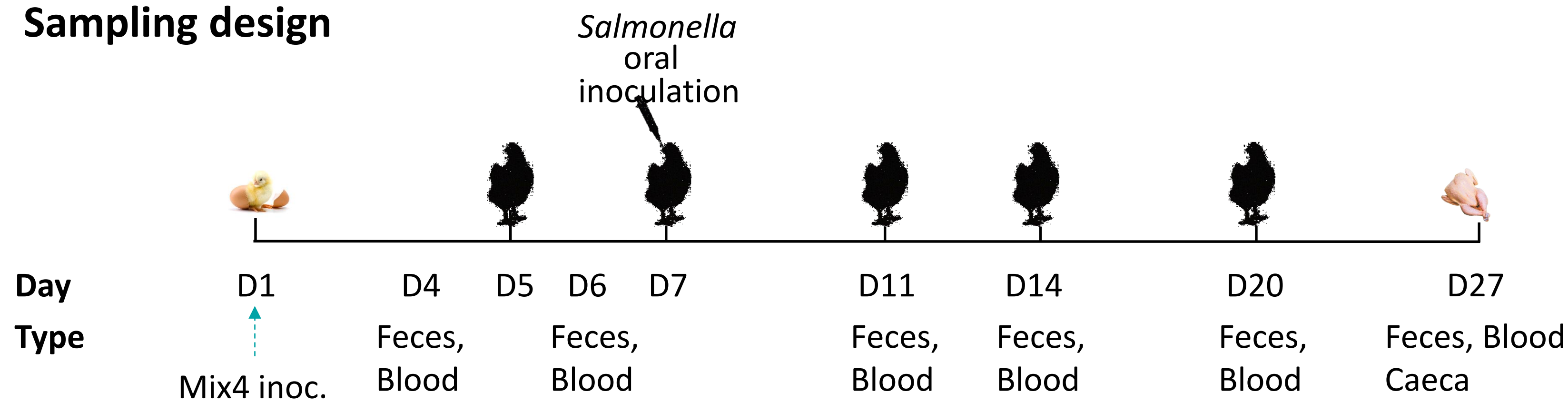
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Introduction

Salmonella infections are diseases of particular medical and economic interest in developed countries. *Salmonella*-infected host animals may present highly heterogeneous shedding levels, depending on the outcome of infection. Several studies have investigated the host-gut microbiota-pathogen interactions and their role in the occurrence of *Salmonella* super-shedding. They suggested the possibility to manipulate the relationships between *Salmonella* and the GM to reduce the fecal shedding.

In this project, we analysed the impact of four commensal bacteria inoculated before infection on: 1- the faecal and caecal microbiota composition ; 2- *Salmonella* colonization 3- the immune status of chicks measured in kinetic in blood.

Sampling design



→ **Isolator 1:** only *Salmonella* inoculation at 7 days of age

→ **Isolator 2:** Mix4 exposure prior *Salmonella* inoculation at 7 days of age

Mix4

- 1x *E. faecium*
- 1x *E. coli* ('Nissle' strain)
- 1x *Lactobacillus rhamnosus*
- 1x *Clostridium butyricum*

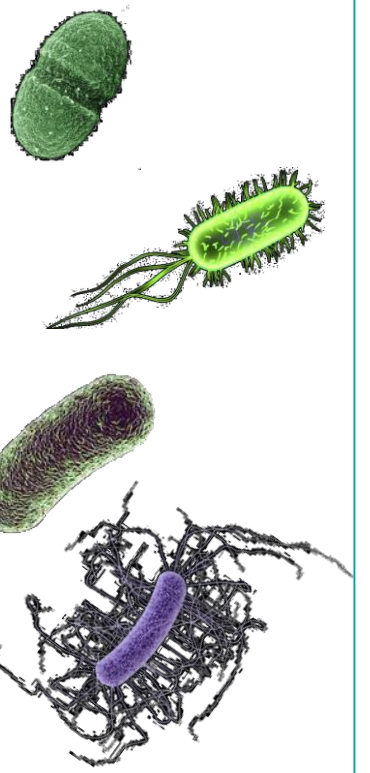


Fig 1) Impact of the Mix4 on gut microbiota β-diversities

Principal coordinate analysis summarizing Unifrac distances among the chicken bred in isolator 1 and 2, at every age category.

In **isolator 1**, 35 chickens were bred until 27 days of age and inoculated with *Salmonella* at 7 days of age. A subset of 14 chickens was kept for the metabarcoding characterization of their gut microbiota.

In **isolator 2**, 35 chickens were bred until 27 days of age, inoculated at 1 day of age with the Mix4, and infected with *Salmonella* at 7 days of age. A subset of 15 chickens was kept for the metabarcoding characterization of their gut microbiota.

We observed significant differences between the Unifrac β-diversities measured in isolator 1 and 2 at every age category except on day 4 (Permanova tests).

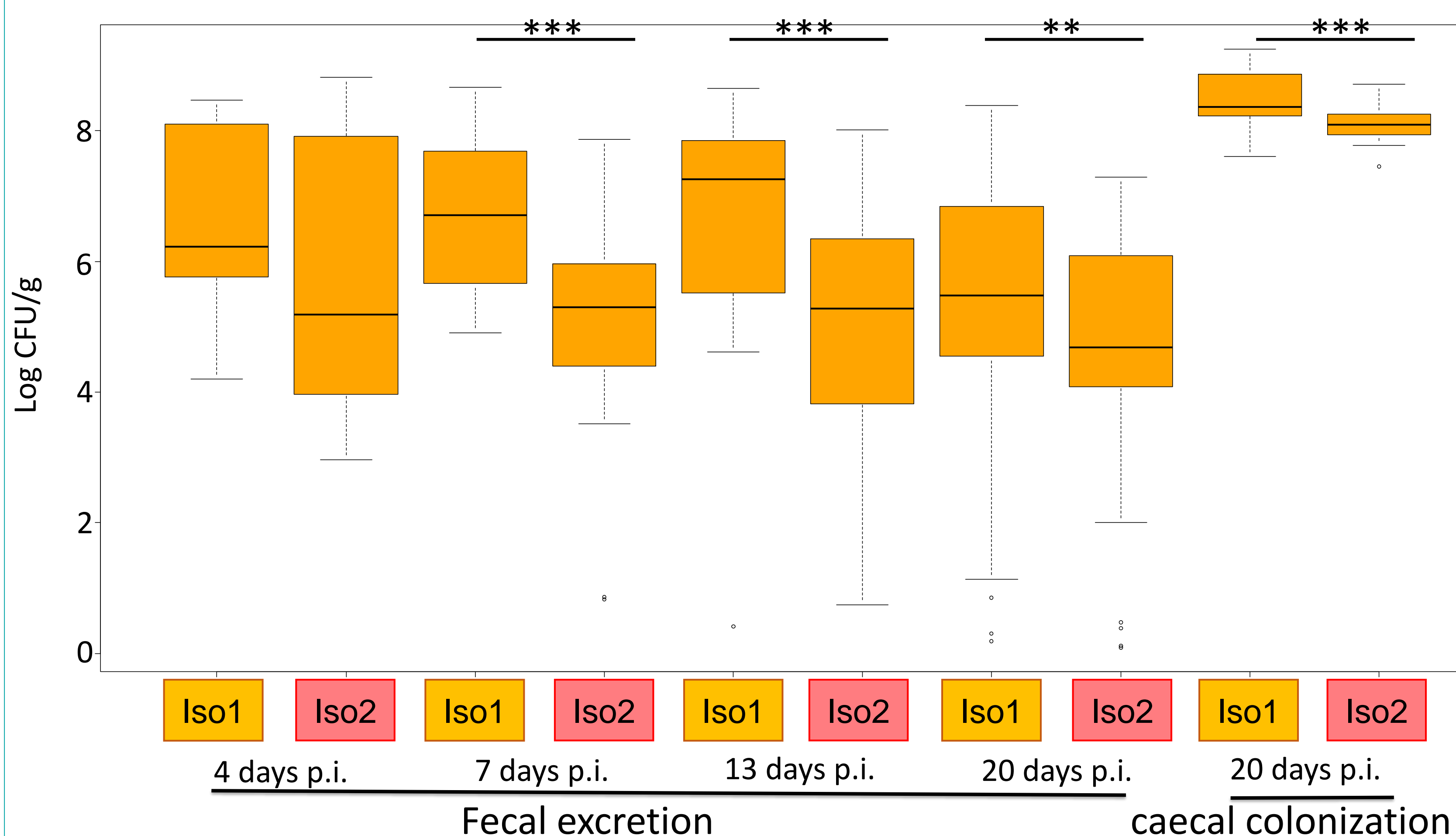
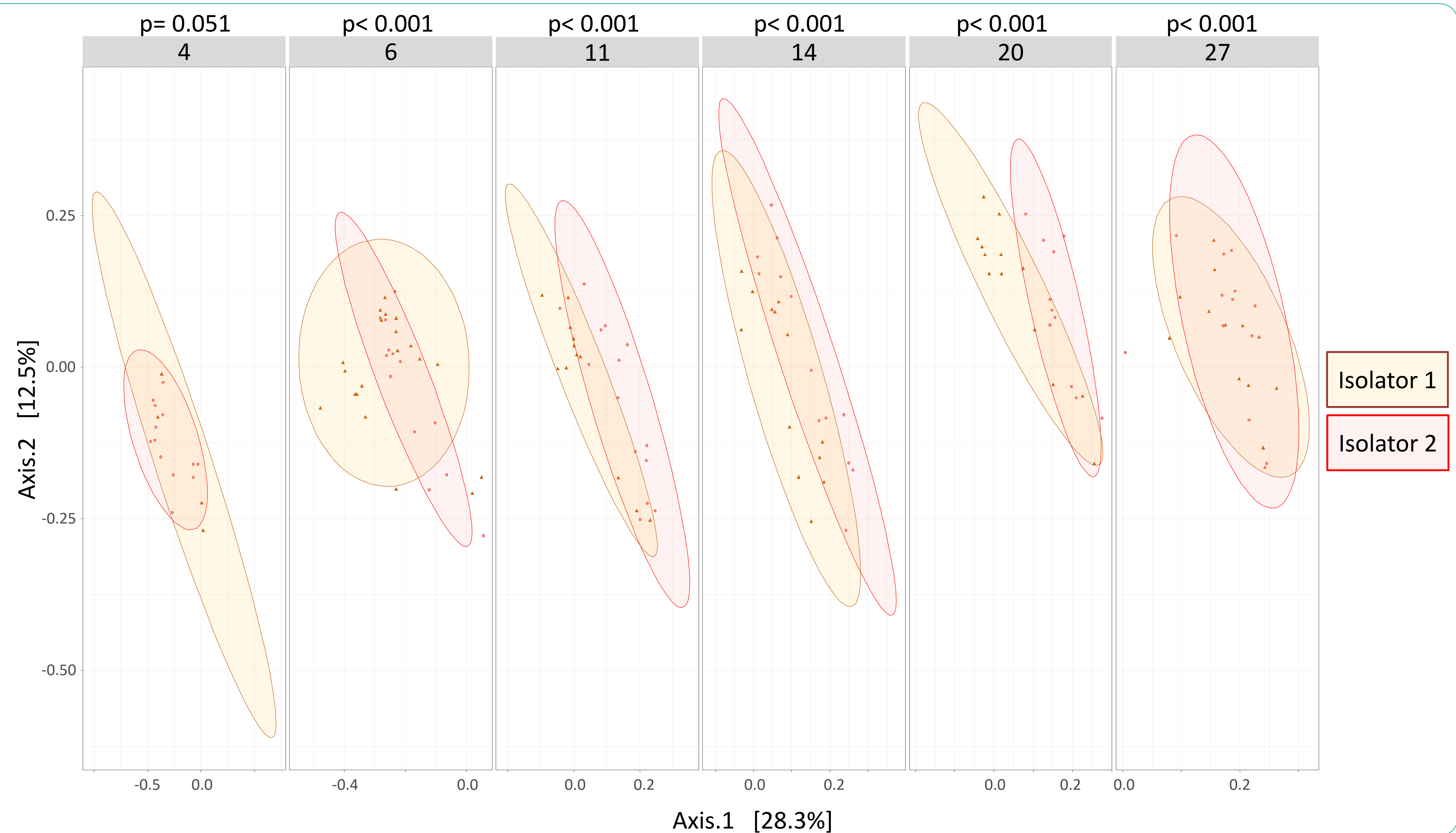


Fig 2) Impact of the Mix4 on *Salmonella* colonization

Levels of *Salmonella* shedding in **isolator 1** and **2** (cf. Fig1) after *Salmonella* infection, assessed using bacteriologic counts. At 14, 20 and 27 days of age, the difference between Isolator 1 and 2 was significant.

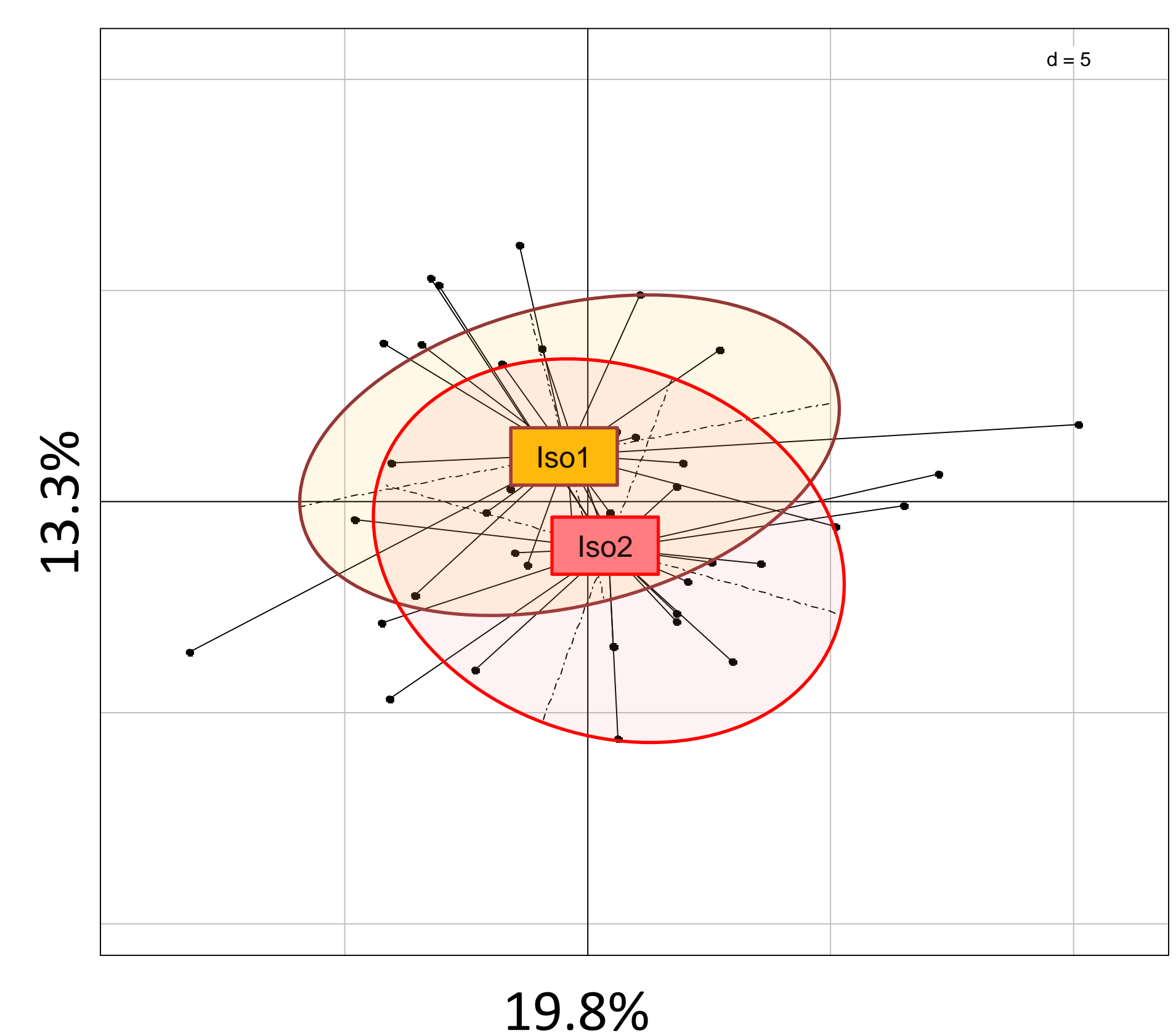


Fig 3) Impact of the Mix4 on the immune response

PCA summarizing the patterns of 66 immune gene expression levels at 11 days of age (i.e. 4 days post infection) in **isolator 1** and **2**. The levels of gene expression were assessed using a medium-throughput qPCR approach (Biomark).

The patterns were slightly different (p=0.046, between group analysis, Monte Carlo permutation test). The same result was found at 14 days of age (p=0.044; not shown).

Conclusion

Inoculation of four commensal bacteria (namely: Mix4) after hatching durably modified gut microbiota composition and *Salmonella* colonization. The inoculation of these commensal bacteria also induces a slightly different immune response against *Salmonella* infection. These results pave the way of the development of bacterial consortia able to increase the resistance of chicks to pathogens.